

Fish Oil (n-3) Polyunsaturated Fatty Acids Beneficially Affect Biliary Cholesterol Nucleation Time in Obese Women Losing Weight¹

(Manuscript received 20 March 2001. Initial review completed 4 May 2001. Revision accepted 23 June 2001.)

Nahum Méndez-Sánchez,* Verónica González,*
Patricia Aguayo,* Juan M. Sánchez,* Miguel A. Tanimoto,*
Javier Elizondo[†] and Misaél Uribe*^{†2}

*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 $5.75 \pm 2.7\%$ /mo (range, 1.5–12.42%/mo) 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. *J. Nutr.* 131: 2300–2303, 2001.

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-

¹ Supported in part by the National Council of Science and Technology of Mexico, and The Ministry of Health, CONACYT-SSA, Project No. M0059-M9602, (N.M.,-S, and M.U.).

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; UDCA, ursodeoxycholic acid.

lestyramine) within 2 wk of entering the trial; or 8) a total bilirubin level >20 mg/L. Any diuretic therapy was discontinued at least 1 d before trial entry.

A total of 42 women, who met all of the entry criteria and agreed to participate, were enrolled in the trial. The study was approved by the Human Subjects Committees at The Medica Sur Clinic and Foundation and The National Institute of Nutrition, as conforming with the ethical guidelines of the 1975 Declaration of Helsinki; written informed consent was obtained from all participants before entry.

Study design. Subjects were assigned to one of three treatment groups in a double-blind fashion, with blocking for body weight (BMI > 30 kg/m²), according to a table of random numbers. One group received a normal diet (5.02 MJ) plus 1200 mg/d of UDCA (Laboratorios Farmasa, S.A. de C.V., Mexico); the second group received a normal diet (5.02 MJ) plus 11.3 g/d of fish oil containing (n-3) PUFA in three doses (Eicosapen; generously provided by Nycomed Arzneimittel GmbH, Munich, Germany); and the third group received the normal diet (5.02 MJ) plus a placebo. All capsules were identical in appearance and number. Treatment was begun on the day on which energy restriction began. Compliance was determined by counting unused capsules each week. Subjects were treated for 6 wk.

Weight-control program. The patients gave the standard personal history statement and were given the standard physical examination 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 the presence of strong intraluminal echoes that were gravity dependent, or that attenuated ultrasound transmission (acoustic shadowing). Sludge was defined as diffuse, low amplitude, homogeneous or heterogeneous nonshadowing echoes forming a fluid-fluid level. At the completion of each woman's participation in the study, all ultrasonographic studies were evaluated again by the same radiologist. No discrepancies were found between the results of the first and second evaluations. In the second evaluation, all studies for each subject were viewed side-by-side without knowledge of group assignment.

Bile analysis. Bile was collected endoscopically from all subjects for analysis, before the start of the diet treatment and at the completion of the trial. Each subject had a routine upper endoscopy while under conscious sedation (midazolam, 2–4 mg) in the morning after an overnight fast. The tip of the endoscope was advanced into the second portion of the duodenum, and Vater's ampulla was visualized. Bile samples were collected by stimulating contraction of the gallbladder using an intraduodenal infusion of 30 mL of an 80 g/L amino acid solution. A 20- to 30-mL sample of concentrated bile was usually obtained. Any dilute bile obtained before the appearance of dark bile was discarded. Bile specimens were collected on ice and immediately transported to the laboratory where they were stored at –20°C.

All bile samples were thawed and analyzed as a single batch. Total phospholipid levels were determined by the method of Bartlett (20). Cholesterol was determined after appropriate dilution of whole bile with an assay kit (Sigma Chemical, St. Louis, MO). The total bile salt concentration was enzymatically analyzed using Turley's (21) method. The CSI of each bile sample was calculated from tables developed by Carey (22). Cholesterol NT were determined as described by Holan et al. (23). For this assay, each sample was filtered through a 0.22- μ m filter (Millipore Continental Water Systems, Bedford, MA), transferred into a 1-mL glass microvial, flushed with nitrogen, sealed and placed in an incubator at 37°C. Time zero of nucleation was defined as 1 h after thermal equilibration. The nucleation time was defined as the interval between time zero and the first detection of solid cholesterol crystals. Samples were checked for cholesterol crystal formation by polarized microscopy, once daily for up to 3 wk.

Statistical analysis. Differences among the groups were evaluated by ANOVA and Bonferroni tests and changes within a group over time were evaluated by paired Student's *t* test (24). The differ-

ences were considered significant when $P < 0.05$. Values in the text are means \pm SD.

RESULTS

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 patients receiving UDCA and (n-3) PUFA. The most common adverse experiences were abdominal bloating and constipation in two women from each group.

The clinical characteristics of subjects at the beginning of the diet trial are given in Table 1. Body weight, measured before the start of treatment for subjects included in the final data analysis, was 81.8 ± 11.0 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 groups before the start of the study.

Weight reduction on the diet program. Maximum weight loss 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 $5.75 \pm 2.7\%$ in their BMI per month during the diet program (range, 1.5–12.42%) (Table 2).

Bile analysis. The CSI did not change ($P = 0.18$ – 0.67) in any of the groups during the experiment. Initial and final values in the groups were as follows: 1.37 ± 0.6 and 1.25 ± 0.4 (UDCA); 1.27 ± 0.5 and 1.04 ± 0.3 [(n-3) PUFA]; and 1.10 ± 0.1 and 0.99 ± 0.1 (placebo). Cholesterol NT decreased significantly in both the UDCA and placebo groups from 13.2 ± 5.0 d to 7.8 ± 3.0 d and from 16.4 ± 5.7 d to 10.3 ± 3.2 d, respectively ($P < 0.05$), but did not change in the (n-3) PUFA group (from 17.1 ± 5.2 d to 14.0 ± 3.7 d; $P = 0.12$) (Fig. 1).

Gallstone formation during weight reduction. None of the women developed gallstones that were visible by ultrasonography by 6 wk.

TABLE 1

Characteristics of obese women at the start of the diet trial¹

Characteristic	UDCA ² (1200 mg/d)	(n-3) PUFA (11.3 g/d)	Placebo
<i>n</i>	12	12	11
Age, y	37.8 ± 8.7	37.0 ± 9.8	39.7 ± 8.4
Body weight, kg	79.8 ± 12.1	84.3 ± 9.8	81.9 ± 11.6
Body mass index, kg/m ²	34.2 ± 4.2	34.2 ± 3.7	33.4 ± 1.6

¹ Values are means \pm SD.

² UDCA, ursodeoxycholic acid; (n-3) PUFA, (n-3) polyunsaturated fatty acids.

TABLE 2

Weight reduction in obese women consuming a weight-loss diet supplemented with ursodeoxycholic acid (UDCA), (n-3) polyunsaturated fatty acids (PUFA) or placebo for 6 wk¹

Variable	UDCA (1200 mg/d)	(n-3) PUFA (11.3 g/d)	Placebo
n	12	12	11
Maximum ² weight loss, kg	5.68 ± 1.9	7.03 ± 4.2	6.13 ± 2.8
Maximum ² decrease in body mass index, kg/m ²	2.4 ± 1	2.85 ± 1.6	2.2 ± 0.7
Rate of weight loss, kg/mo	4.24 ± 1.3	4.95 ± 2.7	3.96 ± 2.2
Decrease in body mass index, %/mo	6.12 ± 2.6	5.86 ± 3.2	5.10 ± 2.6

¹ Values are means ± SD.

² At 6 wk.

DISCUSSION

The results of this study suggest that (n-3) PUFA administered to obese women is associated with the maintenance of the CSI. In addition, (n-3) PUFA improved the composition of the bile as indicated by the maintenance of cholesterol NT.

Several studies suggest that the CSI increases during rapid weight loss (7). This increase can then lead to the development of cholesterol gallstones. In contrast, in this study, the CSI tended to decrease in each group ($P = 0.18-0.67$) during the experiment. Interestingly, no subjects developed gallstones visible by ultrasonography at 6 wk. We also found that cholesterol NT decreased significantly in both the UDCA and placebo groups, but not in the women given (n-3) PUFA. The observed response of the women to UDCA is not consistent with the expected increase in NT and decrease in the CSI (25). However, we believe that this difference can be explained by two factors. The experimental period of this study was shorter (6 wk) than that in the previous studies (12–16 wk) and the NT of subjects treated with UDCA in this study did not decrease as rapidly as had been observed in untreated subjects with cholesterol gallstones (<2 d) (26). Interestingly, there are no data concerning the biliary cholesterol NT in obese subjects who are losing weight.

The aim of this study was to evaluate the effects of (n-3) PUFA on the CSI and cholesterol NT in obese subjects during weight loss. Recently, we had an opportunity to analyze the factors involved in the development of cholesterol gallstones in subjects losing weight (27). In that analysis, we concluded that both a low energy diet and the fat composition of the diet play important roles. The results of the present study confirm that conclusion. In this clinical trial, we examined the role of (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 (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 (n-3) PUFA decrease the CSI of the bile in patients with gallstones (19). But how do (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

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 ± 0.11) compared with the fish oil-fed group (0.86 ± 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 arachidonate by (n-3) PUFA [eicosapentaenoic acid (EPA; 20:5) and docosahexaenoic acid (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 hypersecretion 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 (n-3) PUFA have a beneficial effect on bile composition. The mechanisms by which (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, Berg 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

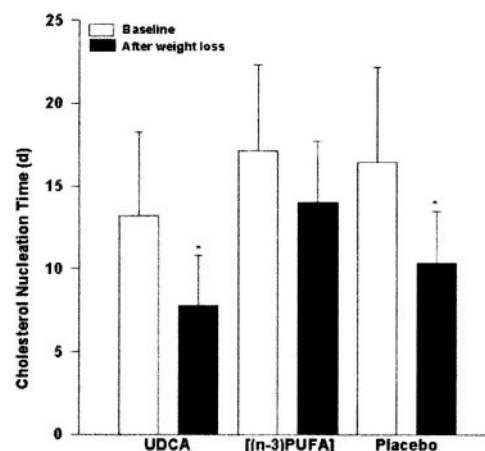


FIGURE 1 Bile cholesterol nucleation time at baseline and after weight loss in obese women consuming a weight-loss diet supplemented with ursodeoxycholic acid (UDCA), (n-3) polyunsaturated fatty acids (PUFA) or placebo for 6 wk. Values are means ± SD, $n = 11-12$. *Different from baseline, $P < 0.05$

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 nucleation time. 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.

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