The Role of Gallbladder Emptying in Gallstone Formation During Diet-Induced Rapid Weight Loss

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Obese persons are at risk for cholesterol gallstones because their bile is saturated with cholesterol. The risk increases during rapid weight loss by means of certain very-low-calorie diets or gastric bypass surgery. Gallstone risk factors during rapid weight loss include increased bile cholesterol saturation index and gallbladder stasis. Obese subjects were randomized to one of two low-calorie liquid diets for rapid weight loss: a 520-kcal diet with less than 2 g fat/d, and a 900-kcal diet with 30 g fat/d (including one 10-g fat meal to stimulate maximal gallbladder emptying). Bile and blood lipids, saturation index, leukocyte 3-hydroxy-3-methylglutaryl coenzyme A (HMG CoA) reductase activity, and ultrasonographic gallbladder emptying were measured repeatedly during dietary treatment. Both diets produced comparable weight loss of 22%. Bile cholesterol saturation index increased during both diets (26%), but fell to 15% below prediet level after weight loss. Compared with subjects' maximal gallbladder emptying fraction of 66%, the 520-kcal diet provided poor gallbladder emptying (35%), whereas the 10-g fat meal of the 900-kcal diet provided maximal emptying. Gallstones developed in four of six 520-kcal subjects and none of seven 900-kcal subjects (*P* = .021), an unanticipated difference that resulted in premature study termination for ethical reasons. Blood lipids and HMG CoA reductase activity in mononuclear leukocytes fell at week 8 during both diets, but recovered while weight was still being lost. The findings suggest that gallstone risk during rapid weight loss may be reduced by maintenance of gallbladder emptying with a small amount of dietary fat. Ultimately, weight loss reduced bile cholesterol saturation and improved high-density lipoprotein (HDL) levels. (HEPATOLOGY 1996; 24:544-548.)

Rapid weight loss in obese subjects may lead to cholesterol gallstones. Gallstones developed in 11% to 26% of obese subjects rapidly losing body weight on very-low-calorie liquid diets, containing less than 2 g of fat per day.[1-4] New gallstones formed in 36% of obese subjects losing weight after gastric bypass surgery.[5] The mechanism(s) for gallstone formation during rapid weight loss is not clear. There are three recognized factors in cholesterol gallstone formation; bile which is saturated with cholesterol is essential for crystals to form, nucleation factors can influence whether crystals form in saturated bile, and gallbladder stasis can allow crystal growth.

Obesity is associated with increased bile cholesterol secretion and saturation index.[6] Several studies suggest that bile saturation index increases further during rapid weight loss.[1,6,7] In one study, 10 subjects losing weight on a 1,000-kcal diet showed reduced output of all bile lipids, but 6 of the 10 increased their bile saturation index.[6] On the other hand, several studies have reported decreased bile saturation index after 6 to 20 days of fasting.[8,9] Cholesterol may be mobilized from tissue stores to be secreted into bile during rapid weight loss.[6] Decreased bile acid synthesis has also been detected during diet-induced weight loss and fasting.[6,9,10] Thus, rapid weight loss may alter secretion of cholesterol and bile acids in a manner conducive to gallstone formation.

Pro- and antinucleating proteins and mucins are described in gallbladder bile, and may predispose to gallstones. These factors could change during rapid weight loss. Shortened crystal nucleation time has been observed during very-low-calorie liquid diets.[7] Mucin and calcium increased in gallbladder bile of obese subjects forming gallstones after gastric bypass.[11,12]

Gallbladder emptying may also participate in gallstone formation.[13] Studies of meal-stimulated gallbladder emptying in obese subjects have given variable results.[14-17] We and others have shown that typical very-low-calorie liquid diets are poor stimuli for gallbladder emptying in both obese and normal-weight individuals.[14,17] Impaired gallbladder emptying has also been reported after gastric bypass surgery.[18]

The current study was planned to evaluate and compare biliary physiology in subjects given two different low-calorie liquid diets. Bile and blood lipids, bile cholesterol saturation index, and gallbladder contraction were serially measured during treatment with a formula that has been associated with gallstone formation and a formula that was designed to promote normal gallbladder emptying. The study was prematurely terminated when a high incidence of gallstones developed in one diet group.

PATIENTS AND METHODS

Subjects. Healthy, obese individuals were recruited from a medical clinic weight loss program, informed of the study protocol and offered a monetary incentive to participate. Twenty-three subjects were screened; 13 volunteers were entered into the study. Volunteers had normal gallbladder ultrasonography, underwent preliminary gallbladder and bile testing, and were randomized to one of two formulae. One subject was taking a stable dose of thyroid replacement; no other significant medical or endocrine disease or lithogenic medications were present in subjects. Study protocol and consent form were approved by the Human Subjects Subcommittee at the Minneapolis VA Medical Center. The study took place from May 1993 to May 1994.

Dietary Treatment. The medically monitored 24-week diet program provided weekly meetings, diary and compliance review by a single registered dietitian experienced in this method of weight loss,

Abbreviations: HDL, high-density lipoprotein; LDL, low-density lipoprotein; HMG CoA, 3-hydroxy-3-methylglutaryl coenzyme A.

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and periodic evaluation by a physician. The treatment protocol involved consuming one of two liquid formula diets for 12 weeks. Diets were prepared by mixing one (or two) packets of powdered formula in 8 to 12 oz of a noncaloric beverage for consumption five times daily. Packets contained no nutritional information or labeling, to blind both subjects and staff to dietary conditions. Compliance was enhanced by weekly behavior modification sessions. Following 12 weeks of liquid formula only, conventional foods were reintroduced and liquid formula reduced. By week 18, subjects consumed 1,000 kcal of solid food. Caloric intake was gradually increased to 1,200 to 1,500 kcal over the next 6 weeks. Several large male subjects (one in each group) were initially allowed six formula meals per day.

The first liquid formula ("2 g fat") contained 520 kcal per day: 50 g protein, less than 2 g fat, and 30 mg cholesterol. Each meal of the formula (Health Management Resources, Boston, MA) contains less than 1 g fat. The diet has previously been associated with gallstone formation.[1,3,4] The second formula ("30 g fat") contained 900 kcal per day: 90 g protein, 30 g fat, and 90 mg cholesterol. Each packet of this noncommercial diet (prepared by Sandoz Nutrition, Minneapolis, MN) contained 5 g fat. One daily meal consisted of two packets in 12 oz of fluid, to provide one 10-g fat meal daily to stimulate maximal gallbladder emptying.[14] Both diets were casein-based and equally supplemented with at least 100% of the United States recommended daily allowance for vitamins and minerals. The fat source for the 30-g fat diet was canola oil. Lab technicians and ultrasonographers were blinded to diet allocation throughout.

Measurements. Before dietary treatment, fasting subjects underwent gallbladder ultrasonography to exclude stones and to measure maximal emptying response to a 20-g fat liquid meal, as previously described and validated.[14,19] Volunteers were then randomized to one of the liquid formulas, and gallbladder emptying response to their assigned liquid meal (<1-g fat meal or 10-g fat meal) was measured by a second prediet test. To assess the effect of substantial weight loss on maximal gallbladder emptying, the 20-g fat stimulus was retested following weight loss (week 24). To ascertain whether an adaptive response to study diets occurred during liquid formula use, gallbladder emptying response to each subject's assigned liquid formula meal was also retested at weeks 2, 4, and 8 of treatment. Gallbladder volumes were calculated by sum-of-cylinders method.[19]

Before dietary treatment, fasting gallbladder bile was sampled via duodenal intubation with a weighted plastic tube, gravity drainage of secretions, and induction of gallbladder contraction with 0.02 μg/kg Kinevac IV (Squibb Diagnostics, Princeton, NJ) over 10 minutes, as described.[20] Gallbladder bile was also obtained at week 8 of liquid formula and after week 24. Bile was collected on ice, diluted with methanol, assayed for bile lipids, and saturation index was calculated using the formula of Carey and Small for 10 g solids per deciliter.[20,21]

Serum cholesterol fractions (total, very-low-density lipoprotein, high-density lipoprotein (HDL), and low-density lipoprotein (LDL) by subtraction) were measured in fasting blood pretreatment and at weeks 8 and 24. HDL was measured using a precipitation method[22] and very low density lipoprotein was derived from triglyceride measurement.[23] Cholesterol was quantitated as described[24] and triglycerides were measured using Sigma Kit 405 (Sigma, St. Louis, MO). At the same intervals, mononuclear leukocytes were isolated from heparinized blood to measure microsomal 3-hydroxy-3-methylglutaryl coenzyme A (HMG CoA) reductase activity as described.[25] HMG CoA reductase is feedback regulated by cholesterol and is generally the rate-determining step in cholesterogenesis.[26] Its activity in mononuclear leukocytes may reflect total body cholesterol synthesis.[25] Total blood cholesterol was also measured at 2-week intervals during liquid diet and then at 4-week intervals. Fatty acid composition of bile and serum phospholipids was measured as previously described,[27] using a Model 5890 GLC fitted with a 30-m × 0.25-mm HP-INNOWAX capillary column (Hewlett-Packard, Palo Alto, CA).

Statistics. Values are mean ± SE unless noted. Based on previously observed changes in bile cholesterol saturation index and gallbladder emptying, we calculated that 10 subjects in each group would be sufficient to detect a 22% difference in gallbladder emptying and a 30% change in saturation index between the two diet treatments at a statistical power greater than 0.80. Data involving multiple measurements were analyzed by repeated ANOVA and means were compared with the Fisher's least significant difference test. Student's paired t test was used for comparisons involving only two groups, where ANOVA was not appropriate. Fisher's exact test was used to evaluate gallstone formation.

TABLE 1. Subjects' Characteristics Before and After Dietary Treatment

	2 g Fat	30 g Fat
Sex	5F/1M	5F/2M
Age (yr)	40 ± 5	40 ± 8
Weight (kg)	105 ± 21	114 ± 29
BMI (kg/m^2)	37 ± 4	36 ± 6
Lowest BMI	29 ± 3	28 ± 3
Weight loss (kg)	23 ± 6	25 ± 13
(% initial weight)	(22)	(22)
Gallbladder		
Fasting volume (cc)	25 ± 9	29 ± 6
Residual volume (cc)*	8 ± 3	10 ± 4
Blood cholesterol (mg/dL)	205 ± 35	220 ± 23
Nadir blood cholesterol (mg/dL)	151 ± 32	165 ± 30
Blood triglyceride (mg/dL)	147 ± 104	164 ± 48

NOTE. Values are means ± SD.

Abbreviation: BMI, body mass index.

* Residual gallbladder volume for this value was measured after a 20-g fat liquid meal.

RESULTS

Three subjects were excluded for silent gallstones, 7 subjects declined entry, and 13 subjects were randomized. Subjects in both diet groups had similar characteristics at entry (Table 1). Both formulas produced equivalent loss of 22% body mass, in spite of differences in caloric content. Incentive and supervision resulted in compliance judged to be very good for both groups.

As shown in Fig. 1, 12 of 13 subjects' gallbladder bile was saturated with cholesterol (index >1.0) before beginning dietary protocol. Although cholesterol saturation index at week 8 rose to a numerically higher value for 30-g fat diet subjects, ANOVA showed no significant diet-specific effect on saturation index ($F_{1,9}$ = 1.333, P = .278). However, Fig. 1 shows that bile saturation index did increase for most subjects by week 8 of respective liquid diets and fell below initial values at week 24. At week 24, saturation index was ≤1.0 in 7 of the 13 subjects. When all subjects were considered, ANOVA showed a highly significant main effect of time on bile saturation index ($F_{2,18}$ = 9.087, P = .0019). Thus, compared with prediet values, saturation index increased 26 ± 11% at week 8 during weight loss (P < .05) and decreased 15 ± 6% at week 24 after weight loss (P < .05).

Bile lipid secretion rates were not measured. However, bile phospholipid molar ratio fell during rapid weight loss (22.0 ± 1.3% to 16.3 ± 0.6%, P < .01), bile acid molar ratio rose (68.9 ± 1.7% to 74.4 ± 1.1%, P < .01) and bile cholesterol did not change. At week 24, the only bile lipid change from prediet was decreased molar ratio of cholesterol (9.4 ± 0.6% to 7.4 ± 0.6%, P < .01).

Also shown in Table 1, both groups had comparable initial gallbladder fasting volumes and comparable residual volumes after a 20-g fat liquid meal. Subjects' maximal meal gallbladder emptying fraction of 66% to 70% is similar to values that we and others have reported for normal subjects.[14,16,17,28] After weight loss (week 24), fasting gallbladder volumes and maximal emptying fractions to 20 g fat were not significantly changed from prediet (22 ± 4 mL and 66 ± 6% for all subjects).

As expected (Fig. 2), the 10-g fat meal stimulated normal gallbladder emptying, whereas the <1 g fat meal produced very poor emptying pretreatment and throughout the weight loss. Both meals were tested in two subjects (64% and 95% for 10 g vs. 0% and 13% for <1 g), confirming that meal content accounted for the differences. Significant formula-specific gallbladder emptying differences persisted at weeks 2, 4, and 8. No significant changes in fasting gallbladder volumes occurred during diet.

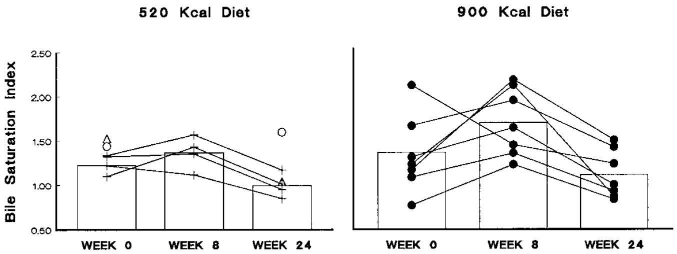

FIG. 1. Bile cholesterol saturation index before treatment, week 8 during liquid formula, and after weight loss. Individual 520-kcal (2-g fat) subjects shown as crosses (two subjects, △ and ○, had only two measurements). Individual 900-kcal (30-g fat) subjects shown (●). Bars represent means.

TABLE 2. Time Course of Blood Lipid Values and Activity of HMG CoA Reductase in Mononuclear Leukocytes (A Reflection of Cholesterol Synthesis) for all Subjects

	Prediet	Week 8	Week 24
Total cholesterol (mg/dL)	213 ± 8	159 ± 8*	210 ± 8
LDL cholesterol (mg/dL)	136 ± 7	93 ± 7*	124 ± 6
HDL cholesterol (mg/dL)	46 ± 4	44 ± 4	61 ± 5*
Triglycerides (mg/dL)	156 ± 21	117 ± 14	125 ± 17
HMG CoA reductase (pmol/mg protein/min)	1.4 ± 0.2	0.9 ± 0.1*	1.0 ± 0.2

NOTE. Values are mean ± SE.
* $P < .05$ versus prediet.

The protocol was intended to study bile and gallbladder physiology and chemistry during liquid formula consumption, rather than gallstone formation. Surprisingly, four subjects developed echogenic foci with acoustic shadowing (indicative of gallstones) during the liquid-formula phase: by week 4 in two subjects, week 8 in one, and between weeks 8 and 13 in one symptomatic subject. One of these subjects showed "sludge" (echogenicity without acoustic shadowing) at week 2, a clean gallbladder at week 4, and gallstones at week 8. All four gallstone subjects were found to have been on the 2-g fat formula. This difference between diets was significant at $P = .021$ by Fisher's exact test. Because two subjects were symptomatic, the Human Subjects Subcommittee encouraged cessation of enrollment.

Subjects developing gallstones remained on diet therapy. They were given one 10-g fat meal per day to provide gallbladder emptying,[24] and treated with 300 mg ursodeoxycholic acid twice daily for 4 to 6 weeks. All gallstones disappeared within 6 weeks of drug and diet modification. Serum and bile lipid data were not gathered during ursodeoxycholic acid treatment.

In Table 1, ANOVA showed no significant diet-specific effect on blood lipid levels before or during weight loss ($F_{1,11}$ = 0.202, $P = .6616$). However, Table 2 shows that there was a highly significant main effect of time of diet on blood cholesterol when all subjects were considered ($F_{2,22} = 23.18$, $P = .0001$). Thus, total and LDL cholesterol fell markedly by week 8 of liquid formula, but returned toward baseline by week 24. Total blood cholesterol rose during the final 4 weeks of very low cholesterol formulas. The only significant change after weight loss was increased HDL; the LDL decrease was $.2 > P > .1$.

Mononuclear leukocyte HMG CoA reductase activity also did not differ by dietary group ($F_{1,9} = 1.096$, $P = .3224$). Shown in Table 2, there was a significant main effect of diet time on reductase activity ($F_{2,18} = 4.78$, $P = .0216$). Surprisingly, reductase activity fell at week 8 of liquid formula, in spite of the very low dietary cholesterol and the low levels of blood cholesterol. Reductase activity after week 24 was less than pretreatment, but the difference was not statistically significant.

No significant changes in fatty acid composition of serum phospholipids were found in either group (data not shown). For both groups, bile phospholipid fatty acids changed modestly at week 8 of liquid formula compared with pretreatment. Arachidonic acid content rose from 7.1% to 9.7% ($P < .01$), palmitic acid rose from 35.4% to 38.7% ($P < .01$), and stearic acid fell from 5.6% to 3.8% ($P < .01$). Composition returned to pretreatment at week 24.

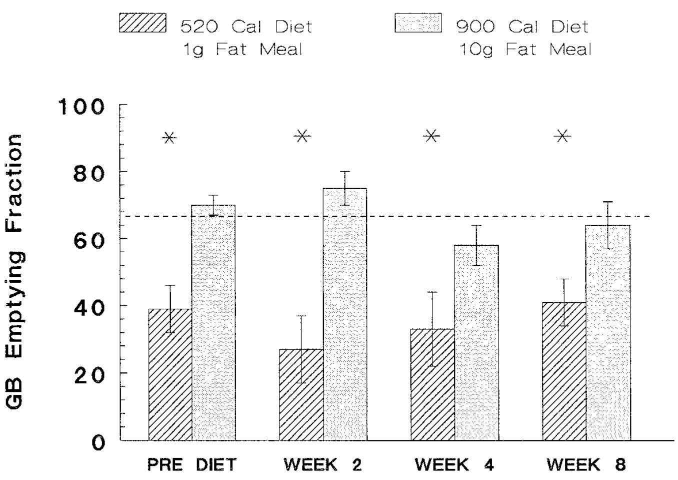

FIG. 2. Gallbladder emptying fraction expressed as:

$$\frac{\text{(fasting volume-low post meal volume)}}{\text{fasting volume}} \times 100\%$$

gallbladder emptying from subjects' specific diet meals (<1-g fat meal for the 520-kcal group, 10-g fat meal for the 900-kcal group) before treatment and at weeks 2, 4, and 8 during weight loss formula. Values are mean ± SE. Broken line represents the mean maximal emptying response to a 20-g fat meal (66%) for all subjects. *$P < .01$ versus the same subject's maximal emptying and versus 10-g fat meal of the 900-kcal group.

DISCUSSION

Obesity is a risk factor for cholesterol gallstones. This study and others indicate that certain weight loss methods may increase gallstone risk, at least during rapid weight loss. Elucidation of the mechanism(s) underlying the increased risk may enable risk reduction strategies. One such strategy has been concurrent use of ursodeoxycholic acid during diet-induced rapid weight loss.[1,4] The current study was intended to evaluate bile lipids and gallbladder emptying as risk factors for cholesterol gallstones, with diet composition as a variable.

Subjects receiving the two dietary treatments, 520 kcal versus 900 kcal, showed very similar maximal and percent weight loss. This observation is consistent with a recent report which found no significant differences in weight loss between very-low-calorie diets supplying 420 kcal, 660 kcal, or 800 kcal.[29] The result could be explained by differences in diet compliance by subjects at the two calorie levels. However, the 22% loss of body mass by the subjects receiving 520 kcal/d was identical to that of other reports for this diet,[1,3,4] and the study dietitian reported equivalent compliance by the two groups.

Our data confirm that increased bile cholesterol saturation index can occur during rapid weight loss.[1,6,7] The reason for the increase could not be ascertained from our study. Dietary cholesterol was not a factor, and cholesterol synthesis, which decreased in mononuclear leukocytes during dieting, did not appear to play a role. Bennion and Grundy suggested that mobilization of tissue cholesterol into bile may occur.[6] Decreased bile acid synthesis and secretion have been observed during weight loss dieting,[6,10] and also noted during fasting.[9] Our finding of reduced bile phospholipid molar ratio at week 8 could be a result of decreased phospholipid secretion during rapid weight loss. Reduced secretion of all bile lipids by 40% to 50% during weight loss has been reported.[6] Increasing phospholipid content of bile by as little as 10% has also been reported to prolong nucleation time.[30] Thus, decreased bile phospholipid content to the extent seen here might shorten crystal nucleation time in addition to raising saturation index.

Once weight was lost and normal eating resumed, bile saturation index and cholesterol molar ratio fell in comparison with the obese state. Bile became unsaturated in 7 of 13 subjects. This confirms an overall benefit of weight reduction.[6]

Cholesterol crystal nucleation factors were not specifically studied, but arachidonic acid content of bile phospholipids increased in all subjects at week 8. As a prostaglandin precursor, arachidonic acid could influence mucin production and facilitate crystal nucleation.[31]

Although weight loss was identical for the two diets and cholesterol saturation index increased in most subjects, with highest levels occurring on 30 g fat, gallstones only developed in the 2-g fat subjects. This suggests that poor gallbladder emptying played an important role in the cholelithiasis. Other diet variables such as calorie or protein content may have been involved, but consistent and significant differences in gallbladder contraction were found. Poor gallbladder emptying is implicated in gallstone formation during total parenteral nutrition and somatostatin therapy.[32-34] Gallstones have been prevented by cholecystokinin injections during parenteral nutrition.[33] Dietary fat may have provided a protective emptying function in our study.

The incidence of gallstone formation in our 2-g fat group was higher than reported for similar diets.[1-4] This may be explained by our frequent ultrasonographic surveillance during the most rapid weight loss. Shiffman et al. observed a 65% incidence of gallstones in subjects losing 15 to 20 pounds per month on this same 520-kcal diet[4]; our subjects averaged 50 pounds lost over 3 months.

Total and LDL blood cholesterol levels initially fell during rapid weight loss, then rebounded during continued low cholesterol formula to return to prediet values after weight loss. Only HDL levels improved after weight loss. Other studies have also reported the transient nature of fall in blood cholesterol during diet-induced rapid weight loss.[35,36]

Mononuclear leukocyte HMG CoA reductase activity was measured to possibly reflect cholesterol synthesis. Reductase activity decreased in the face of very low dietary cholesterol content and low blood cholesterol levels. Although actual cholesterol synthesis may not be adequately reflected by leukocyte reductase in this setting, decreased cholesterol synthesis has been reported during fasting in the rat.[26] Altered levels of hormones (e.g., glucagon) could mediate depressed cholesterol synthesis during weight loss or fasting.[26]

In conclusion, gallstones are a significant risk for obese individuals because their bile is saturated with cholesterol. Saturation can increase during rapid weight loss. Once a reduced weight is obtained, however, saturation index and gallstone risks diminish. Ursodeoxycholic acid has been reported to decrease stone formation if taken during dieting,[1,4] but the drug adds expense. The current study suggests that maintenance of gallbladder emptying, with a modest quantity of dietary fat, may also reduce gallstone risk during low-calorie rapid weight loss. In fact, an abstract has reported no gallstone formation during a very-low-calorie diet with fat, which maintained gallbladder emptying.[37]

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