# Phosphatidylcholine-enriched diet prevents gallstone formation in mice susceptible to cholelithiasis

**Joelle Kasbo,[*,†] Beatriz Tuchweber,[†] Shahid Perwaiz,[*,†] Guylaine Bouchard,[†] Huguette Lafont,[§] Nicole Domingo,[§] Francoise Chanussot,[§] and Ibrahim M. Yousef[1,*,†]**

Departments of Pharmacologie,* Université de Montréal, Montréal, Canada; Centre de Recherche† de l'Hôpital Sainte-Justine, Montréal, Canada; and Unite 476-INSERM,§ Nutrition Humaine et Lipides, Facultè de Medicine 27, Bd Jean Moulin, 13385, Marseille Cedex, France

**Abstract Cholesterol gallstones affect approximately 10–15% of the adult population in North America. Phosphatidylcholine (PC) is considered to be the main cholesterol solubilizer in bile. This study examined the effect of a PC-enriched diet on gallstone incidence in mice susceptible to cholelithiasis. The result obtained showed that the feeding of a lithogenic (LG) diet for 4 weeks or 8 weeks resulted in cholesterol gallstone incidences of 47% and 89%, respectively. These gallstone incidences were either reduced or prevented when the LG diet was enriched with 2% or 6% PC, respectively. The cholesterol saturation index (CSI) was reduced only in mice fed with LG + 6% PC diet as compared with mice fed the LG diet alone. However, in all groups, the CSI was significantly higher than in mice fed Purina chow diet. The biliary anionic polypeptide fraction (APF) was significantly increased in mice fed the LG + 2% PC diet and was reduced in those fed with LG + 6% PC diet. In conclusion, prevention or delay of gallstone formation was not due to a consistent effect on biliary lipid composition, suggesting a direct effect of PC on cholesterol solubilization and/or the effect of an additional nonlipid biliary component such as APF.**—Kasbo, J., B. Tuchweber, S. Perwaiz, G. Bouchard, H. Lafont, N. Domingo, F. Chanussot, and I. M. Yousef. **Phosphatidylcholine-enriched diet prevents gallstone formation in mice susceptible to cholelithiasis. *J. Lipid Res.* 2003.** 44: **2297–2303.**

**Supplementary key words** inbred mice • cholesterol saturation index • gas chromatography/mass spectrometry • electrospray tandem mass spectrometry • anionic polypeptide fractions

Gallstones may be divided into two major types: cholesterol gallstone and pigment stones. Cholesterol gallstones constitute >80% of stones in the Western world and are composed predominantly of cholesterol (1). The mechanism of cholesterol gallstone formation has not been completely elucidated (2). Research interest during the last two decades has been focused on the metabolism of biliary lipids and its relationship to gallstone formation (3, 4). It has become evident that a disturbed metabolism of cholesterol and/or bile salt constitutes a major factor in gallstone formation (5, 6). In bile, bile salts, phospholipids (PLs), and cholesterol are the major lipid components (7) and, depending on their relative concentrations, they interact to form several different biliary lipid micelles (8). These mixed micelles allow the cholesterol molecule to be transported in bile (9), being incorporated into the hydrophobic interior held in a stable thermodynamic state (10, 11). When bile is supersaturated with cholesterol, vesicles are formed and more PLs are needed for the conversion of vesicles into mixed micelles, and the residual vesicles consequently become richer in cholesterol (12). These cholesterol-rich vesicles are thermodynamically unstable and can aggregate, allowing the formation of cholesterol crystals (13).

Biliary anionic polypeptide fraction (APF) is a small peptide (7 kDa) present in both normal and pathological biles (14). It has been demonstrated that APF is involved in the uptake of unesterified cholesterol (15) and stimulates cholesterol and PL transport to the bile (16, 17). It was also recently demonstrated that APF plays an important role in controlling the onset and rates of precipitation of calcium salts, bile pigments, and cholesterol in gallstone formation (18, 19).

Several in vitro studies showed a beneficial effect for phosphatidylcholine (PC) on cholesterol solubility, crystallization, and nucleation time (20, 21). Other in vivo studies have also shown that dietary PC increases the biliary lipid secretion and bile flow, which is a critical step in cholesterol homeostasis (22). However, there has not been a study on the effect of dietary PC on cholelithiasis. The present study was designed to investigate the possible preventive effect of a diet enriched in PC on cholesterol gallstone formation in C57BL/6 mice. These mice have

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[1] To whom correspondence should be addressed.
e-mail: ibrahim.yousef@umontreal.ca

an intermediate susceptibility to cholelithiasis when fed the lithogenic (LG) diet composed of 15% dairy fat, 1% cholesterol, and 0.5% cholic acid.

## EXPERIMENTAL PROCEDURES

### Animals

Male C57BL/6 mice (homozygous for susceptible Lith alleles), 4–6 weeks old, were purchased from the Jackson Laboratory (Bar Harbor, ME) and were housed in a temperature-controlled room (22–23°C) with alternating 12 h light/12 h dark cycles. All animals were kept for 1 week prior to dietary treatment and were provided free access to food and water throughout the experiment. Weight gain and food intake were monitored twice every week during the period of the study. The components for the diets were purchased from ICN Biochemicals, Inc. (Costa Mesa, CA) with the exception of corn oil, sucrose, and DL-α-tocopherol, which were obtained from Sigma (Oakville, Ontario). Sodium cholate and PLs from soya bean PC were obtained from Calbiochem, Diagnostics (San Diego, CA). Xylaket was obtained from Bayer, Inc. (Etobicoke, Ontario). The bile salt standards (lithocholic, deoxycholic, chenodeoxycholic, cholic, and ursodeoxycholic sodium salts) were purchased from Calbiochem.

### Experimental diet

The LG diet was prepared as previously described (23). The diet contained 15% dairy fat, 50% sucrose, 20% casein, 1% corn oil, 5% cellulose, 5% AIN-76 mineral mix, 1% AIN-76 vitamin mix, 1% choline chloride, 0.3% DL-methionine, 0,13% DL-α-tocopherol, 0.5% sodium cholate, and 1% cholesterol. The supplementation with PC replaced a part of the sugar content.

### Experimental protocol

Two experiments were performed. In the first experiment, mice were divided into three groups. Each group contained at least 10 mice. Animals of the first group were fed the LG diet for eight weeks; animals of the second group received the LG diet containing 2% PC for 4 weeks; and animals in the third group received LG containing 2% PC for 8 weeks. In the second experiment, mice were fed a control diet (Purina chow) or the LG diet supplemented with 6% PC for 4 weeks. In preliminary studies, there was no difference obtained between mice fed LG + 6% PC diet for 4 weeks or 8 weeks with regard to the incidence of gallstones; therefore, the group fed LG + 6% PC for 8 weeks was dropped from the protocol of the study. Prior to the experimental procedure, animals were fasted overnight but had free access to water. After weighing, mice were anesthetized by intraperitoneal injection with 4 μl of Xylaket (0.4 mg/g body weight). An additional 1 μl of Xylaket (0.1 mg/g body weight) was given if a mouse showed signs of regaining sensation. Surgery was performed at 9 AM through an upper midline incision, and the gallbladder was removed and examined for the presence of gallstones. The bile duct was then cannulated with a polyethylene catheter (PE-10), and bile was collected for 60 min. Bile flow was determined gravimetrically, assuming a density of 1 g/ml of bile, and bile samples were stored at −20°C until further analysis.

### Analysis of bile acids and biliary lipid

Total bile salt concentration was measured enzymatically by the 3α-hydroxysteroid dehydrogenase method (24). Biliary lipids were extracted as described by Folch and Lees (25), and total PLs were measured as inorganic phosphorus by the method of Bartlett (26). Cholesterol was extracted from bile by the method of Bligh and Dyer (27) and was measured using a commercial kit from Boehringer Mannheim (Montreal, Canada).

### Analysis of bile acids

The methodology used for the determination of total and individual bile acids in bile was similar to that described previously by this laboratory (28) using electrospray tandem mass spectrometry and gas chromatography/mass spectrometry.

### Cholesterol saturation index

Cholesterol saturation index (CSI) was calculated according to the method described by Carey (29).

### APF determination

APF was isolated from bile according to the method described by Lafont et al. (30). In summary, preparation of human APF with minimal denaturation was obtained by ultracentrifugation of fresh bile at 30,000 rpm for 48 h at 10°C at the density of 1.030 g/ml. APF was then purified by serial tangential ultrafiltration through miniultrasette devices (Filtron, Coignières, France) (14, 30). APF was separated from other small peptide fragments and most residual pigments by a preparative hydrophobic HPLC (30). This protocol afforded lipid-free APF, resulting in a 7 kDa band visualized on a 12.5% SDS-PAGE by silver staining. APF was determined by ELISA (sandwich) using polyclonal and murine monoclonal antibody as previously described (14). Based on the standard curve, the assay was sensitive and accurate for an APF concentration range of 0.2–5 μg/ml.

### Statistical analysis

The data were analyzed statistically by ANOVA and Student's *t*-test. $P < 0.05$ was considered to be statistically significant.

## RESULTS

### Body and liver weight

The LG diet and the LG diet containing 2% or 6% PC were all well accepted by the mice. The mice showed progressive weight gain after an initial weight loss. There was no significant difference observed between body and liver weight with time. However, mice fed with Purina chow diet (control) showed a significant increase in the body weight accompanied by decrease in the liver weight (data not shown).

### Cholesterol gallstone formation

**Table 1** shows the incidence of the presence of pregallstone structures (pregallstone structures include precipitation of cholesterol crystals and cholesterol deposits on the gallbladder wall) and incidence of gallstones during 4 weeks and 8 weeks of treatment with the LG diet containing 2% and 6% PC. In mice fed the Purina chow diet, there was no incidence of gallstone formation or pregallstone structures during the 4 weeks or 8 weeks of the study. In mice fed the LG diet, there was a 47% and 89% incidence of gallstone formation at 4 weeks and 8 weeks, respectively. In mice fed LG + 2% PC, 11% of the mice developed pregallstone structures after 4 weeks of treatment, which was increased to 33% after 8 weeks of treatment. However, cholesterol gallstone incidence was 22% and increased to 44% after 4 weeks and 8 weeks, respectively. When mice were fed the LG + 6% PC diet, 10% of the mice developed pregallstone structures at 4 weeks, but no incidence of gallstones was observed.

TABLE 1. Incidence of pregallstone structures and complete gallstones with time in mice fed Purina chow, lithogenic, lithogenic + 2% phosphatidylcholine, or lithogenic + 6% phosphatidylcholine diets

| | Incidence Pregallstone Structures | Incidence Gallstones |
|---|---|---|
| | % | |
| 4-week diet | | |
| Purina chow | 0 | 0 |
| LG | 0 | 47 |
| LG + 2% PC | 11 | 22 |
| LG + 6% PC | 10 | 0 |
| 8-week diet | | |
| Purina chow | 0 | 0 |
| LG | 0 | 89 |
| LG + 2% PC | 33 | 44 |
| LG + 6% PC | ND | ND |

LG, lithogenic; LG + 2% PC, LG supplemented with 2% phosphatidylcholine; LG + 6% PC, LG supplemented with 6% PC; ND, not determined. The table shows the incidence of precholesterol gallstone structures and cholesterol gallstone formation with time in mice fed the Purina chow, LG, LG + 2%, or LG + 6% PC diets. The values represent the percentage (%) of animals with pregallstone or complete gallstone structures present in the gallbladder. Each group contained a maximum of 10 mice, except the LG + 6% PC group, which was dropped from the study protocol after 4 weeks of feeding.

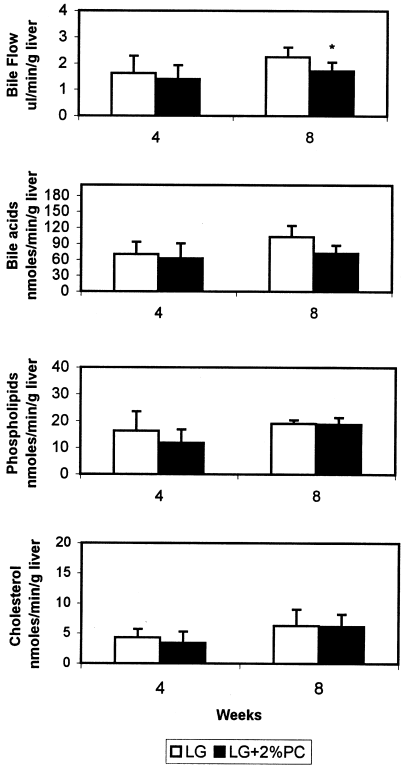


**Fig. 1.** Bile flow and secretion rate of bile acids, phospholipids (PLs), and cholesterol in hepatic bile obtained during 1 h from mice fed lithogenic (LG) and LG + 2% phosphatidylcholine (PC) diets for 4 and 8 weeks. Values are expressed as mean ± SD of 10 mice. * Significantly different from the LG diet group.

### Bile flow and biliary lipid secretion

The LG diet had no significant effect on bile flow and bile acid secretion rate when compared with mice fed a Purina chow diet. However, the secretion of PLs and cholesterol was significantly increased. Feeding an LG + 2% PC diet to the mice for 4 weeks or 8 weeks had no significant effect on bile flow, bile acid, PLs, or cholesterol secretion rate when compared with the LG diet alone (**Fig. 1**). However, feeding with the LG + 6% PC diet for 4 weeks caused a significant increase in bile flow (30%), bile acid (70%), and PL (26%) secretion rates, but did not show a significant effect on cholesterol secretion rate when compared with animals fed the LG diet alone (**Fig. 2**).

### Bile acids profile in hepatic bile

**Table 2** shows the bile salts composition of hepatic bile in mice received the Purina chow diet (control) and mice fed the LG and LG + 2% PC diets (for 4 weeks and 8 weeks), and LG + 6% PC diets (for 4 weeks). In the control group, the major bile acids were tauromuricholic acid (T-Muri) (51%) followed by taurocholic acid (T-CA) (39%) and other taurine-conjugated dihydroxylated bile acids [deoxycholic acid (T-DCA), ursodeoxycholic acid (T-URSO), and chenodeoxycholic acid (T-CDC)], which contributed 10% of the total bile acids. Feeding the LG, LG + 2% PC, or LG + 6% PC diets for 4 weeks increased the percentage of T-CA and T-DOC but reduced the percentage of T-Muri and T-URSO. However, there was no significant change in the percentage of T-CDC acid observed. The pattern of conjugation obtained by the electrospray tandem mass spectrometry showed that taurine-conjugated trihydroxylated bile acid (*m/z* 514) was the major bile acid, followed by taurine-conjugated dihydroxylated bile acid (*m/z* 498). Traces of glycine-conjugated trihydroxylated (*m/z* 464), glycine-conjugated dihydroxylated (*m/z* 448), and glycine-conjugated trihydroxymonosulfated (*m/z* 487) bile acids were also observed, with no significant differences between diets.

### Biliary PLs and fatty acid composition

The PC species obtained by the electrospray tandem mass spectrometry analysis showed that the soya bean PC that was added to the diet was composed mainly of 1-palmitoyl-2-arachidonoyl- (*m/z* 783), 1-oleoyl-2-linoleoyl- (*m/z* 785), 1-palmitoyl-2-linoleoyl- (*m/z* 759), 1-stearoyl-2-linoleoyl- (*m/z* 787), and palmitoleoyl-2-linoleoyl- (*m/z* 757) *sn*-glycerophosphocholines. The PC species obtained in bile of mice fed the LG, LG + 2% PC, and LG + 6% PC diets were similar in all groups, and mainly composed of 1-palmitoyl-2-linoleoyl-*sn*-glycerophosphocholines (*m/z* 759). These data show that dietary fatty acid composition does not influence the fatty acid composition of bile.

### Gallbladder biliary lipids composition

**Table 3** shows the mol% contribution of bile acids, PLs, and cholesterol in gallbladder bile. The LG diet increased the percentage of cholesterol from 1.2% to 4.9% (control vs. LG diet) after 4 weeks of treatment, and there was no significant effect observed in the percent contribution of bile acids and PLs. The addition of 2% PC to the LG diet further increased the percentage of cholesterol from 4.9% to 7.7% (LG vs. LG + 2% PC diet). However, 6% PC significantly de-

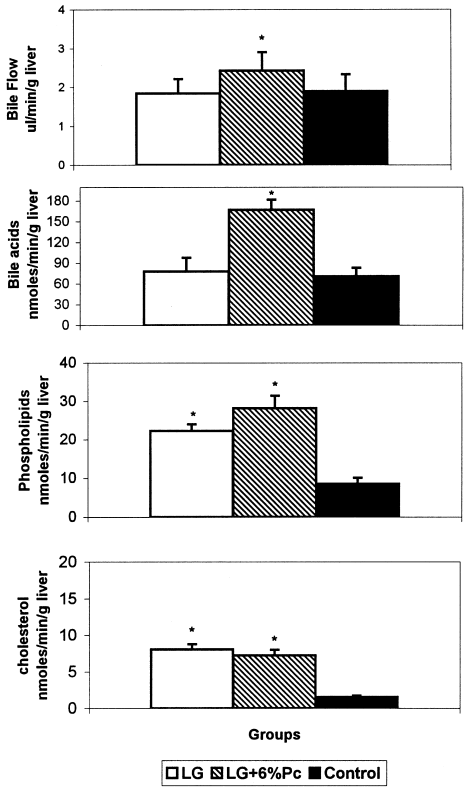


**Fig. 2.** Bile flow and secretion rate of bile acids, PLs, and cholesterol in hepatic bile obtained during 1 h from mice fed LG, LG + 6% PC, and Purina chow (control) diets for 4 weeks. Values are expressed as mean ± SD of 10 mice. * Significantly different from control.

creased the percentage of cholesterol from 7.7% to 4.6% (LG + 2% PC vs. LG + 6% PC diet) but remained significantly higher as compared with control. Although the LG diets increased the biliary cholesterol-to-PL ratio, no effect on the ratio of PL-cholesterol was observed. The gallbladder bile lipid composition after 8 weeks of treatment was not significantly different from that found after 4 weeks of treatment.

### CSI

**Figure 3** shows the CSI in the gallbladder bile obtained from mice fed with control diet, LG, LG + 2% PC, and LG + 6% PC diets. The CSI was significantly increased in the LG diet-fed mice; however, feeding 2% PC and 6% PC in the LG diet reduced the CSI by 25% and 40%, respectively, when compared with gallbladder bile obtained from mice fed the LG diet alone. The CSI in the gallbladder bile obtained from mice fed the LG diet enriched in 2% or 6% PC remained significantly higher than the CSI of the bile obtained from the gallbladder of mice fed the Purina chow diet.

### APF concentration

**Figure 4** shows that the LG and LG + 2% PC diets caused a significant increase in APF concentration in gallbladder bile when compared with the Purina chow diet. However, feeding the LG + 6% PC diet showed a slight (but not significant) increase in APF concentration when compared with the Purina chow diet.

## DISCUSSION

Several factors are associated with cholelithiasis; however, genetic influences and nutrition are the major factors implicated in the formation of cholesterol gallstones. *Lith* genes were reported to control cholesterol gallstone formation susceptibility in certain inbred mice. It has also been reported that cholesterol gallstone prevalence varies between 0% to 100% in different strains of mice, including C57BL/6 mice (31); however, the LG diet is essential for the formation of cholesterol gallstones (23, 32). The characteristics of *Lith* mice include biliary cholesterol hypersecretion, bile supersaturation with cholesterol, and a high prevalence of cholesterol gallstone formation (32, 33). PC, the major PL present in bile, is required for the effective removal of cholesterol from bile. Several studies in rats showed that dietary PC supplementation improved the bile formation by increasing the secretion of bile acids and biliary lipids (34, 35). The objective of this study was to evaluate the effect of PC dietary supplementation on cholesterol gallstone formation in genetically susceptible mice. The results showed that a 2% dietary PC supplement fed for 4 weeks and 8 weeks reduced cholesterol gallstone formation (Table 1). This effect was associated

TABLE 2. Bile acid composition of hepatic bile obtained from mice fed with Purina chow, LG, LG + 2%, or LG + 6%PC diets

| Diet | Week | n | DOC | CDC | CA | URSO | Muri |
|---|---|---|---|---|---|---|---|
| | | | | | % | | |
| Control | 4 | 3 | $4.03^{a,b} \pm 1.38$ | $2.23 \pm 1.87$ | $39.33^{a,b} \pm 8.68$ | $3.14^{a,b} \pm 2.75$ | $51.2^{a,b} \pm 9.73$ |
| LG | 4 | 9 | $13.38^{c} \pm 4.41$ | $2.21 \pm 1.04$ | $78.14^{c} \pm 3.81$ | $0.47^{c} \pm 0.37$ | $5.76^{c} \pm 1.61$ |
| LG + 2% PC 4 | 8 | 8 | $14.28^{c} \pm 4.92$ | $2.39 \pm 1.02$ | $76.75^{c} \pm 5.68$ | $0.91^{c} \pm 0.22$ | $5.68^{c} \pm 1.10$ |
| LG + 6% PC 4 | 4 | 4 | $14.18^{c} \pm 2.26$ | $2.63 \pm 1.12$ | $77.83^{c} \pm 3.15$ | ND | $5.38^{c} \pm 3.35$ |
| LG | 8 | 6 | $16.67 \pm 5.69$ | $1.65 \pm 0.50$ | $74.52 \pm 6.89$ | $1.12 \pm 0.19$ | $6.03 \pm 2.14$ |
| LG + 2% PC 8 | 6 | 6 | $13.68 \pm 2.54$ | $3.53^{a} \pm 1.53$ | $75.20 \pm 3.63$ | $1.02 \pm 0.27$ | $6.6 \pm 1.50$ |

CA, cholic acid; CDC, chenodeoxycholic acid; DOC, deoxycholic acid; URSO, ursodeoxycholic acid. The table shows the bile acids composition of hepatic bile obtained from mice fed with Purina chow, LG, LG + 2%, or LG + 6% PC diets. Values are shown as mean ± SD.

[a] $P < 0.05$ when compared with LG diet group for the same time.

[b] $P < 0.05$ when compared with LG + 6% PC diet group for the same time.

[c] $P < 0.05$ when compared with control diet group for the same time.

TABLE 3. Gallbladder bile lipid composition (mol%) in mice fed with LG, LG + 2%, LG + 6% PC, or Purina chow diets for 4 weeks

| Diet | BA | PL | Cholesterol | Cholesterol-PL | PL/Cholesterol |
|---|---|---|---|---|---|
| LG | 79.80 ± 2.32 | 15.30 ± 1.70 | 4.90 ± 1.46 | 0.32 ± 0.96 | 0.19 ± 0.08 |
| LG + 2% PC | 81.10 ± 2.11 | 11.40 ± 1.69 | 7.70 ± 1.89[a] | 0.68 ± 0.22[a] | 0.14 ± 0.06[a] |
| LG + 6% PC | 81.60 ± 2.80 | 13.90 ± 1.56 | 4.60 ± 2.99 | 0.33 ± 0.14 | 0.17 ± 0.02 |
| Control | 83.00 ± 2.63 | 15.80 ± 1.62 | 1.20 ± 1.25 | 0.08 ± 0.05 | 0.19 ± 0.06 |

BA, bile acid; PL, phospholipid. The table represents the gallbladder bile acids and lipid composition obtained from mice treated with LG, LG + 2%, LG + 6% PC, or Purina chow diets. Values obtained after 8 weeks of similar treatment were not significantly different from the values obtained after 4 weeks of treatment. Values are expressed as mean ± SD.

[a] $P < 0.05$ was considered significant.

with a slight decrease in CSI and a significant increase in hepatic biliary APF concentration (Figs. 3, 4). In mice fed the LG + 6% PC diet, no incidences of gallstones were observed after 4 weeks and 8 weeks of treatment. This was associated with a significant decrease in CSI (Fig. 3) and no significant effect on APF concentration. However, 6% PC in the LG diet increased the bile flow and hepatic secretion of bile acids and PLs (Fig. 2). The LG + 2% PC diet did not show a significant effect on bile flow, bile acids, or in biliary lipid secretion as compared with the LG diet alone. The increase in bile flow in mice fed the LG + 6% PC diet could be explained by the increase in biliary bile acid secretion (36). However, the increase in biliary PL secretion might be due to the fact that dietary PC can stimulate HDL-PC uptake by the liver, and thus PC can be released either from the preformed pool or from the newly synthesized pool (37). It has been observed that in model bile, PC containing saturated fatty acids can delay cholesterol crystallization time, while PC containing unsaturated fatty acids accelerates this process (38). Our data showed that species and fatty acid composition of biliary PC were not influenced by the LG or the LG + PC diets. As reported by Fuchs et al. (39), the *Lith* allele increases the biliary cholesterol hypersecretion in response to the LG diet in genetically susceptible mice. The data obtained from the present study confirm that cholesterol secretion was significantly higher in mice fed the LG diet as well as the LG + 2% and LG + 6% PC diets in comparison with values obtained from mice fed the Purina chow diet. However, dietary supplementation with PC decreased cholesterol hypersecretion when compared with values obtained from mice fed the LG diet alone (Figs. 1, 2).

In general, T-Muri acid is the predominant bile acid in mice bile; however, T-CDC is also present (40, 41). Dietary supplementation with T-CA caused an increase in biliary T-CA excretion and a decrease in T-CDC as well as T-Muri acids (42). Thus, T-CA became the major bile acid in mice fed the LG or the LG + PC diets. There was also a significant decrease in the percentage of T-Muri acid in bile obtained from mice fed the LG or the LG + PC diets; however, there was no change in the percent contribution of T-CDC. Several studies have shown an increase in biliary DOC in cholesterol gallstone patients (43, 44), and it has also been shown that an increased DCA content in bile promotes rapid cholesterol crystallization and the formation of cholesterol gallstones. (45). In fact, it was demonstrated that DOC, which is highly hydrophobic, was associated with human cholelithiasis (46). In this study, we observed a significant increase in biliary T-DOC in mice fed the LG diet. This is to be expected because of the higher percentage of T-CA in the bile acid pool of these mice, and because T-DOC is the secondary bile acid pro-

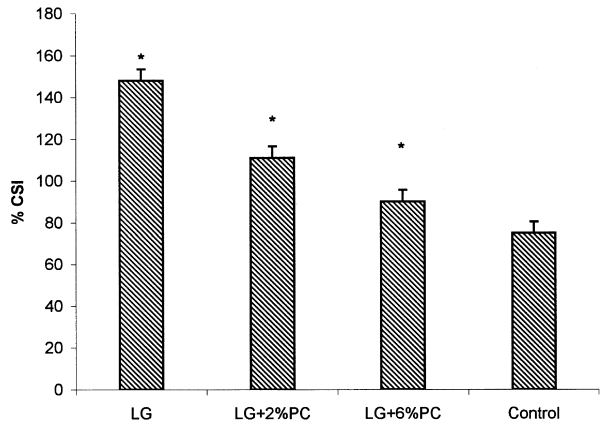


**Fig. 3.** Cholesterol saturation index in gallbladder bile obtained from mice fed with LG, LG + 2% PC, LG + 6% PC, and Purina chow (control) diets. Values are expressed as mean ± SD of six mice. * $P < 0.01$, significantly different from the LG group. There was no significant difference observed between the values obtained after 8 weeks and 4 weeks of treatment.

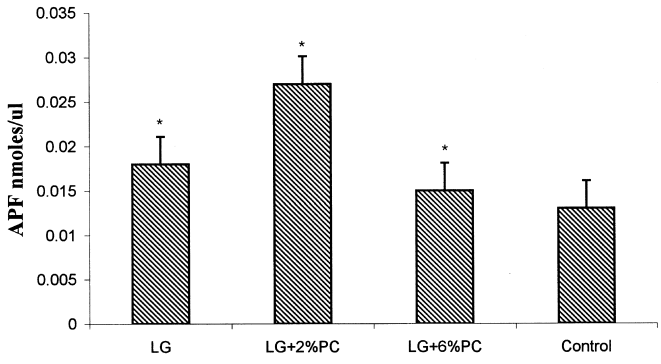


**Fig. 4.** Concentration of anionic polypeptide fraction in gallbladder bile obtained from mice fed LG, LG + 2% PC, LG + 6% PC, and Purina chow (control) diets for 4 weeks. Values are expressed as mean ± SD of six mice. * $P < 0.01$, significantly different from the LG group. There was no significant difference observed between the values obtained after 8 weeks and 4 weeks of treatment.

duced from T-CA. However, PC supplementation of the LG diet did not affect the percent contribution of T-DOC in comparison with the LG diet (Table 2). According to Alexander and Portman (47), at least 93% of bile acids were taurine conjugated before LG diet consumption. However, glycine-conjugated bile acids were not detected in C57BL/6 mice. Our results show that dietary PC supplementation did not affect the conjugation pattern of bile acids. Thus, the above data suggest that the effect of PC on gallstone formation is not related to consistent changes in biliary lipid or bile acid secretion.

APF was originally described as part of a vesicular bile lipoprotein complex ($d$ = 1.030 g/ml) (17). AFP is present in higher concentrations (0.4 g/l) in biliary vesicles compared with the biliary micellar phase (0.3 g/l) (the APF-PL ratio was reported to average 219 in vesicles vs. 30 in micelles) (48). In mice fed the LG + 2% PC diet, although the APF level was increased, bile lipids were unchanged. Thus, the APF-lipid ratios were increased. This observation suggests that such a diet favors the predominance of a bile vesicular phase enriched with APF. This phase exhibits a major regulatory effect on the gallstone formation process. Thus, APF could act in this situation as a protective molecule in the first step of cholesterol gallstone formation. The much more effective dose of 6% PC results in a significant increase in bile salts (such an increase could be associated with the ability to induce the micellar phase, as described elsewhere) (49) and a significant decrease in the CSI, suggesting a regulatory effect of the CSI in the effect of this level of PC supplementation. The decrease in APF level was shown previously in rats infused with cholic acid (50). Thus, we speculate that increasing the micellar phase may act by lowering the APF level and vesicular phase. The reduced CSI and formation of a biliary vesicular form containing APF seem to be protective parameters efficient in preventing the lithiasis process in a diet supplemented with PC.

In conclusion, this study demonstrates that dietary PC supplementation reduced cholesterol gallstone formation in genetically susceptible mice fed the LG diet. This reduction is associated with a decrease in CSI as well as an increase in the secretion of APF in mice supplemented with 2% PC in the LG diet. When the percentage of PC in the diet increases, it seems that the reduction in the CSI and the increase of the micellar phase are more important regulatory factors for gallstone formation than is an increase in APF concentration. The data also suggest that high bile flow, bile salt, and PL secretion in the 6% PC- and 2% PC-fed mice may interfere with the biliary secretion of APF.

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