Fish Oil (n-3) Polyunsaturated Fatty Acids Beneficially Affect Biliary Cholesterol Nucleation Time in Obese Women Losing Weight

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Nahum Méndez-Sánchez,* Verónica González,* Patricia Aguayo,* Juan M. Sánchez,* Miguel A. Tanimoto,* Javier Elizondo† and Misael Uribe*†

*Departments of Biomedical Research, Gastroenterology and Radiology, Medica Sur Clinic and Foundation, Mexico City, Mexico and †Department of Gastroenterology and Endoscopy, The National Institute of Nutrition, Mexico City, Mexico

ABSTRACT It has been reported that intake of (n-3) polyunsaturated fatty acids (PUFA) reduces the risk of coronary heart disease and decreases biliary cholesterol saturation in the bile of gallstone patients. We investigated the effect of n-3 PUFA on cholesterol saturation index (CSI) and nucleation time (NT) in obese subjects who were losing weight. This was a double-blind, placebo-controlled clinical trial. Obese women (n = 35) with a body mass index (BMI) ≥ 30 kg/m², with no prior history of gallstones or cholecystectomy by ultrasound were first studied to ensure absence of stones or biliary sludge. The women were then assigned to a hypocaloric regimen [5.02 MJ (1200 kcal)/d] and to receive 1200 mg/d of ursodeoxycholic acid (UDCA), 11.3 g/d of (n-3) PUFA or a placebo for 6 wk. BMI, CSI and NT were recorded at baseline and at the end of the experimental period. BMI decreased 5.75 ± 2.7%mo (range, 1.5–12.42%) during the experiment. The CSI did not change in any of the groups. Cholesterol NT decreased significantly in the UDCA and placebo groups, but not in the (n-3) PUFA group. None of the women had developed gallstones at 6 wk. These results suggest that (n-3) PUFA maintain the CSI and NT in obese women during rapid weight loss, which probably results in the prevention of cholesterol gallstone formation.


KEY WORDS: obesity ♦ bile acids ♦ cholesterol ♦ (n-3) PUFA ♦ gallbladder ♦ gallstones

Gallstone disease is a major cause of morbidity in the United States (1), other Western countries (2) and Latin American countries, such as Chile (3) and Mexico (4), where the economic impact of gallstone disease is high (1,2). Effective prevention of gallstone formation is, therefore, an important objective. Epidemiologic studies have identified risk factors for cholesterol gallstones (2,5,6). Obesity is the most consistent and important of these. In addition, rapid loss of weight induced by diet or surgery in obese patients results in the frequent and rapid formation of cholesterol gallstones that are often symptomatic (7–9). The risk of developing gallstones during weight reduction is now well accepted (10,11). Approximately 10–25% of people experiencing rapid weight reduction through very low energy intake quickly develop gallstones (7,8,10), and 35% of patients with morbid obesity develop gallstones as they lose weight after bariatric surgery (12–14).

Several diet and drug strategies have been developed to prevent cholesterol gallstone formation in obese subjects during weight loss. Heshka et al. (15) studied 70 obese patients with a mean body mass index (BMI)3 of 28.9 ± 2.8 kg/m², who lost an average of 5.1 ± 3.6 kg of body weight while consuming a normal diet [5.02 MJ (1200 kcal)/d]. No gallstone formation was observed by ultrasonography during the 16-wk study period. However, it is important to note that this study did not measure the cholesterol saturation index (CSI) or nucleation time (NT). Ursodeoxycholic acid (UDCA) is a drug used as prophylaxis in gallstone disease (7,16). Intake of (n-3) polyunsaturated fatty acids (PUFA) has been associated with a reduced risk of coronary heart disease (17). Interestingly, the same (n-3) PUFA-consuming populations also appear to have a relatively low prevalence of cholesterol gallstone disease (18). It has also been reported that (n-3) PUFA decrease biliary cholesterol saturation in the bile of patients with gallstones (19). The aim of this study was to assess the effects of (n-3) PUFA on the CSI and cholesterol NT in obese subjects during weight loss.

SUBJECTS AND METHODS

Subjects. We recruited obese women from two medical centers in Mexico City (The Medica Sur Clinic and Foundation and The National Institute of Nutrition). They were informed of the risk of gallstone formation while dieting, and were asked whether they would participate in a study of gallstone prevention. To be accepted into this trial, subjects required the following: 1) a BMI ≥ 30 kg/m²; 2) an age between 20 and 60 y; 3) a willingness to participate in the diet plan for 6 wk; and 4) normal serum potassium and calcium levels. Women of childbearing age further required a negative serum pregnancy test result. Patients were excluded from the study if they had any one of the following: 1) previous cholecystectomy; 2) gallstone or gallbladder sludge diagnosed by ultrasonogram on entry to the trial; 3) hypersensitivity to bile acids or fish oil; 4) a history of hypothyroidism or Cushing's syndrome; 5) an eating disorder or other psychological problem that might interfere with participation in the diet program; 6) use of oral bile acid preparations, aluminum-based antacids or lithium; 7) long-term use of nonsteroidal anti-inflammatory agents (including aspirin) or antihyperlipidemic agents (including cho-

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2 To whom correspondence should be addressed.

E-mail: nmendez@medicasur.org.mx.

3 Abbreviations used: BMI, body mass index; CCK, cholecystokinin; CSI, cholesterol saturation index; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; NT, nucleation time; PUFA, polyunsaturated fatty acids; UCDA, ursodeoxycholic acid.
was discarded. Bile specimens were collected on ice and immediately
acid solution. A 20- to 30-mL sample of concentrated bile was usually
bladder using an intraduodenal infusion of 30 mL of an 80 g/L amino
second portion of the duodenum, and Vater’s ampulla was visualized.

The patients gave the standard per-

Body mass index, 

Body weight, measured

Weight-control program. The patients gave the standard personal
statement and were given the standard physical exam-
ination of the Department of Program Control, as well as laboratory
tests, including a complete blood count, measurement of electrolytes,
assessment of liver chemistry, measurement of fasting lipids, thyroid-
function tests and electrocardiography. They consumed the normal

diet consisting of 20% of energy as fat, 60% as carbohydrates and 20%
as protein, with 1 L of water daily.

Experimental procedures. Real-time ultrasonographic studies
were done while the patients were fasting. Gallstones were defined by

characteristics of obese women at the start of the diet trial

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>UDCA2 (1200 mg/d)</th>
<th>(n-3) PUFA (11.3 g/d)</th>
<th>Placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>12</td>
<td>12</td>
<td>11</td>
</tr>
<tr>
<td>Age, y</td>
<td>37.8 ± 8.7</td>
<td>37.0 ± 9.8</td>
<td>39.7 ± 8.4</td>
</tr>
<tr>
<td>Body weight, kg</td>
<td>79.8 ± 12.1</td>
<td>84.3 ± 9.8</td>
<td>81.9 ± 11.6</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>34.2 ± 4.2</td>
<td>34.2 ± 3.7</td>
<td>33.4 ± 1.6</td>
</tr>
</tbody>
</table>

1 Values are means ± sd.
2 UDCA, ursodeoxycholic acid; (n-3) PUFA, (n-3) polyunsaturated fatty acids.

A total of 42 women were enrolled in the trial. Of these, seven withdrew prematurely and were not included in the final
analysis. The major reason for early termination was voluntary withdrawal from the program [n = 4 (9.5%)]. The percentage
of subjects who voluntarily withdrew from the trial did not differ among the three groups. Three women were excluded
after becoming pregnant during the trial [n = 3 (7.1%)]. The percentage of subjects who became pregnant did not differ
among the three treatment groups.

UDCA and (n-3) PUFA were well tolerated. Only one woman in the (n-3) PUFA group reported a fish taste. The frequency of reported adverse experiences was similar in pa-
tients receiving UDCA and (n-3) PUFA. The most common adverse experiences were abdominal bloating and constipation in
women from each group.

The clinical characteristics of subjects at the beginning of the
diet trial are given in Table 1. Body weight, measured 6.1 kg (range, 67.5–105.6 kg). The initial BMI was 34.0 ± 3.3 kg/m² (range, 30.4–44.5 kg/m²).

Body weight and BMI did not differ among the three treatment

A weekly dietary history was obtained throughout the study, and
the intake of fat, protein, carbohydrates and fiber was calculated.

Mazur et al. (22). The subjects were divided into three groups:

by weight. Weight reduction on the diet program. Maximum weight
and maximum decrease in BMI for all subjects of the three
groups participating in the diet trial were 6.3 ± 2.9 kg (range, 3.2–14.5 kg) and 2.5 ± 1.1 kg/m² (range, 1.3–5.7 kg/m²), respectively (Table 2). These variables did not differ among the
groups. The rate of weight loss was 4.4 ± 2 kg/mo (range, 1.5–9.4 kg/mo). All subjects had a decrease of 5.75 ± 2.7% in
their BMI per month during the diet program (range, 1.5–12.42%) (Table 2).

Gallstone formation during weight reduction. None of the women developed gallstones that were visible by ultra-
sonography by 6 wk.
The effects of dietary fish oil on biliary lipid secretion and cholesterol gallstone formation. They found that 67% of monkeys fed a lard diet developed cholesterol gallstones compared with only 22% of those fed a fish oil diet \( (P = 0.08) \). The CSI of gallbladder bile also tended to be higher \( (P = 0.06) \) in the lard-fed group \( (1.15 \pm 0.11) \) compared with the fish oil–fed group \( (0.86 \pm 0.09) \). On the other hand, it has since been reported that cholesterol gallstone formation in prairie dogs is accompanied by an increase in the percentage of biliary phospholipids containing arachidonic acid, and an increase in gallbladder prostaglandin synthesis. Booker et al. (32) reported that dietary fish oil significantly influences biliary phospholipids and decreases the incidence of cholesterol monohydrate crystal formation in prairie dogs. They suggested that these effects are probably due to the replacement of arachidonic by \((\text{n}-3)\) PUFA \((\text{eicosapentaenoic acid (EPA)}; 20:5)\) and docosahexaenoic acid \((\text{DHA}; 22:6)\). More recently, Abei et al. (33) observed that fish oil inhibits two pathways of mucus glycoprotein secretion in gallbladder epithelial cells. This observation is important because gallbladder mucus glycoprotein secretion is a critical factor in the pathogenesis of gallstones. Furthermore, it has been demonstrated in rats that dietary fish oil changes intrahepatic cholesterol transport and the hyper-secretion of cholesterol into bile \((34)\). Dietary fish oil also increases the deposition of cholesterol into bile by potentiating bile acid–dependent cholesterol secretion, presumably by facilitating the recruitment of cholesterol.

We contend that \((\text{n}-3)\) PUFA have a beneficial effect on bile composition. The mechanisms by which \((\text{n}-3)\) PUFA improve the bile involve changes in fatty acid composition, enrichment with EPA- and DHA-containing phospholipids, and depletion in linoleic- and arachidonic acid–containing species. Interestingly, in both monkeys \((31)\) and humans \((19)\), dietary fish oil reduced the CSI. By linear path analysis, Berri et al. (19) found a positive correlation between the relative amount of cholesterol in human gallbladder bile and the contribution of oleic and arachidonic acids to biliary phospholipids, whereas negative relationships with linoleic and palmitoleic acids were found. However, EPA and DHA were not included in this analysis.

Another factor that may be involved in maintaining the CSI and cholesterol NT during the weight reduction period is the rate of weight loss. Several studies have shown that the rate of weight loss affects the composition of the bile (28,29). In a recent study, we focused on bile composition and motility (28,29). The results of the present study confirm that conclusion. In this clinical trial, we examined the role of \((\text{n}-3)\) PUFA in the diet on the CSI and cholesterol NT. Other studies investigated the role of diet composition on gallbladder motility (28,29), whereas in this study, we focused on bile composition.

To compare the effects of a drug such as a UDCA with \((\text{n}-3)\) PUFA, we designed the present randomized study with a placebo control. UDCA has been proven safe in both preventing and treating gallstones \((7,30)\). Furthermore, it has been proposed that UDCA has an effect on bile composition that results from a reduction in cholesterol secretion \((30)\), whereas \((\text{n}-3)\) PUFA decrease the CSI of the bile in patients with gallstones \((19)\). But how do \((\text{n}-3)\) PUFA maintain cholesterol nucleation time? The available data come from experimental studies, including that of Scobey et al. \((31)\) who studied two groups of adult male African green monkeys to assess the

### TABLE 2

<table>
<thead>
<tr>
<th>Variable</th>
<th>UDCA</th>
<th>((\text{n}-3)) PUFA</th>
<th>Placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td>(n)</td>
<td>12</td>
<td>12</td>
<td>11</td>
</tr>
<tr>
<td>Maximum weight loss, kg</td>
<td>5.68 ± 1.9</td>
<td>7.03 ± 4.2</td>
<td>6.13 ± 2.8</td>
</tr>
<tr>
<td>Maximum decrease in body mass</td>
<td>2.4 ± 1</td>
<td>2.85 ± 1.6</td>
<td>2.2 ± 0.7</td>
</tr>
<tr>
<td>Rate of weight loss, kg/mo</td>
<td>4.24 ± 1.3</td>
<td>4.95 ± 2.7</td>
<td>3.96 ± 2.2</td>
</tr>
<tr>
<td>Decrease in body mass index, %/mo</td>
<td>6.12 ± 2.6</td>
<td>5.86 ± 3.2</td>
<td>5.10 ± 2.6</td>
</tr>
</tbody>
</table>

\(^1\) Values are means ± sd.
\(^2\) At 6 wk.
the type of diet that we used in this study. In fact, the composition of the diet in terms of fat has been proposed as an important factor in preventing gallstone formation. This is because when the diet contains adequate stimuli for duodenal cholecystokinin (CCK) release (at least 10 g triglyceride/d), normal gallbladder motor function is maintained (35), and neither biliary sludge nor gallstones develop (7,8). CCK given intravenously completely abolishes the risk of biliary sludge and stone formation during total parenteral nutrition (36), which supports the view that gallbladder stasis contributes to gallstone development. In this study, the diet consisting of 20% of energy as fat, 60% as carbohydrates and 20% as protein.

In conclusion, (n-3) PUFA administered to obese subjects is associated with the maintenance of the CSI. In addition, (n-3) PUFA improve the composition of the bile through maintenance of the cholesterol saturation level. Because obesity and rapid weight loss are modifiable risk factors for gallstone disease, our results suggest that it is possible to make dietary recommendations for gallstone prevention at this time.

LITERATURE CITED