Digestive Diseases and Sciences, Vol. 43, No. 2 (February 1998), pp. 335–343

Human Gallbladder Mucosal Function

Effects on Intraluminal Fluid and Lipid Composition in Health and Disease

STEFANO GINANNI CORRADINI, MD, PhD, GUNJI YAMASHITA, MD, HANNU NUUTINEN, MD, ANN CHERNOSKY, CARLUMANDARLO WILLIAMS, Lab Tech, LEIGH HAYS, Lab Tech, MITCHELL L. SHIFFMAN, MD, R. MATTHEW WALSH, MD, JOAR SVANVIK, MD, PAOLA DELLA GUARDIA, MD, LIVIO CAPOCACCIA, MD, and R. THOMAS HOLZBACH, MD

Gallbladder mucosal absorption of fluid during fasting is a well-known process. Indirect *in vivo* and recent *in vitro* evidence for physiologically relevant gallbladder absorption of cholesterol and phospholipids from bile has been observed in humans. The present study explored and compared by indirect means the relative efficiences of human gallbladder mucosal absorption of fluid and lipids in health and disease. Biliary lipids and pigment content were measured in fasting gallbladder bile samples obtained from gallstone-free controls and from four study groups: multiple and solitary cholesterol gallstone patients, and morbidly obese subjects with and without gallstones. Bile salts and pigment content were significantly greater in gallstone-free controls than in all other disease study groups. This was interpreted as evidence of more effective gallbladder mucosal fluid absorption in nonobese gallstone-free controls compared to that in all other groups. Correlation plot analyses of biliary lipids showed lower concentrations of phospholipids than expected from the index bile salt concentrations. The same was found for cholesterol concentrations but only in supersaturated samples. These findings were much more pronounced in gallstone free-controls and were accordingly interpreted as evidence of more efficient gallbladder absorption of both phospholipids and cholesterol in controls compared with that found in each of the disease study groups. Moreover, impaired gallbladder mucosal function, while invariably associated with cholesterol gallstone disease, was not found to be a necessary consequence of the physical presence of stones. It is concluded that efficient gallbladder mucosal absorption of both fluid and apolar lipids from bile is a normal physiological process that is often seriously impaired in the presence of either cholesterol gallstone disease or at least one of its precursor forms.

KEY WORDS: gallbladder mucosa; mucosal fluid absorption; lipid absorption; cholesterol gallstones; obesity.

The bile salt concentration in normal human gallbladder bile samples, obtained under fasting conditions, averages 150–200 mmol/liter as a result of the well-known fluid absorption capacity of the gallbladder mucosa (1–4). Under physiologic conditions, bile salts are only minimally absorbed by the gallbladder

Manuscript received March 20, 1997; revised manuscript received August 13, 1997; accepted October 6, 1997.

From the Gastrointestinal Research Unit, Research Institute, Departments of Gastroenterology and General Surgery, Cleveland Clinic Foundation, One Clinic Center, 9500 Euclid Ave., Cleveland, Ohio 44195; and Department of Gastroenterology, Box 711, MCV Station, Virginia Commonwealth University, Richmond, Virginia 23298.

This work was supported by Research Grant DK-17562 from the USPHS National Institutes of Health. A portion of the work was presented in preliminary form at the annual meeting of the American Association for the Study of Liver Disease, Chicago, Illinois, November 5, 1993, and published as an abstract in *Hepatology* 18: 97A, 1993. Dr. Ginanni Corradini (Italy) was the recipient of a National Institutes of Health Fogarty International Research Fellowship. Drs. Yamashita and Nuutinen were supported in part by the Research Institute, Cleveland Clinic Foundation. Dr. Nuutinen was also partially supported by the Finnish Cultural Foundation and by the Paulo Foundation (Helsinki).

and comprise, by mass, the largest single component of total biliary lipids (5–7). A reduced total lipid concentration in the gallbladder bile during fasting in the presence of cholesterol gallstone disease is a common finding (2, 3, 8, 9). In addition, the fasting gallbladder to hepatic bile ratio for bile salt concentration, a rough estimate of the efficiency of fluid absorption by the gallbladder, is several times higher in subjects free of gallbladder disease when compared to that for cholesterol gallstone patients (10). The most likely explanation for these findings is an impairment of the highly efficient normal mucosal fluid absorption process that occurs at some stage in the evolution of cholesterol gallstone disease. Conclusive evidence for this impairment has not yet been provided, and it remains unclear whether such a defect, if present, can precede or is always a consequence of the presence of the stones. This problem, however, is very difficult to investigate prospectively in humans.

Biliary cholesterol and phospholipid absorption by the gallbladder mucosa likewise has been previously demonstrated in a number of settings, eg, *in vitro* by using human gallbladder fragments or cultured epithelium (11–14), as well as in various *in vivo* animal studies (15–17). In addition, one study of gallstone-free morbidly obese patients has raised the possibility that concomitant mucosal absorption *in vivo* of fluid, cholesterol, and phospholipids occurs in this population (18). Recent data obtained *in vitro* in intact human gallbladders show that major cholesterol and phospholipid mucosal absorption from bile occurs in the absence of cholesterol gallstone disease but that this process is significantly decreased when cholesterol gallstone disease is present (19).

The purpose of the present study was to explore gallbladder mucosal function as inferred from differences in fasting bile solute concentrations measured in a single sample obtained from a large number of control and four separate disease study groups, including one of morbidly obese gallstone-free subjects, a population known to be at high risk for gallstone development. The concentrations of nonabsorbable solutes, ie, biliary pigments and bile salts, were considered as indexes of the efficiency of fluid absorption. Correlation plots of the concentrations of absorbable biliary lipids, ie, cholesterol and phospholipids, versus concentrations of bile salts were taken as indicative of the degree of cholesterol and phospholipid absorption in the control and the four disease study groups.

MATERIALS AND METHODS

Subjects and Sampling of Bile. With the approval of the Cleveland Clinic Foundation's Research Projects and Institutional Review Committee Regarding Human Studies, gallbladder bile was obtained by aspirating the gallbladder during surgery after an overnight fast. The following subject groups were included: Group A included 26 nonobese gallstone-free subjects undergoing intraabdominal surgery for gastric ulcer (3), duodenal ulcer (2), esophageal reflux (2), gallbladder adenomyoma (1), gastric carcinoma (7), early gastric cancer (4), colon cancer (5), or small hepatocellular carcinoma in the absence of chronic liver disease (2). This heterogeneous control group is referred to here as the gallstone-free control group; no chemotherapy was administered prior to surgery in any of the patients. In group B were 13 nonobese patients with multiple symptomatic cholesterol gallstones found at laparoscopic cholecystectomy; in group C, 9 nonobese patients with symptomatic solitary cholesterol gallstones found at laparoscopic cholecystectomy; in group D, 10 morbidly obese subjects with no previous history of biliary disease found to have cholesterol gallstones (9 multiple and 1 solitary) by intraoperative ultrasound at surgery for gastric bypass; and in group E, 20 morbidly obese gallstone-free patients at surgery for gastric bypass.

In patients without gallstones, bile was aspirated after the needle was passed through the anterior lip of the liver and inserted into the gallbladder. In the gallstone groups, bile was sampled immediately after the cystic duct was clamped. Care was taken to reduce stratification of bile before aspiration (20). No significant difference was present in the mean aspirated volume among groups ($P = 0.63$). Absence of gallstones in the stone-free groups was confirmed by either intraoperative ultrasonography or gallbladder palpation. Gallstones were classified as cholesterol in type by conventional morphological criteria (2) and by the presence of cholesterol crystals in the sediment after centrifugation of fresh bile (15,000 *g* for 30 min).

Samples were excluded if they showed a positive test for blood contamination (Hemoccult, Smith Kline Diagnostics, Inc., San Jose, California) or if evidence for nonneoplastic liver disease, cholestasis, cystic duct obstruction, or biliary sepsis was present. A total lipid concentration of less than 5 g/dl and/or nonopacification of the gallbladder at oral cholecystography have been used as exclusion criteria in previous studies (2–4, 8, 9). These were selected as exclusion criteria as a nonopacifying gallbladder on oral cholecystography and a very low total lipid concentration in gallbladder

Dr. Ginanni Corradini's, Della Guardia's, and Capocaccia's present address: Universitá degli Studi di Roma I, Dipartimento di Medicina Clinica, Divisione di Gastroenterologia, Universitá "La Sapienza," Viale dell'Universitá 37, 00158 Rome, Italy.

Dr. Yamashita's present address: First Department of Internal Medicine, Hiroshima University School of Medicine, Kasumi 1-2-3, Hiroshima 734, Japan.

Dr. Nuutinen's present address: Second Department of Medicine, University of Helsinki, 00290 Helsinki, Finland.

Dr. Shiffman's present address: Hepatology Section, Department of Gastroenterology, Box 711, MCV Station, Virginia Commonwealth University, Richmond, Virginia 23298.

Dr. Svanvik's present address: Department of Surgery, University of Linköping, S-581 85 Linköping, Sweden.

Address for reprint requests: Dr. Stefano Ginanni Corradini, Via Asmara 9-B, 00199 Rome, Italy.

bile coexist when the cystic duct is obstructed (21). It was decided not to apply these exclusion criteria as we found in the present study that 25% of our morbidly obese gallstone-free subjects had a total lipid concentration of less than 5 g/dl.

Bile samples were carefully mixed, divided into aliquots, immediately frozen at −80°C, and protected from light exposure until analyzed. Every assay run was performed to ensure that an equal proportion of randomly selected samples from each study group was represented. Histological examination of the removed gallbladders failed to reveal evidence of either acute or marked chronic cholecystitis using conventional histological criteria.

Lipid Analysis. Bile was thawed, vortexed thoroughly, and extracted in methanol (1:100 v/v) for bile salt and in isopropanol (1:30 v/v) for cholesterol and phospholipid measurements. Extracted samples were centrifuged (3000 *g* for 10 min), and the supernatant was assayed. Total bile salt concentration was measured by the 3α-hydroxysteroid dehydrogenase method (22). Phospholipid concentrations were measured by a kit modification that uses the enzymatic method of Takayama (PI-Kit K, Nippon Shoji Kaisha, Ltd., Osaka, Japan) (23, 24). Cholesterol concentrations were assayed enzymatically using a commercially available assay kit (Boehringer-Mannheim Corp., Indianapolis, Indiana) (25). Every assay was performed in triplicate and also included a sample blank to correct for possible artifacts arising from differences in pigment content among samples.

Lipid Regression Analysis. Regression plots of individual gallbladder bile sample concentrations of cholesterol and phospholipids as a function of bile salt concentrations were used to derive their regression lines, where possible, according to an approach recently introduced by Schiffman et al (18). As reported by these authors, depending on the slopes of the regression lines obtained, it was observed that for a given 100% increase in the level of sample bile salt concentrations (taken as an endogenous nonabsorbed marker), there were variable but lesser increases (ie, relative decreases) in the cholesterol and phospholipid concentrations, to as low as only a 70–80% relative increase. Similar plots relating bile salt, cholesterol, and phospholipid concentrations (expressed as relative moles per 100 ml for a given sample) were also found useful. In the present study, we further segregated the sample analysis into cholesterol supersaturated and cholesterol undersaturated subgroups. An underlying assumption in the use of this procedure is that the disproportionately lower concentrations of cholesterol and phospholipid in relation to bile salts over the range of these plots primarily represents variations in mucosal absorption of the lipids. A limitation of this new approach is that data are not derived from either simultaneous gallbladder and common duct bile samples or repeated samples from each gallbladder at timed intervals to permit a kinetic analysis. However, by studying reasonably large and well-defined patient groups, this constraint has been largely overcome. Increases in bile salt concentrations and cholesterol saturation index observed with differing durations of fasting in a comparable study (18), for example, were very similar to those observed in individual patients over varying time intervals (26). Owing to the nature of the present method, however, interpretive statements concerning mucosal function must be construed as operative and inferential based on the necessary indirectness of the data analysis method employed.

Pigment Content. Pigment content was estimated in duplicate as follows: bile was thawed, vortexed thoroughly, and extracted 1:100 (v/v) in methanol. After centrifugation (2000 *g* for 5 min), the supernatant was isolated and absorption at 450 nm was determined spectrophotometrically. Although the method we used to measure pigment content of bile is crude, it is suitable for a semiquantitative comparison among groups.

To further assess whether different levels of pigment content could interfere with the cholesterol assay, we performed additional "spiking" and recovery experiments for cholesterol. Cholesterol was added in increasing amounts to two different bile samples. These samples were mainly characterized by a major difference in pigment content: one had a high (OD at 450 nm = 2.75) and the other a low pigment content (OD at 450 nm = 0.81), but both had a low concentration of endogenous cholesterol.

Statistical Analysis. Intergroup differences were analyzed first by the Kruskal-Wallis test. The variables found to differ significantly between groups by this analysis were then submitted to multiple pairwise comparisons using the Wilcoxon rank-sum test. To control for overall error, Bonferroni's correction was used to adjust the alpha level to 0.005 for intergroup differences found significant by the Wilcoxon test. Regression lines were fitted to the data using simple linear regression and the regression lines were compared using the *F* test (27). *P* values of less than 0.05 were considered significant for the regression equations of the lipid plots (28). Calculations were performed using the SAS software package (SAS Institute, Inc., Cary, North Carolina).

RESULTS

Patient Demographics. Patient demographics are shown in Table 1. The morbidly obese patients with and without gallstones were younger than the subjects in the other study groups. The gallstone-free control group had relatively more males than the other study groups. No difference was found in any of the lipid and pigment values when the gallstone-free control group was divided into two subgroups according to sex or diagnosis, ie, noncancer or cancer (data not shown).

Lipid and Pigment Concentrations. A comparison of biliary lipid variables (means ± SD) in samples from the patient study groups is shown in Table 2. The absolute concentrations of cholesterol and phospholipids did not significantly differ among groups. The gallstone-free control group had a significantly higher bile salt concentration, nearly twofold greater compared to all other groups (Figure 1). The gallstone-free control group also had a significantly higher bile salt molar percent than all other groups. This group is also characterized by a significantly lower phospholipid molar percent than the two mor-

TABLE 1. DEMOGRAPHIC CHARACTERISTICS OF 78 PATIENTS FROM WHOM GALLBLADDER BILE WAS ASPIRATED

	Nonobese			*Morbidly obese*	
	Gallstone-free (N = 26; mean ± SD)	*Multiple gallstones (N = 13; mean ± SD)*	*Solitary gallstones (N = 9; mean ± SD)*	*Gallstone-free (N = 20; mean ± SD)*	*Gallstones (N = 10; mean ± SD)*
Age (yr)	51.4 ± 9.2	51.5 ± 4.7	51.9 ± 12.9	37.9 ± 7.3*	33.4 ± 8.2*
Sex (M/F)	18 / 8	5 / 8	5 / 4	5 / 15	2 / 8
Weight (kg)	65.2 ± 9.3	69.2 ± 8.6	67.7 ± 4.3	136.1 ± 28.7†	137.3 ± 12.2†

*$P < 0.005$ versus all nonobese groups.
† $P < 0.001$ versus all nonobese groups.

bidly obese groups and the nonobese solitary stone group. In addition, a significantly lower cholesterol molar percent is found in the gallstone-free control group compared to the nonobese multiple stone group. No difference in the cholesterol-to-phospholipid molar ratio was seen among any of the groups. The cholesterol-to-bile salt molar ratio was significantly higher in the nonobese multiple gallstone patients and in the morbidly obese gallstone subjects than in the gallstone-free controls. All the disease study groups had a higher phospholipid-to-bile salt molar ratio than the gallstone-free controls (Table 2). The gallstone-free control group had a significantly increased pigment content compared to all other groups except for the nonobese solitary gallstone group (Figure 2). The failure of the nonobese solitary gallstone group to reach a statistically significant difference is probably due to the fact that pigment content measurement for this group was only available from seven samples (possible type II statistical error).

Lipid Correlation Studies. Figure 3A depicts a plot of cholesterol and bile salt concentrations in samples from the four disease study groups, all of which had low bile salt and pigment levels. All these groups showed a similar pattern. Significant positive correlations were present for cholesterol supersaturated ($r = 0.81$, $P < 0.0001$) and undersaturated samples ($r = 0.91$, $P < 0.0001$). From the regression equation for the supersaturated samples ($y = 4.09 + 0.12x$), it can be calculated that for every 100% increase in bile salt concentration (taking as a starting point a bile salt concentration of 100 mmol/liter) proportionate concentration increases for cholesterol are only 75%.

TABLE 2. COMPARISON OF GALLBLADDER BILIARY LIPID VARIABLES IN 78 PATIENTS

	Nonobese			*Morbidly obese*	
	Gallstone-free (N = 26; mean ± SD)	*Multiple gallstones (N = 13; mean ± SD)*	*Solitary gallstones (N = 9; mean ± SD)*	*Gallstone-free (N = 20; mean ± SD)*	*Gallstones (N = 10; mean ± SD)*
Cholesterol (mM)	17.06 ± 9.17	15.72 ± 5.24	13.25 ± 8.15	13.74 ± 9.2	10.08 ± 5.65
Phospholipids (mM)	35.48 ± 15.43	32.72 ± 16.81	39.42 ± 18.25	36.71 ± 17.8	23.2 ± 12.3
Bile salts (mM)	187.07 ± 57.01	97.32 ± 49.91[c]*	103.83 ± 57.14[a]	102.88 ± 55.06[c]	72.85 ± 58.0[c]
Total Lipids (g/dl)	12.54 ± 3.46	7.87 ± 3.24b	8.6 ± 3.92	8.37 ± 4.25[b]	5.9 ± 3.76[c]
Cholesterol (mol/100 ml)	7.26 ± 3.33	11.38 ± 2.78a	8.83 ± 4.89	9.09 ± 3.86	10.78 ± 4.71
Phospholipids (mol/100 ml)	15.19 ± 5.75	23.22 ± 10.83	26.83 ± 13.36[a]	24.84 ± 3.61[c]	24.49 ± 5.94[b]
Bile salts (mol/100 ml)	77.56 ± 7.35	65.40 ± 12.07[a]	64.34 ± 12.0[a]	66.07 ± 5.57[c]	64.72 ± 10.04[b]
Cholesterol saturation index	1.31 ± 0.64	1.90 ± 0.79	1.33 ± 0.088	1.25 ± 0.52	1.71 ± 0.75
Cholesterol/phospholipid (molar ratio)	0.539 ± 0.34	0.62 ± 0.41	0.417 ± 0.32	0.37 ± 0.16	0.433 ± 0.12
Cholesterol/bile salt (molar ratio)	0.097 ± 0.05	0.185 ± 0.07[a]	0.14 ± 0.08	0.142 ± 0.07	0.182 ± 0.12[a]
Phospholipids/bile salt (molar ratio)	0.204 ± 0.1	0.4 ± 0.27[b]	0.474 ± 0.37[b]	0.38 ± 0.8[a]	0.403 ± 0.18[a]
Pigment content (453 nm O.D.)	1.864 ± 0.91	0.914 ± 0.52[a]	1.43 ± 0.70†	0.713 ± 0.38[c]	0.808 ± 0.53[a]

*[a], $P < 0.005$; [b], $P < 0.001$; [c], $P < 0.0005$, comparison with gallstone-free normals.
† Measurements available in 7 samples.

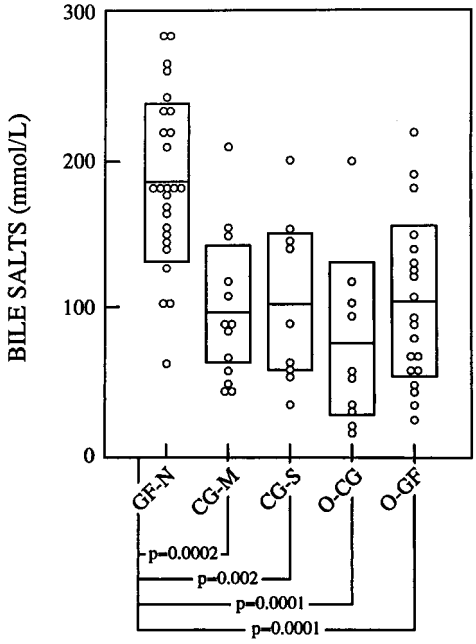

Fig 1. Bile salt concentrations (including means ± SD) in bile samples from gallstone-free normals (GF-N) compared with those from the four disease study groups: patients with multiple gallstones (CG-M) or with solitary stones (CG-S), morbidly obese subjects with gallstones (O-CG), and morbidly obese subjects without gallstones (O-GF). The bile samples from gallstone-free normals had significantly higher bile salt concentrations than all other groups.

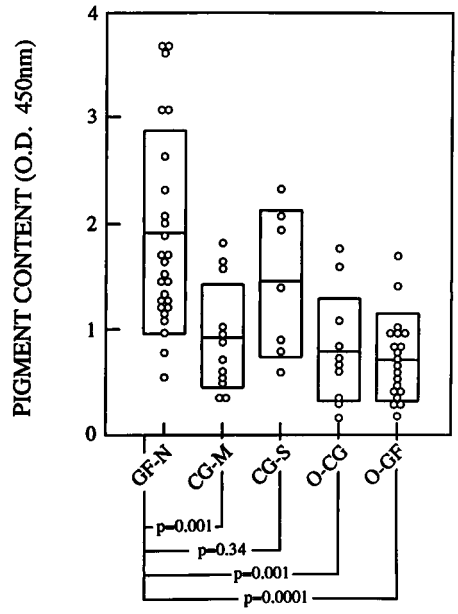

Fig 2. Pigment content (including means ± SD) in bile samples from gallstone-free normals (GF-N) compared with those from the four disease study groups: patients with multiple gallstones (CG-M) or with solitary stones (CG-S), morbidly obese subjects with gallstones (O-CG), and morbidly obese subjects without gallstones (O-GF). For sample measurement details, see Materials and Methods section. The bile samples from gallstone-free normals had significantly higher pigment content than patients with multiple gallstones and morbidly obese subjects either with or without gallstones.

However, when the same calculation is performed with the undersaturated samples using the relevant equation ($y = -0.61 + 0.08x$), a given 100% increase in bile salt concentrations is accompanied by a parallel 108% proportional increase in cholesterol.

When the cholesterol and bile salt concentrations were plotted for the gallstone-free control group (Figure 3B), the undersaturated samples showed a pattern similar to that of the undersaturated samples of all the other groups. Again, a positive correlation ($r = 0.91$, $P < 0.01$) was present. From the regression equation ($y = 0.27 + 0.04x$), it can be calculated that for every 100% increase in bile salt concentration, a similar increase in cholesterol concentration (94%) occurs. Unexpectedly, a correlation was not found between bile salt and cholesterol concentrations in the supersaturated samples obtained from gallstone-free control subjects.

Figure 4A shows the relation between phospholipid and bile salt concentrations for all groups except gallstone-free controls. The supersaturated samples show a positive correlation ($r = 0.74$, $P < 0.001$). From the regression equation for this line ($y = 8.7 + 0.25x$), it can be calculated that for every 100% increase in bile salt concentration, an increase in phospholipid concentration of only 74% occurs. The regression line for undersaturated samples is nearly the same as that for supersaturated samples. In the gallstone-free control group (Figure 4B), no distinct patterns or correlations between phospholipid and bile salt concentrations were found in either the supersaturated or undersaturated samples.

Figure 5A shows the relation between the cholesterol molar percent and the bile salt concentration of samples from all study groups. In the cholesterol-supersaturated samples, a negative correlation was present ($y = 13.62 + -0.03x$, $r = -0.51$, $P < 0.001$) indicating that as bile salt concentration increased, the cholesterol molar percent decreased. The undersaturated samples, however, showed no correlation, indicating that the cholesterol molar percentage was stable over the entire range of bile salt concentrations.

In Figure 5B, the relation between the phospholipid molar percent and the bile salt concentration is shown. In both cholesterol supersaturated ($y = 29.78 + -0.08x$, $r = -0.63$, $P < 0.0001$) and undersaturated samples ($y = 38.57 + -0.1x$, $r = -0.74$, $P <$

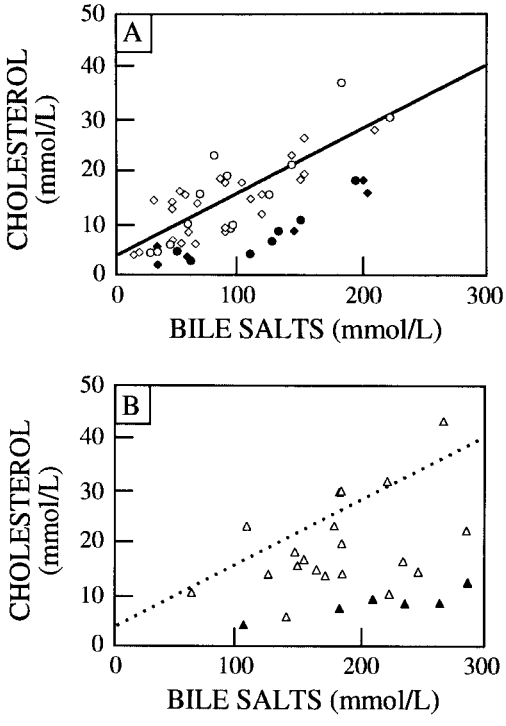

Fig 3. (A) Correlation plot of cholesterol and bile salt concentrations in bile samples from the four disease study groups. Open symbols: cholesterol supersaturated samples; solid symbols: cholesterol undersaturated samples; circles: morbidly obese subjects without gallstones, diamonds: the three groups with gallstones, ie, morbidly obese subjects, and nonobese gallstone patients with either multiple or solitary stones. The regression line for the supersaturated samples ($y = 4.09 + 0.12x$, $r = 0.81$, $P < 0.0001$) is depicted. (B) Correlation plot of cholesterol and bile salt concentrations in bile samples from the gallstone-free normal group. Open triangles: supersaturated samples; solid triangles: undersaturated samples. The interrupted line represents the regression for the corresponding plots in the cholesterol supersaturated samples from all of the disease study groups (see Figure 3A).

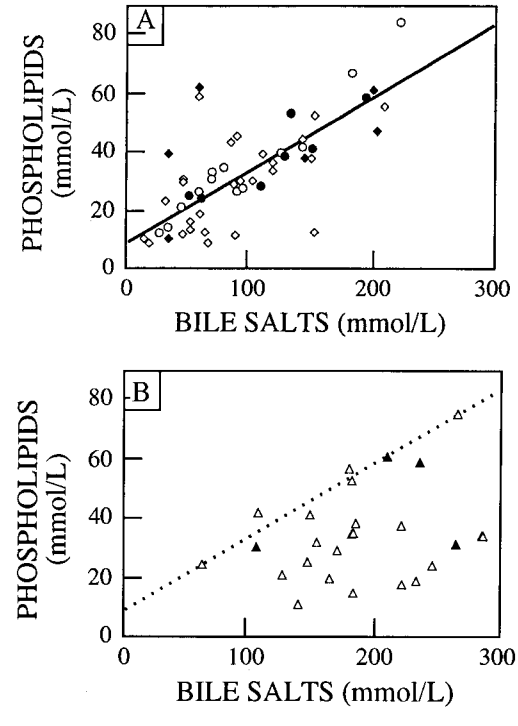

Fig 4. (A) Correlation plot of phospholipid and bile salt concentrations in the bile samples from the four disease study groups. Open symbols: cholesterol supersaturated samples; solid symbols: cholesterol undersaturated samples; circles: morbidly obese subjects without gallstones, diamonds: the three groups with gallstones, ie, morbidly obese subjects, and nonobese gallstone patients with either multiple or solitary stones. The regression line for the supersaturated samples ($y = 8.7 + 0.25x$, $r = 0.74$, $P < 0.001$) is depicted. (B) Correlation plot of phospholipid and bile salt concentrations in the samples from the gallstone-free normal group. Open triangles: supersaturated samples; solid triangles: undersaturated samples. The interrupted line represents the regression for the corresponding plot in the cholesterol supersaturated samples from all of the disease study groups (see Figure 4A).

0.001), a negative correlation was present, indicating that the phospholipid molar percent decreased as bile salt concentration increased. The possibility of interference from pigment content with the cholesterol assay was excluded by additional "spiking" and recovery experiments for cholesterol. In the physiological range of cholesterol concentrations (5–40 mmol/liter), no difference in the recovered amounts of spiked cholesterol was observed, as indicated by the almost identical regression lines obtained with the samples containing either a high ($y = -0.46 + 1.16x$) or low ($y = -1.07 + 1.20x$) level of pigment.

DISCUSSION

Our data show that fluid absorption by the gallbladder is impaired in the presence of gallstone disease. The defect is also observed despite the absence of gallstones when morbid obesity, a known risk factor for gallstones, is present (29). Fluid absorption by the gallbladder is very difficult to quantitate *in vivo* prospectively in humans. In the present study we indirectly derived information on the efficiency of fluid absorption in different settings by measuring the concentrations of nonabsorbable bile solutes, ie, biliary pigments and bile salts, in a single sample of gallbladder bile obtained from a large number of subjects (5–7, 30). We found that, after overnight fasting, nonobese stone-free control subjects have both a significantly higher gallbladder bile salt concentration and pigment content than all the other study groups. Our explanation for these differences is that gallbladders in the control group absorb fluid more efficiently than those of the other study groups.

It is highly unlikely that the striking differences we found in nonabsorbable biliary solute (ie, conjugated

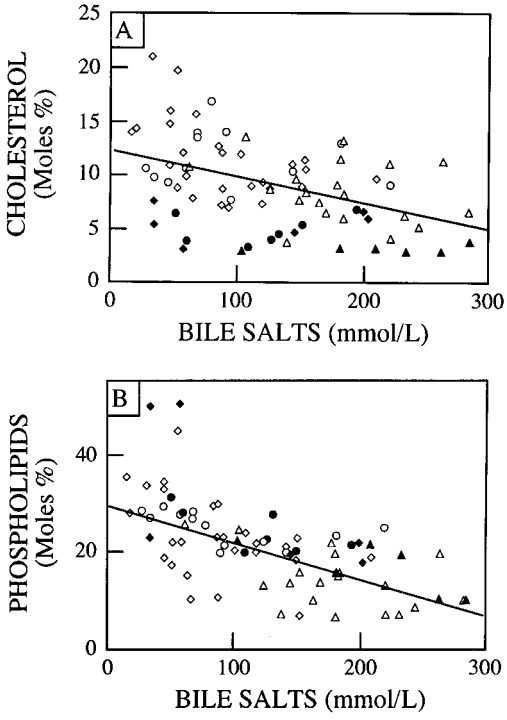

Fig 5. (A) Correlation plot of cholesterol molar percent and bile salt concentrations in bile samples from all of the study groups combined. Open symbols: supersaturated samples; solid symbols: undersaturated samples; triangles: gallstone-free normals; circles: morbidly obese subjects without gallstones; diamonds: the three groups with gallstones, ie, morbidly obese subjects and nonobese gallstone subjects with either multiple or solitary stones. The regression line for the supersaturated samples ($y = 13.62 + -0.03x$, $r = -0.51$, $P < 0.001$) is depicted. (B) Correlation plot of phospholipid molar percent and bile salt concentrations in the same samples as are represented in A using the same symbols. The regression line for the supersaturated samples ($y = 29.78 + -0.08x$, $r = -0.63$, $P < 0.0001$) is depicted.

bile salts and pigments) concentrations in gallbladder bile between the non-obese stone-free control and the other four study groups can be explained by differences in their intraluminal accumulation rather than in fluid absorption. There are several reasons for this. First, although bile salt and bilirubin secretion rates of hepatic bile are known to be independent (31–33), our results show a similar pattern of variations for both bile salts and total pigments for each of the study groups. Second, the bile salt concentration in common duct bile sampled after overnight fasting of subjects free of gallstone disease is significantly lower than in cholesterol gallstone patients, while in gallbladder bile an opposite trend is observed (10). Third, even if decreased rates of bile salt hepatic secretion in gallstone patients have been reported, this was not the case for obese gallstone-free subjects (34). Despite this, we found the same reduction in nonabsorbable biliary solute concentrations in all the disease study groups, including obese gallstone-free subjects.

An implication of our finding of markedly impaired fluid absorption leading to a total lipid concentration of less than 5 g/dl, even in the absence of gallstone disease (25% of our morbidly obese gallstone-free patients), is that, in the absence of cystic duct obstruction and/or chronic inflammatory gallbladder sclerosis with atrophy, the exclusion criterion in clinical studies of a total lipid concentration of less than 5 g/dl and/or of nonopacification of the gallbladder at oral cholecystography is no longer necessary.

The second finding of the present study was the evidence, albeit indirect, that the gallbladder mucosa absorbs significant amounts of both cholesterol and phospholipids during fasting. This process was much more efficient in many gallstone-free controls than in either gallstone patients or morbidly obese gallstone-free subjects. When the samples with cholesterol supersaturation are separately considered for correlation analysis, both the cholesterol (Figure 3) and phospholipid (Figure 4) concentrations increase linearly in relation to the bile salt concentration in all the disease study groups, indicating that about 25% of both cholesterol and phospholipids had been absorbed from bile during fasting. The linear relationships that emerged between lipid concentrations in gallbladder bile confirms previous data on gallbladder bile (18) and on hepatic bile obtained in T-tube patients (35, 36). However, most of published data on hepatic secretion obtained by duodenal sampling under continuous gallbladder contraction have shown hyperbolic lipid relationships (37). The most likely explanation for this apparent discrepancy is that when the enterohepatic circulation is interrupted, as after bile diversion, or decreased, as during an overnight fasting, the bile salt output is diminished to a range where the secretion of both cholesterol and phospholipids is linearly related to that of bile salts.

Our data showing no lipid correlation in the supersaturated samples obtained from nonobese gallstone-free controls can be explained by the fact that half the samples in this group had much lower relative (%) concentrations of both cholesterol and phospholipids (Figures 3B and 4B). Our interpretation is that in about half of the gallstone-free control subjects a highly efficient but variable mucosal absorption of cholesterol and phospholipids occurs.

When the cholesterol-undersaturated samples are analyzed in comparable lipid plots, the phospholipid but not the cholesterol pattern resembles that found in the supersaturated samples (Figure 4A and B). In

undersaturated samples, cholesterol and bile salt concentrations positively correlated in all groups including controls (Figure 3A and B) but showed no evidence for cholesterol absorption. Both this unexpected finding in undersaturated samples of all groups, as well as the results in the supersaturated samples of the control group showing a highly variable lipid absorption could be explained if the composition of the bile entering the gallbladder lumen were a major determinant of cholesterol absorption. In keeping with this hypothesis, it has previously been shown *in vitro* that cholesterol absorption by the gallbladder increases with increasing cholesterol concentration (11, 12) and saturation of bile (12, 38).

Our data are amenable to more than one interpretation. We believe it is most likely that our lipid plots express a less efficient gallbladder mucosal lipid absorption process in the four disease study groups compared to controls and that this malfunction is concomitant with the fluid absorption defect. However, it cannot be excluded that our lipid plots are, other than an expression of gallbladder lipid absorption, at least partially influenced by differences in hepatic bile composition between controls and all the disease study groups. It would have been useful to compare lipid plots in hepatic and gallbladder bile in all groups of our study, including controls. However, as common duct bile sampling is unethical in gallstone-free subjects, the comparison would have been possible only in gallstone patients, who represent only a minor part of our study population. Nevertheless, in our opinion, the role played by gallbladder lipid absorption is of major importance for the following reason: we have recently directly measured, *in vitro*, lipid absorption from a supersaturated bile by the intact human gallbladder (19). Within 5 hr, 23% of biliary cholesterol and 30% of phosphatidylcholine was absorbed by the gallbladder in the absence of cholesterol gallstone disease. Moreover, the rate of lipid absorption was found to be significantly reduced (about half) in gallbladders with cholesterol gallstone disease. These values, obtained within 5 hr, ie, approximately half the time span of nocturnal fasting, are in keeping with the conclusions of the present paper.

To conclude, the findings of this study clearly document that fasting gallbladder bile samples from nonobese stone-free control subjects are consistently more concentrated than those of cholesterol gallstone patients and morbidly obese subjects with or without gallstones. Indirect evidence of gallbladder mucosal absorption of appreciable amounts of cholesterol and phospholipid was also found, this process being more pronounced in controls than in the disease study groups. Impaired gallbladder mucosal function is not necessarily a consequence of gallstone disease.

REFERENCES

1. Svanvik J: Role of gallbladder in modifying hepatic bile composition. *In* Hepatic Transport and Bile Secretion Physiology and Pathophysiology. N Tavoloni, PD Berk (eds). New York, Raven Press, 1994, pp 607–618
2. Holan KR, Holzbach RT, Hermann RE, Cooperman AM, Claffey WJ: Nucleation time: A key factor in the pathogenesis of cholesterol gallstone disease. Gastroenterology 77:611–617, 1979
3. Sahlin S, Danielsson A, Angelin B, Reihnér E, Henriksson R, Einarsson K: Mucin in gall bladder bile of gall stone patients: Influence of treatment with chenodeoxycholic acid and ursodeoxycholic acid. Gut 29:1506–1510, 1979
4. Gallinger S, Harvey PRC, Petrunka CN, Ilson RG, Strasberg SM: Biliary proteins and the nucleation defect in cholesterol cholelithiasis. Gastroenterology 92:867–875, 1987
5. Ostrow JD: Absorption by the gallbladder of bile salts, sulfobromophthalein, and iodipamide. J Lab Clin Med 74:482–494, 1969
6. Ostrow JD: Absorption of organic compounds by the injured gallbladder: J Lab Clin Med 78:255–264, 1971
7. Svanvik J, Allen B, Pellegrini C, Bernhoft R, Way L: Variations in concentrating function of the gallbladder in the conscious monkey. Gastroenterology 86:919–925, 1984
8. Strasberg SM, Toth JL, Gallinger S, Harvey PRC: High protein and total lipid concentration are associated with reduced metastability of bile in an early stage of cholesterol gallstone formation. Gastroenterology 98:739–746, 1990
9. Chijiiwa K, Hirota I, Noshiro H: High vesicular cholesterol and protein in bile are associated with formation of cholesterol but not pigment gallstones. Dig Dis Sci 38:161–166, 1993
10. Nakano K, Chijiiwa K: Reduced cholesterol metastability of hepatic bile and its further decline in gallbladder bile in patients with cholesterol gall stones. Gut 34:702–707, 1993
11. Ross PE, Butt AN, Gallacher C: Cholesterol absorption by the gallbladder. J Clin Pathol 43:572–575, 1990
12. Jacyna MR, Ross PE, Bakar MA, Hopwood D, Bouchier IAD: Characteristics of cholesterol absorption by human gall bladder: Relevance to cholesterolosis. J Clin Pathol 40:524–529, 1987
13. Ulissi A, Purdum PP, Moore EW: Vesicular phospholipid transport in cultured human gallbladder epithelia is dependent on vesicular size, epithelial confluency, and presence of oxygen. Hepatology 18:95A, 1993 (abstract)
14. Purdum PP III, Shamburek RD, Hylemon PB, Moore EW: Rapid phospholipid (PL) transfer across cultured human gallbladder epithelia. Gastroenterology 102:A871, 1992 (abstract)
15. Neiderhiser DH, Harmon CK, Roth HP: Absorption of cholesterol by the gallbladder. J Lipid Res 17:117–124, 1976
16. Neiderhiser DH, Morningstar WA, Roth HP: Absorption of lecithin and lysolecithin by the gallbladder. J Lab Clin Med 82:891–897, 1973
17. Booker ML, Scott TE, LaMorte WW: Effect of dietary cholesterol on phosphatidylcholines and phosphatitylethanolamines

in bile and gallbladder mucosa in the prairie dog. Gastroenterology 97:1261–1267, 1989
18. Shiffman ML, Sugerman HJ, Moore EW: Human gallbladder mucosal function. Gastroenterology 99:1452–1459, 1990
19. Ginanni Corradini S, Della Guardia P, Giovannelli L, Ripani C, Elisei W, Quartini A, Corsi A, Codacci Pisanelli M, Chirletti P, Caronna R, Ziparo V: Biliary cholesterol and phospholipid absorption by the gallbladder mucosa is impaired in cholesterol gallstone disease. A study in the isolated *in vitro* perfused human gallbladder. Hepatology 24:323A, 1996 (abstract)
20. Tera H: Stratification of human gallbladder bile *in vivo*. Acta Chir Scand Suppl 256:1–85, 1960
21. Strasberg SM, Robert P, Harvey PRC, Hofmann AF: Bile sampling, processing and analysis in clinical studies. Hepatology 12:176S–182S, 1990
22. Turley SD, Dietschy JM: Re-evaluation of the 3α-hydroxysteroid dehydrogenase assay for total bile acids in bile. J Lipid Res 19:924–928, 1978
23. Takayama M, Itoh S, Nagasaki T, Tanimizu I: A new enzymatic method for determination of serum choline-containing phospholipids. Clin Chim Acta 79:93–98, 1977
24. Gurantz D, Laker MF, Hofmann AF: Enzymatic measurement of choline-containing phospholipids in bile. J Lipid Res 22:373–376, 1981
25. Allain CC, Poon LS, Chan CS, Richmond W, Fu PC: Enzymatic determination of total serum cholesterol. J Clin Chem 20:470–475, 1974
26. Bloch HM, Thornton JR, Heaton KW: Effects of fasting on the composition of gallbladder bile. Gut 21:1087–1089, 1980
27. Neder J, Wasserman W, Kutner MH: Applied Linear Statistical Models, 3rd ed. Homewood, Illinois, Irwin Publishing, 1990
28. Woolson RF: Statistical Methods for the Analysis of Biomedical Data. New York, John Wiley & Sons, 1987
29. Everhart JE: Contribution of obesity and weight loss to gallstone disease. Ann Intern Med 119:1029–1035, 1993
30. Ostrow JD: Absorption of bile pigments by the gall bladder. J Clin Invest 46:2035–2052, 1967
31. Kesäniemi YA, Miettinen TA: Biliary secretion of bilirubin and lipids in chronic cholestatic liver disease. Scand J Gastroenterol 20:433–438, 1975
32. Raedsch R, Stiehl A, Gundert-Remy U, Walker S, Sieg A, Czygan P, Kommerell B: Hepatic secretion of bilirubin and biliary lipids in patients with alcoholic cirrhosis of the liver. Digestion 26:80–88, 1983
33. Harvey PRC, Toth JL, Upadhya GA, Ilson RG, Strasberg SM: Total protein output during rapid reduction of bile salt secretion rates in man. Gut 30:118–122, 1989
34. Bennion LJ, Grundy SM: Effects of obesity and caloric intake on biliary lipid metabolism in man. J Clin Invest 56:996–1011, 1975
35. Carulli N, Loria P, Bertolotti M, Ponz de Leon M, Menozzi D, Medici G, Piccagli I: Effects of acute changes of bile acid pool composition on biliary lipid secretion. J Clin Invest 74:614–624, 1984
36. Alvaro D, Angelico M, Cantafora A, Di Biase A, De Santis A, Bracci F, Minervini G, Ginanni Corradini S, Attili AF, Capocaccia L: Biliary secretion of phosphatidylcholine and its molecular species in cholecystectomized T-tube patients: Effects of bile acid hydrophilicity. Biochem Med Metab Biol 36:125–135, 1986
37. Carey MC, Mazer NA: Biliary lipid secretion in health and in cholesterol gallstone disease. Hepatology 4:31S–37S, 1984
38. Hayashi A, Lee SP: Bidirectional transport of cholesterol between gallbladder epithelial cells and model bile. Am J Physiol 271:G410–G414, 1996