Effect of the type of dietary fat on biliary lipid composition and bile lithogenicity in humans with cholesterol gallstone disease

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Abstract

Objective: The effect of the type of dietary fat on bile lipids and lithogenicity is unclear. This study compared the effects of two dietary oils that differed in fatty acid profile on biliary lipid composition in humans.

Methods: Female patients who had cholesterol gallstones and were scheduled for elective cholecystectomy were studied. For 30 d before surgery, subjects were kept on diets that contained olive oil (olive oil group, n = 9) or sunflower oil (sunflower oil group, n = 9) as the main source of fat. Gallbladder bile and stones were sampled at surgery. After cholecystectomy, duodenal samples were collected by nasoduodenal intubation during fasting and after administration of mixed liquid meals that included the corresponding dietary oil. Duodenal and gallbladder bile samples were analyzed for cholesterol, phospholipids, and total bile acids by established methods. Individual bile acid conjugates in gallbladder bile were measured by high-performance liquid chromatography. Gallstones were analyzed by semiquantitative polarizing light microscopy.

Results: Despite marked differences in the absolute concentration of biliary lipids and total lipid content, manipulation of dietary fat ingestion did not influence the cholesterol saturation or the profile of individual bile acids in gallbladder bile obtained from patients who had gallstones. All but one subject had mixed cholesterol stones. A cholesterol saturation index of hepatic bile in fasted cholecystectomized patients was similar in both dietary groups and indicative of supersaturation. In response to the test meal, the cholesterol saturation index decreased significantly in patients given the olive oil diet, reaching values lower than one at 120 min postprandially. In contrast, hepatic bile secreted by patients who consumed sunflower oil appeared supersaturated (cholesterol saturation index >1.5) throughout the experiment.

Conclusions: Our results suggest that the type of dietary fat habitually consumed can influence bile composition in humans. In gallbladder, this influence was noted in the presence of more concentrated bile in the olive oil group. However, this was not translated into a modification of cholesterol saturation, which is likely due to the fact that cholesterol gallstones were present by the time the dietary intervention started. The finding that a typical postprandial variation in hepatic bile lithogenicity occurred only in olive oil patients was revealing. While keeping in mind the methodologic limitations of this part of the study, some gastrointestinal and metabolic mechanisms for this effect are discussed. © 2005 Elsevier Inc. All rights reserved.

Keywords: Dietary fat; Biliary lipids; Cholesterol saturation; Gallstone disease; Cholecystectomy

Introduction

The overall effect of the type of dietary fat on bile lipids and lithogenesis in humans is unclear, although epidemiologic, clinical, and animal studies have indicated an important role for this dietary component. A key limitation of animal studies is the existence of marked differences in biliary lipid homeostasis from those in humans, i.e., most animal species readily convert any excess cholesterol to bile acids, so biliary cholesterol concentration seldom approaches the saturation point [1].
As for the human studies, the investigation of the effect of dietary fats on biliary composition has yielded equivocal results. A first reason is the small number of available studies, possibly because of the difficulty and risks of sample collection. Second, gender and inherent differences in metabolism at the time of dietary intervention may affect the outcome. The third reason concerns the variety of dietary protocols, including differences in the amount of dietary fat and in the duration of treatment. Thus, consuming for 2 to 4 wk “prudent” diets with less cholesterol and saturated fat and more polyunsaturated fat than the previous “normal” diets tended to increase biliary cholesterol saturation in healthy women [2] but induced no change in “normal” diets with less cholesterol and dietary fat and in the duration of treatment. Thus, consuming for 2 to 4 wk “prudent” diets with less cholesterol and saturated fat and more polyunsaturated fat than the previous “normal” diets tended to increase biliary cholesterol saturation in healthy women [2] but induced no change in gallbladder bile of healthy men despite modifications in the relative proportion of biliary lipids [3]. Dietary supplementation with ω-3 fatty acids over 6 wk decreased the cholesterol saturation index (CSI) in healthy subjects [4]. A similar effect was found in lithiasic patients in association with an increased proportion of long ω-3 fatty acids and a decreased proportion of ω-6 fatty acids in biliary phospholipids [5]. In another study in patients who had gallstone, no change in lipid composition or decrease in cholesterol saturation was observed after supplementing the diet with linoleic acid or soybean lecithin for 3 wk [6]. Although the effects of polyunsaturated fat rather than the typical Western diet have been investigated in several studies, the influence of monounsaturated fats has been even less explored, although Schlierf et al. [7] found no significant difference in biliary lithogenicity in healthy males who received for 2 wk two lipid-lowering diets, one rich in polyunsaturated fatty acids and the other rich in oleic acid.

The present study compared the effects of two dietary oils that differ markedly in fatty acid profile (virgin olive oil and sunflower oil) on biliary lipid composition in human subjects. These oils are used preferentially in our geographic area. Further, olive oil is a major component of the Mediterranean diet, and its role in human health currently is being actively debated. The study group, which comprised 18 female patients who had gallstones and have been referred to elective cholecystectomy, was kept on the experimental diets for 30 d before surgery. This design made possible the collection of fasting gallbladder bile and stones (at surgery). In addition, fasting and postprandial hepatic bile (postcholecystectomy) levels were examined to rule out that a possible influence of the dietary intervention was masked by the occurrence of pre-existing gallstones.

Materials and methods

Subjects

Eighteen non-smoking, non-alcoholic women who had cholelithiasis, showed current clinical signs or symptoms, and were scheduled for elective surgery (cholecystectomy) were selected within the population served by the Hospital Clinico, University of Alicante (Alicante, Spain). None of them had undergone previous pancreatic, gastric, or biliary tract surgery. Cases of cholecholelithiasis and asymptomatic cholelithiasis were excluded from the study. The subjects had no history of systemic or gastrointestinal disease. None was receiving medication known to influence gastrointestinal secretions, motility, or postprandial hormonal responses. The study was conducted in conformance with the Helsinki Declaration and the experimental protocol was approved by the ethics committee of the Hospital Clinico. All subjects gave written consent after being fully informed of the nature and procedures of the study. Patients were randomized into two experimental groups, an olive oil group and a sunflower oil group, according to their dietary habits and specifically to the type of dietary fat habitually consumed before the study. This information was gained from a dietary history interview performed at the beginning of the study. Each group comprised nine patients and had the following characteristics (mean ± standard error): 54.4 ± 4.04 y of age and 27.3 ± 1.2 kg/m² for body mass index for the olive oil group and 41.7 ± 3.85 y of age and 28.4 ± 8.0 kg/m² for the sunflower oil group. These parameters showed no significant differences between groups.

Experimental protocol and diets consumed before surgery

Subjects from both groups were told at the start of the study to consume their habitual diets for the 30-d period immediately before surgery, with these requirements: 1) the only source of dietary fat used to prepare their meals had to be olive oil (olive oil group) or sunflower oil (sunflower oil group); 2) subjects had to avoid eating food items high in saturated fat (butter, sausages, etc.). Although patients with gallstone are usually very inclined to adhere to dietary recommendations, compliance with the diets was checked by completion of four 7-d dietary records. These were collected during weekly visits to the hospital, the data were quantified, and the energy and nutrient intakes were assessed with our computer program Nutrition and Health (Institute of Nutrition and Food Technology, University of Granada, Spain; General Asde, Valencia, Spain). The database of this program is the Spanish Food Composition Tables [8]. As shown in Table 1, the two dietary groups differed primarily in relation to intakes of polyunsaturated and monounsaturated fats, whereas the composition of the rest of the diet was similar.

Collection of gallbladder bile and stone samples

All patients fasted from 12 AM and underwent surgery between 9 and 10 AM. Gallbladders were removed with a laparoscopic cholecystectomy procedure. Bile samples were stored as aliquots at −80°C until analyzed for biliary lipid...
the same meal as the day before. Complete feeding and sampling procedure was repeated on ingestion of the liquid meal (200 mL ingested over 30 min). The gallbladder bile and duodenal content was collected at the distal aspiration site, separate aspiration of gastric and duodenal contents. Duodenal samples (2 mL) were taken immediately before and at 30, 60, 120, and 180 min after beginning the ingestion, which was placed in the third or fourth duodenal segment. Adequate positioning of the tube was confirmed by radiologic control at different times throughout the investigation. A radiopaque two-lumen nasoduodenal tube that enabled a semiquantitative polarizing light microscopy. Sections

Postcholecystectomy study

The experiments were performed 48 h after surgery, once it had been confirmed that subjects had recovered gastrointestinal motility. The test meals (pH 6.33, 294 mosm/L) and at 30, 60, 120, and 180 min after beginning the ingestion which was composed. Gallstones were preserved under sterile conditions for further microscopic examination.

Table 1
Daily energy and nutrient intake by gallstone patients during the 30-d dietary adaptation period before cholecystectomy*

<table>
<thead>
<tr>
<th></th>
<th>Olive oil group</th>
<th>Sunflower oil group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy (kcal/d)</td>
<td>1623.5 ± 153.3</td>
<td>1485.5 ± 297.9</td>
</tr>
<tr>
<td>Energy ratio</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Protein (%)</td>
<td>18.6 ± 1.7</td>
<td>18.2 ± 2.1</td>
</tr>
<tr>
<td>Carbohydrate (%)</td>
<td>39.4 ± 2.5</td>
<td>38.8 ± 3.9</td>
</tr>
<tr>
<td>Fat (%)</td>
<td>41.6 ± 2.5</td>
<td>42.6 ± 3.3</td>
</tr>
<tr>
<td>Fatty acids (g/d)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Monounsaturated fatty acids</td>
<td>40.1 ± 2.6</td>
<td>26.2 ± 2.9</td>
</tr>
<tr>
<td>Polysaturated fatty acids</td>
<td>8.3 ± 0.8</td>
<td>19.8 ± 2.5</td>
</tr>
<tr>
<td>Saturated fatty acids</td>
<td>20.6 ± 3.8</td>
<td>18.8 ± 3.2</td>
</tr>
</tbody>
</table>

* Subjects consumed diets containing virgin olive oil or sunflower oil as the main source of dietary fat. Data are presented as mean ± standard deviation of four 7-d dietary records per subject (nine subjects per group).

Sample analysis

Gallbladder bile and duodenal samples were analyzed for cholesterol, phospholipid, and total bile acid concentrations after removing protein and pigments [10]. Cholesterol levels were measured by the CHOD-PAP method (Boehringer Mannheim, Mannheim, Germany). An enzymatic colorimetric test (Boehringer Mannheim) was used to determine phospholipid concentration. Total bile acid concentration was measured by the 3α-hydroxysteroid dehydrogenase procedure [11].

A modification of the technique described by Tietz et al. [12] was used to measure individual glycine and taurine bile acid conjugates in gallbladder bile by reverse phase high-performance liquid chromatography with an isotropic solvent system. The chromatograph (Beckman Instruments, Fullerton, CA, USA) was equipped with a Waters µBondapak octadecysilane column (30 cm long, 3.9 mm in inner diameter, 10-μm particles; Waters Associates, Milford, MA, USA). Detection was accomplished at 200 nm. The mobile phase (flow rate 2 mL/min) was 10% acetonitrile and 90% of a mixture of methanol and 0.1 M monobasic potassium phosphate (60:40, v/v, pH 4.50). Before use, the solvent was filtered through a 0.45-μm filter (type HV, Millipore, Bedford, MA, USA). An elution profile of conjugated bile acid standards (Sigma, St. Louis, MO, USA) was obtained by injecting 20 μL of a standard methanolic mixture that contained 20 μg of each bile acid. We also established a standard curve for each standard bile acid by plotting concentration injected versus eluted peak area. Before analysis, gallbladder bile samples were diluted 1:3 (v/v) in isopropanol, heated for 10 min at 75°C, centrifuged at 450g for 15 min and the supernatant filtered through a Gelman Acrodisc filter (0.45-μm pore, Pall Gelman Sciences, Ann Arbor, MI, USA). Of this solution, 100 μL was evaporated under nitrogen and redissolved in methanol. The injection volume was 20 μL.

Gallstone sections (20- to 30-μm thick) were analyzed by semiquantitative polarizing light microscopy. Sections

Table 2
Fatty acid composition of the liquid test meals administered to cholecystectomized patients*

<table>
<thead>
<tr>
<th>Fatty acid composition</th>
<th>Olive oil group</th>
<th>Sunflower oil group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oleic acid (18:1)</td>
<td>61.89 ± 2.00*</td>
<td>29.03 ± 0.66</td>
</tr>
<tr>
<td>Linoleic acid (18:2ω-6)</td>
<td>5.06 ± 0.12*</td>
<td>42.16 ± 1.70</td>
</tr>
<tr>
<td>Monounsaturated fatty acids</td>
<td>63.08 ± 1.95*</td>
<td>29.67 ± 0.66</td>
</tr>
<tr>
<td>Polysaturated fatty acids</td>
<td>8.19 ± 0.14*</td>
<td>44.62 ± 1.55</td>
</tr>
<tr>
<td>Saturated fatty acids</td>
<td>29.03 ± 1.86</td>
<td>25.93 ± 1.93</td>
</tr>
<tr>
<td>U/S</td>
<td>2.51 ± 0.23</td>
<td>2.99 ± 0.30</td>
</tr>
</tbody>
</table>

U/S, ratio of unsaturated to saturated fatty acids

* Patients were kept on diets with olive oil or sunflower oil as the main source of dietary fat for 30 d before surgery. Results are expressed as grams per 100 g of total fatty acids. Values are mean ± standard error (n = 6 for both groups).

† P < 0.05 versus sunflower oil group.
were prepared by the Microscopy Service of the University of Granada (Granada, Spain). Briefly, gallstones were embedded in polyester resin (Estratil A-116, Glaspol Coposites, Valencia, Spain) for 30 min under vacuum and then stored in the freezer for 24 h. Next, samples were cut into 2- to 5-mm sections, polished, and mounted on glass slides impregnated with polyester resin. After drying, sections were reduced to a thickness of approximately 300 μm on a diamond cut-off saw. Sections were then ground and polished to the final thickness.

Data analysis and statistical evaluation

The CSI was defined as the ratio of the actual molar percentage of cholesterol in a given sample to that sample’s maximum capacity for cholesterol solubility. The latter was calculated according to the method of Metzger et al. [13]. In gallbladder bile samples, maximum cholesterol solubility was also obtained from Carey’s critical tables [14]. Unless otherwise stated, results are expressed as mean ± standard error. Statistical analysis was done with SPSS 11.0.1 for Windows (SPSS, Inc., Chicago, IL, USA). Differences between dietary groups in fatty acid composition of meals and in gallbladder and hepatic bile parameters were tested for significance by the independent samples Student’s t test. For statistical comparisons within groups (above or below fasting values) in the postcholecystectomy study, a one-way analysis of variance was done. P < 0.05 was considered statistically significant.

Results

Gallbladder bile and gallstones

Table 3 presents the composition of gallbladder bile specimens obtained at surgery. There was a significantly (P < 0.05) greater concentration (millimoles per liter) of cholesterol and bile acids in the olive oil group than in the sunflower oil group. The concentration of phospholipids was slightly higher in the olive oil group. As a consequence, gallbladder bile from patients who consumed olive oil had a higher total lipid content (11.48 ± 1.22 g/dL versus 7.77 ± 1.59 g/dL in the sunflower oil group, borderline significance with P = 0.063 with nine subjects in each group). When the composition of bile was expressed relative to total biliary lipids (molar percentages), we found that the proportions of cholesterol and bile acids were similar in both groups, which contrasts with a significantly (P < 0.05) lower molar percentage of phospholipids in the olive oil group. Among the linkage coefficients, only the molar ratio of cholesterol to phospholipids was found to differ between groups (P < 0.05), with higher values in the olive oil group. These differences, however, did not translate into bile lithogenicity, as shown by comparable values of cholesterol saturation (CSI; Table 3).

| Table 3 | Biliary lipid composition of gallbladder bile sampled at the time of surgery* |
|---------|---------------------------------|-----------------|-----------------|
|         | Olive oil group | Sunflower oil group |
| Total bile acids | | |
| mM/L | 142.23 ± 15.05 | 87.95 ± 20.42 |
| mol% | 66.47 ± 0.56 | 60.37 ± 3.11 |
| Cholesterol | | |
| mM/L | 17.60 ± 1.78 | 9.92 ± 1.94 |
| mol% | 8.33 ± 0.52 | 7.54 ± 1.20 |
| Phospholipids | | |
| mM/L | 54.06 ± 6.00 | 42.55 ± 7.94 |
| mol% | 25.20 ± 0.25 | 32.09 ± 2.43 |
| Linkage coefficients | | |
| Cholesterol/bile acids | 0.126 ± 0.009 | 0.132 ± 0.027 |
| Phospholipids/bile acids | 0.379 ± 0.006 | 0.553 ± 0.072 |
| Cholesterol/phospholipids | 0.331 ± 0.021 | 0.236 ± 0.034 |
| CSI‡ | 0.835 ± 0.032 | 0.857 ± 0.167 |
| CSI§ | 0.906 ± 0.069 | 1.013 ± 0.176 |
| Total lipids (g/dL) | 11.48 ± 1.22 | 7.77 ± 1.59 |

CSI, cholesterol saturation index
* Patients were kept on diets with either olive oil or sunflower oil as the main source of dietary fat for 30 d prior to surgery. Data are mean ± standard error (n = 9, for both groups).
† P < 0.05 versus sunflower oil group.
‡ Calculated according to Metzger et al. [13].
§ Calculated according to Carey [14].

Bile acid composition of gallbladder bile is shown in Fig. 1. No significant differences were noted in the proportion of six major individual bile acid conjugates. The molar ratios of glycine-to-taurine conjugates and dihydroxylated-to-trihydroxylated bile acids were similar in the olive oil group (3.19 ± 0.44 and 1.69 ± 0.07, respectively) and the sunflower oil group (3.81 ± 0.74; 2.04 ± 0.18, respectively; n = 9 for both groups in all parameters). Values for total (glycine plus taurine conjugates) cholic acid were 37.33 ± 1.02 mol% for the olive oil group, and 33.40 ± 1.74 mol% for the sunflower oil group and those for chenodeoxycholic acid were 43.52 ± 1.09 mol% for the olive oil group and 41.17 ± 3.10 mol% for the sunflower oil group (n = 9 for both groups in all parameters).

Gallstones from all patients were analyzed. Using a 70% minimum cholesterol content to define mixed cholesterol, the following results were obtained. In the sunflower oil group, one of nine patients had a pure cholesterol stone and the rest had mixed cholesterol stones composed of: cholesterol and sodium palmitate (one of eight), cholesterol and bilirubin (five of eight), and cholesterol, bilirubin, and sodium palmitate (two of eight). All patients in the olive oil group had mixed cholesterol stones composed of: cholesterol and sodium palmitate (one of nine), cholesterol and bilirubin (five of nine), and cholesterol, bilirubin, and sodium palmitate (three of nine).

Postcholecystectomy study

No differences were noted between groups in the percent composition of biliary lipids in fasting duodenal samples.
cholesterol to bile acids (Fig. 3A, B) followed a similar trend in the olive oil group. At 60 and 120 min, cholesterol values were significantly different compared with the fasting level, whereas a clear decrease was noted in the sunflower oil group after food intake, whereas a significant decrease was noted in the olive oil group (Fig. 2A). Molar percentage of cholesterol in any group, and there were no significant differences between them (Fig. 2C). No appreciable changes from fasting values were recorded for the molar percentage of bile acids in the sunflower oil group after the test meal in contrast to a significant increase at 60 min postprandially in the olive oil group (Fig. 2A). However, this different response did not result in appreciable changes in the ratio of cholesterol to phospholipids and of cholesterol to bile acids (Fig. 3C). As measured by the CSI (Fig. 3C), hepatic bile secreted by patients in the sunflower oil group appeared supersaturated throughout the experiment (mean CSI > 1.5), whereas the degree of cholesterol saturation in bile obtained from patients in the olive oil group decreased from a fasting level of 2.051 ± 0.290 (n = 18, supersaturated) to a significantly lower value of 1.048 ± 0.152 (n = 18) at 60 min postprandially and to a minimum of 0.821 ± 0.201 (n = 18) at 120 min (P < 0.05) that was indicative of undersaturated bile. CSI showed a significant (P < 0.05) difference between dietary treatments at 120 min after the meal.

Discussion

Manipulation of dietary fat ingestion did not influence the cholesterol saturation of gallbladder bile sampled at the time of surgery. To a certain extent, this was an expected finding because our subjects were patients with pre-existing gallstones. Data on the effects of dietary fats and fatty acids on gallbladder bile in patients with established cholelithiasis are very limited. In one study, fish oil decreased the CSI by 25% after a 5-wk treatment period [5]. In contrast, dietary supplementation with linoleic acid for 3 wk did not alter the lipid composition or cholesterol saturation [6], in good agreement with the results of the present study. The occurrence of a similar CSI in our two groups is also in line with the homogeneity in the composition of the stones. Concerning the profile of individual bile acids in gallbladder bile, we observed no clear influence of the type of fat ingested. Both groups of patients displayed a modest “cheno” profile, i.e., bile had slightly more chenodeoxycholic acid than cholic acid. This is a common feature in patients who have cholesterol gallstone. In comparison, cholic species predominated in healthy human bile [1, 15].

When gallbladder bile composition was analyzed in terms of absolute concentrations of lipids, marked differences were found between our study groups. Concentrations of cholesterol and total bile acids were enhanced in patients who received the olive oil diet. Gallbladder bile from the olive oil group also had considerably higher total lipid content. A decreased total lipid concentration has been reported in samples of gallbladder bile collected from patients who had cholesterol gallstones [16]. It is unclear why this feature was noted only in our patients who received the sunflower oil diet. One possibility is that gallbladders in the sunflower oil group absorbed fluid less efficiently than did those in the olive oil group. Gallbladder fluid absorption is usually determined indirectly by calculating the ratio of gallbladder to hepatic bile for bile acid concentration. Unfortunately, we were unable to collect paired gallbladder and hepatic samples at surgery, but the concentrations of bile acids (as non-absorbable bile solutes) and total lipids in gallbladder bile provide a rough estimate [17]. A proposed...
mechanism for differential gallbladder fluid absorption is that the activity of the membrane-bound enzyme Na\(^+\)-K\(^+\)-adenosine triphosphatase, the key factor in this process, is affected by dietary-induced alterations in phospholipid acyl chain composition, as shown in other tissues [18]. Whether this is also true for epithelial cells of the gallbladder membrane awaits investigation. It cannot be excluded that these results are, other than an expression of gallbladder fluid absorption, at least partly influenced by differences in hepatic bile composition. However, our observation that the absolute concentration of bile acids in fasting duodenal samples obtained after cholecystectomy from patients who received olive oil (data not shown) was significantly lower than in those who received sunflower oil (although the opposite was found in gallbladder bile) seems to support the first hypothesis.

Previous work in our laboratory [19] investigated the effects of feeding dogs diets high in olive oil or sunflower oil on bile secretion. The animals, with intact gallbladders, were prepared with chronic biliary cannulas and secretion was studied at rest and after food intake. Patterns of biliary response to food suggested a greater involvement of the gallbladder in animals fed the olive oil diet, as most clearly shown by a marked increase in bile acid output soon after eating compared with a continuous decrease in dogs fed sunflower oil. These differences [19] could not be explained by changes in bile flow rate, which was similar during the initial postprandial hours, but by dramatic differences in the concentration of bile acids in the bile secreted. Although this might be in part a consequence of differences in gallbladder emptying [20], the existence of a more concentrated gallbladder...
bile after olive oil feeding (as shown in the present study) is also compatible with the results in dogs.

The investigation of biliary homeostasis is very difficult to perform in humans. In the second part of this study, hepatic bile was obtained from cholecystectomized patients. The rationale for these experiments may be questioned because there is the concept that gallbladder bile aspirated at surgery after an overnight fast is much more representative of the situation of the bile salt pool and other biliary lipids. We agree in part with this idea but also believe that examination of hepatic bile composition after cholecystectomy was crucial in this study to confirm that a possible effect of dietary fat type on biliary lipids was detected. Analysis of only gallbladder bile could have masked the influence of the dietary intervention because of the presence of established gallstones. We collected hepatic bile samples by duodenal intubation. This technique does not allow measurement of biliary lipid output unless a non-absorbable marker is used, but this approach would have complicated even more an already demanding study. However, duodenal sampling provided a mechanism by which the proportion of biliary lipids (relative to total lipids) in hepatic bile could be quantified, offering interesting data that would otherwise be difficult to obtain in humans in vivo.

No differences were noted in molar percentages, molar ratios, or cholesterol saturation (CSI) between our groups of fasted cholecystectomized patients. CSI in all patients was indicative of cholesterol supersaturation. This is a well-recognized physiologic phenomenon [21–24] that results from the curvilinear relation between cholesterol and bile acid output [23,24]. During overnight fasting, which interrupts the enterohepatic circulation, bile acid flux rate is very
low and bile becomes supersaturated with cholesterol. Conversely, accelerated recirculation of intestinal bile acids in response to a meal increases bile acid output and hepatic bile reverts to a less saturated state. This trend, which has been observed in healthy, lithiasic, and cholecystectomized subjects [21–24], was, surprisingly, non-existent in our patients in the sunflower oil group (Figs. 2 and 3). Without biliary output data, this finding is amenable to more than one interpretation. The postprandial response observed in this study is consistent with an attenuated increase in bile flow and bile acid output in the sunflower oil group compared with a normal response in the olive oil group. Sustained output of bile acids in response to a meal depends on the return of bile acids to the liver, which, in cholecystectomized patients, is strongly influenced by intestinal motility and transit time [23,24]. The ingestion (chronic, acute, or both) of the two types of fat used in this study might have produced different feeding motility patterns through differential stimulation of humoral agents, in turn affecting choleresis. A second possibility is that patients in the sunflower oil group have normal bile acid secretion in response to food but that this is coupled with a supranormal cholesterol secretion rate and persistence of cholesterol supersaturation of bile. In the rat, a species lacking the gallbladder, diets rich in ω-6 polyunsaturated fatty acids versus saturated fats have been shown to increase the secretion of cholesterol into bile [25,26]. Biliary cholesterol is derived principally from circulating lipoproteins [1,27], mainly high-density lipoprotein [1,28,29]. This particle is captured by the hepatic scavenger receptor class B type I [30,31]. Interestingly, diets rich in ω-6 polyunsaturated fatty acids upregulate hepatic expression of scavenger receptor class B type I [32] and enhance binding of high-density lipoprotein to liver membranes [33] compared with saturated and monounsaturated fats, respectively. This explains the lowering effect of plasma high-density lipoprotein cholesterol by dietary ω-6 polyunsaturated fatty acids, an effect not consistently shared by monounsaturated fatty acids [34,35]. According to this metabolic hypothesis, our results in the sunflower oil group may have been the result of increased transfer of cholesterol back to the liver and subsequently more secretion into bile. It remains unknown why cholesterol hypersecretion occurred only postprandially. It seems clear that the nature of the delivery and route of transit of cholesterol from the blood through the liver into bile need to be clarified.

Duodenal sampling in the present work was conducted 48 h after surgery, so we should caution readers that the results of the postcholecystectomy study might reflect abnormal postoperative changes. However, it is more likely that the differences found in biliary lipid composition after the test meals can be explained by changes in dietary fat intake. There are two main reasons for this. First, cholecystectomy was performed by laparoscopic means. This procedure is usually accompanied by a very short recovery of gastrointestinal motility [36,37]. Before the experiments, we confirmed in all patients by clinical observations that postoperative atony was non-existent. Second, our patients tolerated a high degree not only of the test meals but also the rest of the food supplied (once each experiment was completed) to suit their daily nutritional requirements.

In conclusion, the present results indicate that type of dietary fat (olive oil versus sunflower oil) affects bile composition in humans with established cholelithiasis. Although manipulation of dietary fat ingestion did not influence the cholesterol saturation or the profile of individual bile acids in gallbladder bile, there were marked differences in terms of absolute concentrations of lipids and total lipid content. After cholecystectomy, cholesterol saturation of hepatic bile decreased typically in response to a test meal only in patients given the olive oil diet and remained unchanged, i.e., supersaturated, in those given sunflower oil. Further research is required to define the precise mechanisms of the reported effects and to confirm whether a similar behavior occurs in healthy human subjects.

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