Published in final edited form as:

J Surg Res. 2008 June 15; 147(2): 290–297. doi:10.1016/j.jss.2007.04.042.

Steatocholecystitis: The Influence of Obesity and Dietary Carbohydrates

Abhishek Mathur, MD, Hayder H. Al-Azzawi, MD, Debao Lu, MD, Kyle W. Yancey, MD, Deborah A. Swartz-Basile, PhD, Attila Nakeeb, MD, and Henry A. Pitt, MD

Department of Surgery, Indiana University School of Medicine, Indianapolis, IN

Abstract

Introduction—We have recently demonstrated that obese and lean mice fed a high fat diet have increased gallbladder wall fat and decreased gallbladder contractility, cholecystosteatosis. Animal and human data also suggest that diets high in refined carbohydrates lead to gallstone formation. However, no data are available on the role of dietary carbohydrates on gallbladder wall fat and inflammation. Therefore, we tested the hypothesis that both obesity and dietary carbohydrates would increase gallbladder fat and cytokines, steatocholecystitis.

Methods—At eight weeks of age 47 lean and 22 obese female mice were fed a 45% carbohydrate (CHO) diet while an equal number of lean and obese mice were fed a 75% CHO diet for four weeks. All mice underwent cholecystectomy, and the gallbladders were snap frozen. Individual and total lipids were measured by gas chromatography. Interleukin (IL)-1 β , tumor necrosis factor (TNF)- α and IL-6 were measured by ELISA. Data were analyzed by ANOVA and Tukey test.

Results—Gallbladder total fat, triglycerides and cholesterol were maximum ($p < 0.001$) in obese mice on the 75% CHO diet. Gallbladder TNF- α and IL-1 β as well as serum cholesterol levels showed a similar pattern ($p < 0.001$). Gallbladder saturated free fatty acids and IL-6 levels were highest ($p < 0.001$) in obese mice on the 45% CHO diet.

Conclusions—These data suggest that 1) both obesity and dietary carbohydrates increase gallbladder total fat, triglycerides, cholesterol, TNF- α and IL-1 β and 2) obesity also increases gallbladder free fatty acids and IL-6. Therefore, we conclude that obesity is associated with steatocholecystitis and that a high carbohydrate diet exacerbates this phenomenon.

Keywords

carbohydrate; cholecystosteatosis; steatocholecystitis; gallstones

INTRODUCTION

Obesity has become an epidemic in the United States with 40% of Americans being overweight (BMI > 25) and/or obese (BMI > 30).¹ Obesity leads to fat infiltration of multiple organs including the heart, kidney and liver. In the liver, fat infiltration has been termed nonalcoholic

Corresponding Author: Henry A. Pitt, MD, Department of Surgery, Indiana University School of Medicine, 535 Barnhill Drive, RT 130D, Indianapolis, IN 46202, Phone: (317) 274-2304, Fax: (317) 274-4554, Email: hapitt@iupui.edu.

To be presented at the Association for Academic Surgery, February 6–9, 2007, Phoenix, AZ

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

fatty liver disease (NAFLD) which affects 20 to 30% of adults in the United States.² A recent murine study from this laboratory suggested that congenital obesity and a high fat/high cholesterol diet are associated with a similar phenomenon of fatty infiltration of the gallbladder, cholecystosteatosis.³ In addition, we have demonstrated that patients with acalculous and calculous cholecystitis have increased gallbladder wall fat.⁴ Gilloteaux et al using electronmicroscopy, also demonstrated that patients with cholecystitis have an increased accumulation of fat in individual gallbladder smooth muscle fibers.⁵ In obesity, adipose tissue produces adipokines which increase local macrophages and proinflammatory cytokines resulting in organ dysfunction.^{2,6,7} We believe that this process also occurs in the gallbladder.

Diet is an important environmental factor which predisposes to cholesterol gallstone formation. In 1892 Osler first postulated that sweet foods may play a role in gallstone development.⁸ In 1999 Misciagna et al in a population-based case-control study demonstrated a positive association between intake of refined carbohydrates and the incidence of gallstones.⁹ Refined sugars also have been documented to increase the biliary cholesterol saturation index in humans.⁸ In addition, diets rich in carbohydrates have been shown to produce gallstones in various animal models.^{10,11} A recent study from our laboratory also documented that a high (refined) carbohydrate diet decreased gallbladder volume and enhanced biliary cholesterol crystal formation.¹² However, no data are available on the interaction among obesity, dietary carbohydrates, gallbladder wall fat and inflammation. Therefore, we tested the hypothesis that both obesity and a high carbohydrate diet would increase gallbladder wall fat and cytokines resulting in steatocholecystitis.

MATERIALS AND METHODS

Animals and Diets

Ninety-four lean control (C57BL/6J) and 44 obese leptin-deficient (Lep^{ob}) female mice were obtained from Jackson laboratory (Bar Harbor, Maine). At eight weeks of age 47 lean C57BL/6J and 22 obese leptin-deficient (Lep^{ob}) female mice were fed a low carbohydrate diet (45% carbohydrate, 15% fat and 40% protein) (Dyets Inc., Bethlehem, Pennsylvania) while the remaining 47 lean and 22 obese mice were fed a high carbohydrate diet (75% carbohydrate, 15% fat and 10% protein) (Dyets Inc., Bethlehem, Pennsylvania) for four weeks. The carbohydrates (CHO) in the 45% diet were 35% sucrose and 10% cornstarch. The 75% CHO diet had 65% sucrose and 10% cornstarch. In both diets the fat was anhydrous milk fat, and the protein was exclusively casein. Both the diets were reinforced with essential vitamins and minerals, and the high carbohydrate low protein diet although modestly protein deficient had four times the protein content of a protein energy malnutrition diet. Both the animals and the food were weighed weekly to determine growth and dietary intake. All protocols for these animal studies were approved by the Indiana University Institutional Animal Care and Use Committee.

Tissue and Serum Collection

At 12 weeks of age, after an overnight fast with water allowed ad libitum, the mice were anesthetized with an intraperitoneal injection of xylazine (15 mg/kg) and ketamine (50 mg/kg). The animals were weighed and then underwent laparotomy and cholecystectomy. All gallbladders from the four groups were snap frozen to -80°C. For gallbladder wall lipid analysis, 35 gallbladders from both groups of lean mice were pooled (seven/pool, n= five pools) and 10 gallbladders from both groups of obese mice were pooled (two/pool, n= five pools). Twelve gallbladders from each of the four groups were pooled (two/pool, n= six pools) for measuring gallbladder wall cytokines. Blood was aspirated from the heart and centrifuged to isolate serum.

Gallbladder Lipid Analysis

Gallbladder lipids were analyzed in five pools in each group by thin layer and gas chromatography by the Mouse Metabolic Phenotyping Center at Vanderbilt University Medical Center, as previously described by Goldblatt et al.³

Cytokine Analysis

Cytokines interleukin (IL)-1 β , tumor necrosis factor-alpha (TNF- α) and IL-6 were measured in six pools from each group by employing the quantitative sandwich enzyme immunoassay technique. The ELISA kits for this purpose were obtained from R&D Systems, Inc, Minneapolis, MN, U.S.A.

Serum Analysis

Whole blood was spun at 15,000 rpm for 5 minutes to separate serum. Serum was pooled to give six pools each for lean mice on the 45% and 75% CHO diets. Five pools were obtained for obese mice on the 45% and 75% CHO diet. Serum cholesterol and triglycerides were determined by using an enzymatic colorimetric method for their quantitative determination. The kits for these analyses were obtained from Wako Chemicals USA, Inc, Richmond, VA, U.S.A and Stanbio labs, Boerne, TX, U.S.A., respectively.

Statistical Analysis

Statistical analyses were performed using Sigma Stat Statistical Software (Jandel Corp., San Rafael, California). All data are expressed as mean $\pm$ SEM. Differences in animal body weight, food intake, serum lipids, gallbladder lipids and cytokines data were tested for statistical significance by ANOVA and Tukey test. A p value of less than 0.05 was considered statistically significant.

RESULTS

Animal Weights and Dietary Intake

All mice appeared healthy, well groomed and were moving freely about the cage. Data for initial and final animal weights and average CHO consumption/100 gm body weight are presented in Table 1. At eight weeks of age no significant difference in weight was observed between the two groups of lean mice or the two groups of obese mice. No difference in final weight existed between the two groups of lean mice. Obese mice on the 75% CHO diet gained less weight than those on the 45% CHO diet; and as a result, final weights of the obese mice on the 75% CHO diet were significantly lower ($p<0.001$) than those on the 45% CHO diet. As expected, the obese mice weighed significantly more ($p<0.001$) than the lean mice both initially and after four weeks. On the 45% CHO diet the mean CHO consumption for lean and obese mice were 40 and 20gm/100 gram body weight, respectively ($p<0.001$). The average CHO consumption per 100 gram body weight for the lean and obese mice on the 75% CHO diet was 68 and 32, respectively ($p<0.001$). The average CHO consumption per 100 gram body weight was significantly higher ($p<0.001$) for lean and obese mice on the 75% CHO diet. Additionally, average CHO consumption per 100 gram body weight was significantly lower for the obese compared to the lean mice on either diet ($p<0.001$).

Gallbladder Wall Lipid Analysis

Total Fat and Triglycerides

Gallbladder wall lipids and total fat are shown in Table 2. Gallbladder triglycerides are presented in Table 2 and Figure 1A. By ANOVA, both strain ($p<0.02$) and diet ($p<0.01$) influenced total fat and triglycerides. The highest levels of total fat and triglyceride ($p<0.05$)

were found in obese mice on the 75% CHO diet. No differences in total fat and triglyceride levels were found between the two groups of lean mice, and no strain differences existed on the 45% CHO diet.

Phospholipids and Free Fatty Acids

Phospholipids did not differ among the four groups (Table 2). Gallbladder total free fatty acids (FFA), unsaturated FFA and individual FFA chains are shown in Table 2. Saturated FFA also are presented in Figure 2A. By ANOVA, strain ($p<0.001$) but not diet influenced free fatty acid levels. Gallbladder total free fatty acids, unsaturated FFA, palmitoleic, oleic and linoleic acid were all significantly elevated ($p<0.05$) in the obese mice on both diets compared to their lean counterparts. Saturated FFA, myristic and palmitic acid were maximally increased in obese mice on the 45% CHO diet ($p<0.05$ vs other groups).

Total Cholesterol and Cholesterol Esters

Gallbladder total cholesterol and cholesterol esters are shown in Table 2. By ANOVA, total cholesterol was increased by diet ($p<0.001$) and the combination of diet and strain ($p<0.001$), whereas cholesterol esters were increased ($p<0.01$) by both diet and strain. Both were maximally increased in obese mice on the 75% CHO diet ($p<0.05$ vs other groups).

Total Cholesterol/Phospholipid Ratio

Total cholesterol/phospholipid ratio is shown in Table 2. By ANOVA, the cholesterol phospholipid ratio was increased by diet ($p<0.001$) and the combination of diet and strain ($p<0.05$).

Serum Data

Serum cholesterol and triglyceride levels are shown in Table 3. By ANOVA, serum cholesterol was increased by strain ($p<0.001$), diet ($p<0.001$) and the combination of both ($p<0.03$) (Figure 3A). By ANOVA, serum triglycerides were decreased by the 75% CHO diet ($p<0.001$) and the combination of strain and diet ($p<0.02$).

Cytokine Analysis

Cytokine levels of TNF- α , IL-6 and IL-1 β are shown in Figures 1B, 2B and 3B, respectively. By ANOVA, gallbladder TNF- α was increased by both strain ($p<0.001$) and diet ($p<0.001$) with the two being additive, a pattern identical to triglycerides (Figure 1A). By ANOVA, gallbladder IL-6 was increased ($p<0.001$) by strain and decreased by diet ($p<0.001$), a pattern similar to FFAs and saturated FFAs (Table 2) (Figure 2A). By ANOVA, gallbladder IL-1 β increased ($p<0.001$) by strain and further by diet ($p<0.001$) in the obese mice, a pattern similar to that for serum cholesterol (Table 3) (Figure 3A).

DISCUSSION

In this study 47 lean C57BL/6J and 22 leptin deficient obese Lep^{ob} female mice were fed a low (45%) carbohydrate diet, and an equal number of lean and obese mice were fed a high (75%) carbohydrate diet for four weeks. The average carbohydrate consumption was greater for both lean and obese mice on the 75% carbohydrate versus the 45% carbohydrate diet. The obese mice on the high carbohydrate diet had maximal gallbladder levels of total fat, triglycerides, total cholesterol, cholesterol/phospholipid ratio, TNF- α , IL-1 β and serum cholesterol. The obese mice also had higher tissue levels of free fatty acids, both saturated and unsaturated, than the lean mice. However, free fatty acids were not increased on the 75% carbohydrate diet. The gallbladder levels of IL-6 followed a similar pattern. No differences were observed in gallbladder phospholipid levels among the four groups.

A significant fallout of the burgeoning obesity epidemic is fatty infiltration of multiple organs. Visceral fat has been implicated in dysfunction of various organs including the heart, kidney and liver.^{2,7} Goldblatt et al also have demonstrated that obese leptin-deficient mice have fatty infiltration of the gallbladder, cholecystosteatosis, and have increased gallbladder volumes with decreased response to neurotransmitters compared to lean controls.^{3,13,14} Moreover, feeding a high fat diet to lean mice also resulted in cholecystosteatosis and decreased gallbladder contractility.³ In addition, administration of leptin to obese leptin-deficient mice caused weight loss, decreased gallbladder volume and ameliorated impaired gallbladder contractility.¹⁵ Further evidence of the deleterious effects of cholecystosteatosis comes from human data suggesting that patients with acalculous and calculous cholecystitis have increased gallbladder wall fat and poor gallbladder emptying.⁴ Moreover, we have recently shown that giving ezetimibe to lean mice fed a high fat diet results in amelioration of gallbladder cholecystosteatosis and restores *in vivo* contractility.¹⁶

White adipose tissue (WAT) is now known to be an endocrine organ which plays a pivotal role in the regulation of metabolism, inflammation and energy intake in addition to fat storage.⁶ Adipose tissue performs these functions by secreting cytokines, chemokines and hormones which are collectively called adipocytokines. Human obesity results in increased leptin and decreased adiponectin production by adipocytes. These adipokines influence macrophage infiltration into fat which, in turn, increase proinflammatory cytokines such as TNF- α , IL-6 and IL-1 β .^{6,17} Thus, visceral steatosis causes a local inflammatory process resulting in organ dysfunction with nonalcoholic steatohepatitis being a well established example of this phenomenon.² Moreover, gallbladder inflammation is known to occur early in gallbladder disease and has been partially attributed to the effects of absorbed lipid from the bile.¹⁸

Hypertrophied adipocytes in the obese are known to produce increased amounts of TNF- α .¹⁹ Moreover, these adipocytes develop a decreased sensitivity to insulin which results in an increased accumulation of triglycerides and a consequent enlargement of the adipocyte.^{20,21} Hirata et al have suggested that cytokine secretion increases as fat cell size increases.²¹ They found that increased triglyceride synthesis in the adipocytes was associated with a concomitant increase in TNF- α expression. Moreover, inhibiting triglyceride synthesis ameliorated this enhanced production of TNF- α . We have shown a similar phenomenon with gallbladder triglyceride levels paralleling TNF- α protein expression (Figure 4). Tumor necrosis factor- α also causes a dysfunction in smooth muscle contractility, absorption, insulin resistance and lipid peroxidation.^{5,22,23} In addition, TNF- α increases secretion of mucin, which is a cholesterol pronucleating agent.²⁴ Gallbladder wall inflammation and mucin hypersecretion have been shown to occur before gallstone formation.²⁵ These observations also are corroborated by a recent study from our laboratory demonstrating that a high carbohydrate diet enhances nucleation of cholesterol monohydrate crystals in obese mice.¹²

Serum FFA levels in obese humans have been shown to correlate with serum IL-6 levels.^{26,27} Additionally, among the FFAs only saturated palmitic and myristic acid have been shown to correlate with IL-6 levels.²⁶ This correlation is reinforced by animal studies showing an induction of IL-6 secretion in adipocytes by palmitate.²⁸ We have shown that gallbladder total saturated FFAs, palmitic and myristic acid are elevated in the obese mice on the 45% CHO diet (Figure 4). Additionally, IL-6 is maximally elevated in this group. The repercussions of elevated IL-6 levels are an increase in insulin resistance, lipid peroxidation and smooth muscle dysfunction.^{6,29} Xiao et al have documented that gallbladder inflammation results in elevated measures of oxidative stress.³⁰ The potential mechanism for this observation could be IL-6 induced lipid peroxidation. Additionally, hyperinsulinemia, secondary to increased insulin resistance, has independently been shown to increase oxidative stress in tissues.⁶ Oxidative stress, in turn, causes damage to transmembrane receptors like those for cholecystokinin, which would result in decreased contractility.³⁰

The deleterious effects of hypercholesterolemia have been well documented in the literature on atherosclerosis.^{31,32} Moreover, this disease is known to be accompanied by a local inflammatory response.^{31–33} Lin et al have documented that human aortic smooth muscle cells on exposure to cholesterol, incorporate it and cause a dose-dependent increase in IL-1 β secretion.³⁴ In our study both cholesterol and IL-1 β were maximally elevated in gallbladders of obese mice on the 75% CHO diet. Interestingly, obese mice on the 45% CHO diet had elevated IL-1 β compared to their lean counterparts, despite similar tissue cholesterol levels (Figure 4). Obesity is known to be associated with macrophage infiltration of the adipose tissue.^{6,17} The elevated levels of IL-6 in this group could have stimulated increased IL-1 β release by macrophages.^{6,33} Interleukin-1 β , in addition to potentiating the effects of IL-6 on smooth muscle contractility and insulin resistance, also affects gallbladder absorption.^{22,29,36} Rege et al showed that cytokines of the IL-1 family can alter gallbladder absorption and secretion.²² Normal concentration and acidification of bile is protective against gallstone formation.³⁷ Therefore, IL-1 β by altering absorption could potentiate gallstones.

Increased membrane cholesterol/phospholipid ratio has been shown to influence membrane fluidity and membrane bound protein function.³⁸ Gallbladders from patients with gallstones have been shown to have increased cholesterol and cholesterol/phospholipid ratio and decreased contractility.³⁹ Moreover, elimination of the excess cholesterol from the gallbladder smooth muscles normalized membrane fluidity and muscle contractility.³⁹ Earlier data from our lab suggest that obese, leptin-deficient mice have decreased responses to neurotransmitters compared to lean mice.¹⁴ Data from our laboratory also suggest that feeding a high fat/high cholesterol diet to lean mice increases the cholesterol/phospholipid ratio and decreased gallbladder contraction.³ In the current study we have again demonstrated an increase in gallbladder wall cholesterol/phospholipid ratio in the obese mice on the 75% CHO diet versus those on the 45% CHO diet. Presumably, this finding would correlate with decreased gallbladder emptying which was not studied in this experiment.

In 1983, Thornton et al demonstrated that humans consuming a diet high in refined sugars had a significantly higher biliary cholesterol saturation index (CSI) compared to subjects on unrefined carbohydrates.⁸ In one of the larger epidemiologic studies investigating the effects of diet on gallstones, Moerman et al looked at the 25 year incidence of clinically diagnosed gallstones and dietary intake in 860 men.⁴⁰ They showed a significant positive correlation for monosaccharide and disaccharide sugars and gallstones. More recently, Tsai et al. in two epidemiologic studies of 70,000 nurses and 51,529 men documented that a high carbohydrate diet is associated with an increased cholecystectomy rate.^{41,42} Animal data also support the role of dietary carbohydrates in gallstone formation. Dam et al reported an elevated incidence of cholesterol gallstones in animals fed a high refined carbohydrate diet.¹⁰ Interestingly, no gallstones formed when the dietary carbohydrates were comprised of only unrefined sugars. Moreover, we have recently documented that a high carbohydrate diet increased biliary cholesterol crystal formation.¹²

Another interesting finding in this study was that the average carbohydrate consumption per 100 grams of body weight was significantly higher in the lean, compared to the obese mice on both diets. Despite this decreased carbohydrate consumption, the obese mice developed more steatosis of their gallbladders. Recent studies from our laboratory have demonstrated that when these congenitally obese leptin-deficient mice are administered leptin, this adipokine decreases the expression of enzymes responsible for lipid metabolism including fatty acid synthase, HMG CoA reductase, and diacylglycerol acyltransferase (unpublished data). This observation suggests that obese mice have an inherent defect in gallbladder lipid metabolism which makes them more susceptible to diet-induced steatosis, despite having lower weight adjusted carbohydrate consumption than their lean counterparts.

In conclusion, this study documents that obesity and dietary carbohydrate increases gallbladder total fat, triglycerides and cholesterol while simultaneously elevating gallbladder TNF- α and IL-1 β . In addition, obesity increases free fatty acids and IL-6. These findings suggest that obesity increases gallbladder fat and cytokines and that a high carbohydrate diet exacerbates this phenomenon. Steatocholecystitis may, in turn, alter biliary motility and absorption leading to biliary dyskinesia and eventually gallstone formation.

Acknowledgements

Supported by NIH grant R-01 DK44279

References

1. Mokdad AH, Ford ES, Bowman BA, Dietz WH, Vinicor F, Bales VS, et al. Prevalence of obesity, diabetes, and obesity-related health risk factors, 2001. *JAMA* 2003;289:76–79. [PubMed: 12503980]
2. Farrell GC, Larter CZ. Nonalcoholic fatty liver disease: From steatosis to cirrhosis. *Hepatology* 2006;43:S99–S112. [PubMed: 16447287]
3. Goldblatt MI, Swartz-Basile DA, Al-Azzawi HH, Tran KQ, Nakeeb A, Pitt HA. Nonalcoholic fatty gallbladder disease: The influence of diet in lean and obese mice. *J Gastrointest Surg* 2006;10:193–201. [PubMed: 16455450]
4. Al-azzawi HA, Nakeeb A, Saxena R, Maluccio MA, Pitt HA. Cholecystosteatosis: An Explanation for Increased Cholecystectomy Rates. *J Gastrointest Surg*. 2007in press
5. Gilloteaux J, Tomasello LM, Elgison DA. Lipid deposits and lipo-mucosomes in human cholecystitis and epithelial metaplasia in chronic cholecystitis. *Ultrastructural Pathology* 2003;27:313–321. [PubMed: 14708722]
6. Greenberg AS, Obin MS. Obesity and the role of adipose tissue in inflammation and metabolism. *Am J Clin Nutr* 2006;83:461S–465S. [PubMed: 16470013]
7. Schaffer JE. Lipotoxicity: When tissues overeat. *Curr Opin Lipidol* 2003;14:281–7. [PubMed: 12840659]
8. Thornton JR, Emmett PM, Heaton KW. Diet and gallstones: Effects of refined and unrefined carbohydrate diets on bile cholesterol saturation and bile acid metabolism. *Gut* 1983;24:2–6. [PubMed: 6293939]
9. Misciagna G, Centonze S, Leoci C, Guerra V, Cisternino AM, Ceo R, Trevisan M. Diet, physical activity, and gallstones: A population-based, case-control study in southern Italy. *Am J Clin Nutr* 1999;69:120–6. [PubMed: 9925133]
10. Dam H, Christensen F. Alimentary production of gallstones in hamsters: Influence of different carbohydrate sources on gallstone formation, diarrhea, and growth. *Z Ernährungsw* 1961;2:91–102.
11. Dam H, Christensen F, Prange I. The relationship between diet and composition of gallbladder bile in mice. *Z Ernährungsw* 1969;9:200–208.
12. Mathur A, Marine M, Al-Azzawi HH, Lu D, Swartz-Basile D, Nakeeb A, Pitt HA. High dietary carbohydrates decreases gallbladder volume and enhance cholesterol crystal formation. *Surgery*. 2007Accepted
13. Goldblatt MI, Swartz-Basile DA, Svatek CL, Nakeeb A, Pitt HA. Decreased gallbladder response in leptin-deficient obese mice. *J Gastrointest Surg* 2002;6:438–42. [PubMed: 12022998]
14. Tran KQ, Goldblatt MI, Swartz-Basile DA, Svatek CL, Nakeeb A, Pitt HA. Diabetes and hyperlipidemia correlate with gallbladder contractility in leptin-related murine obesity. *J Gastrointest Surg* 2003;7:857–863. [PubMed: 14592658]
15. Phillips J, Tran KQ, Goldblatt MI, Swartz-Basile DA, Nakeeb A, Pitt HA. Leptin ameliorates the gallbladder's response to neurotransmitters in congenitally obese mice. *Gastroenterology* 2002;123:9.
16. Mathur A, Walker JJ, Al-Azzawi HH, Lu D, Swartz-Basile D, Nakeeb A, Pitt HA. Ezetimibe ameliorates cholecystosteatosis. *Surgery*. 2007Submitted

17. Weisberg SP, McCann D, Desai M, Rosenbaum M, Leibel RL, Ferrante AW Jr. Obesity is associated with macrophage accumulation in adipose tissue. *J Clin Invest* 2003;112:1796–808. [PubMed: 14679176]
18. Ginanni Corradini S, Ripani C, Della Guardia P, Giovannelli L, Elisei W, Cantafora A, Codacci Pisanelli M, Tebala GD, Nuzzo G, Corsi A, Attili AF, Capocaccia L, Ziparo V. The human gallbladder increases cholesterol solubility in bile by differential lipid absorption: A study using a new in vitro model of isolated intra-arterially perfused gallbladder. *Hepatology* 1998;28:314–22. [PubMed: 9695992]
19. Hotamisligil GS, Shargill NS, Spiegelman BM. Adipose expression of tumor necrosis factor- α : Direct role in obesity-linked insulin resistance. *Science* 1993;259:87–91. [PubMed: 7678183]
20. Molina JM, Ciaraldi TP, Brady D, Olefsky JM. Decreased activation rate of insulin-stimulated glucose transport in adipocytes from obese subjects. *Diabetes* 1989;38:991–5. [PubMed: 2666204]
21. Hirata T, Unoki H, Bujo H, Ueno K, Saito Y. Activation of diacylglycerol O-acyltransferase 1 gene results in increased tumor necrosis factor- α gene expression in 3T3-L1 adipocytes. *FEBS Lett* 2006;18;580:5117–21. [PubMed: 16956609]
22. Rege RV. Inflammatory cytokines alter human gallbladder epithelial cell absorption/secretion. *J Gastrointest Surg* 2000;4:185–92. [PubMed: 10675242]
23. Pazdrak K, Shi XZ, Sarna SK. TNF α suppresses human colonic circular smooth muscle cell contractility by SP1- and NF- κ B-mediated induction of ICAM-1. *Gastroenterology* 2004;127:1096–109. [PubMed: 15480988]
24. Finzi L, Barbu V, Burgel PR, Mergey M, Kirkwood KS, Wick EC, Scoazec JY, Peschaud F, Paye F, Nadel JA, Housset C. MUC5AC, a gel-forming mucin accumulating in gallstone disease, is overproduced via an epidermal growth factor receptor pathway in the human gallbladder. *Am J Pathol* 2006;169:2031–41. [PubMed: 17148666]
25. Rege RV, Prystowsky JB. Inflammation and a thickened mucus layer in mice with cholesterol gallstones. *J Surg Res* 1998;74:81–5. [PubMed: 9536979]
26. Fernandez-Real JM, Broch M, Vendrell J, Ricart W. Insulin resistance, inflammation, and serum fatty acid composition. *Diabetes Care* 2003;26:1362–8. [PubMed: 12716789]
27. Ghanim H, Aljada A, Hofmeyer D, Syed T, Mohanty P, Dandona P. Circulating mononuclear cells in obese are in a proinflammatory state. *Circulation* 2004;110:1564–71. [PubMed: 15364812]
28. Ajuwon KM, Spurlock ME. Palmitate activates the NF- κ B transcription factor and induces IL-6 and TNF α expression in 3T3-L1 adipocytes. *J Nutr* 2005;135:1841–6. [PubMed: 16046706]
29. Cao W, Cheng L, Behar J, Fiocchi C, Biancani P, Harnett KM. Proinflammatory cytokines alter/reduce esophageal circular muscle contraction in experimental cat esophagitis. *Am J Physiol Gastrointest Liver Physiol* 2004;287:G1131–9. [PubMed: 15271650]
30. Xiao ZL, Andrada MJ, Biancani P, Behar J. Reactive oxygen species (H_2O_2): effects on the gallbladder muscle of guinea pigs. *Am J Physiol Gastrointest Liver Physiol* 2002;282:G300–6. [PubMed: 11804851]
31. Stokes KY, Cooper D, Tailor A, Granger DN. Hypercholesterolemia promotes inflammation and microvascular dysfunction: role of nitric oxide and superoxide. *Free Radic Biol Med* 2002;33:1026–36. [PubMed: 12374614]
32. Stokes KY. Microvascular responses to hypercholesterolemia: The interactions between innate and adaptive immune responses. *Antioxid Redox Signal* 2006;8:1141–51. [PubMed: 16910762]
33. Juge-Aubry CE, Henrichot E, Meier CA. Adipose tissue: a regulator of inflammation. *Best Pract Res Clin Endocrinol Metab* 2005;19:547–66. [PubMed: 16311216]
34. Lin SJ, Yen HT, Chen YH, Ku HH, Lin FY, Chen YL. Expression of interleukin-1 β and interleukin-1 receptor antagonist in oxLDL-treated human aortic smooth muscle cells and in the neointima of cholesterol-fed endothelial-denuded rabbits. *J Cell Biochem* 2003;88:836–47. [PubMed: 12577317]
35. Gabay C. Interleukin-6 and chronic inflammation. *Arthritis Res Ther* 2006;8(Suppl 2):S3. [PubMed: 16899107]
36. Jager J, Gremeaux T, Cormont M, Le Marchand-Brustel Y, Tanti JF. Interleukin-1 (β)-induced insulin resistance in adipocytes through down-regulation of insulin receptor substrate-1 expression. *Endocrinology* 2007;148:241–51. [PubMed: 17038556]

37. Rege RV, Moore EW. Evidence for H⁺ secretion by the in vivo canine gallbladder. *Gastroenterology* 1987;92:281–9. [PubMed: 3098620]
38. Gleason MM, Medow MS, Tulenko TN. Excess membrane cholesterol alters calcium movements, cytosolic calcium levels, and membrane fluidity in arterial smooth muscle cells. *Circ Res* 1991;69:216–227. [PubMed: 2054935]
39. Chen Q, Amaral J, Biancani P, Behar J. Excess membrane cholesterol alters human gallbladder muscle contractility and membrane fluidity. *Gastroenterology* 1999;116:678–685. [PubMed: 10029627]
40. Moerman CJ, Smeets FW, Kromhout D. Dietary risk factors for clinically diagnosed gallstones in middle-aged men. A 25-year follow-up study (the Zutphen Study). *Ann Epidemiol* 1994;4:248–54. [PubMed: 8055126]
41. Tsai CJ, Leitzmann MF, Willett WC, Giovannucci EL. Dietary carbohydrates and glycaemic load and the incidence of symptomatic gall stone disease in men. *Gut* 2005;54:823–8. [PubMed: 15888792]
42. Tsai CJ, Leitzmann MF, Willett WC, Giovannucci EL. Glycemic load, glycemic index, and carbohydrate intake in relation to risk of cholecystectomy in women. *Gastroenterology* 2005;129:105–12. [PubMed: 16012940]

Figure 1A.

Figure 1B.

Figure 1.

Figure 1A. Gallbladder wall triglyceride in micrograms per deciliter

1B. Gallbladder wall Interleukin-1 β in picograms/milligram

Figure 2A.

Figure 2B.

Figure 2.

Figure 2A. Gallbladder wall saturated free fatty acids in micrograms per deciliter.

2B. Gallbladder wall Interleukin-6 in picograms/milligram

Figure 3A.

Figure 3B.

Figure 3.

Figure 3A. Gallbladder wall total cholesterol/phospholipids ratio

3B. Gallbladder wall tumor necrosis factor- α in picograms/milligram

Steatocholecystitis

Figure 4.

Schematic of the link between individual gallbladder wall fats and cytokines, which may alter gallbladder contraction and absorption

Table 1

Animal Weights (gm) and Average Carbohydrate (CHO) Consumption/100 gm. body weight

Study Group	Initial Weight (gm)	Final Weight (gm)	Average CHO Consumption/100 gms. body wt
Lean 45%CHO	17 ± 0.2	17 ± 0.1	40 ± 2
Lean 75%CHO	17 ± 0.2	17 ± 0.2	68 ± 2 ^Δ
Obese 45%CHO	45 ± 0.8 [*]	50 ± 0.9 [*]	20 ± 2 [*]
Obese 75%CHO	44 ± 0.3 [*]	45 ± 0.4 ^{*†}	32 ± 3 ^{*†}

*
p<0.001 vs. Lean,†
p<0.001 vs. Obese 45% CHO,Δ
p<0.001 vs Other groups

Table 2

Gallbladder Wall Lipids

Lipid Type	Lean 45% CHO	Lean 75% CHO	Obese 45% CHO	Obese 75% CHO
Total Fat	58 ± 3	68 ± 7	74 ± 10	117 ± 11 ^{*†Δ}
Triglyceride	21 ± 4	25 ± 5	21 ± 6	62 ± 10 ^{*†Δ}
Phospholipid	26 ± 2	27 ± 4	27 ± 4	29 ± 3
FFA	8 ± 3	10 ± 3	23 ± 4 ^{*†}	19 ± 1 [†]
Total XOL	4 ± 0	5 ± 1 [*]	3 ± 0	7 ± 1 ^{*†Δ}
XOL Esters	1 ± 0	2 ± 0	2 ± 0	6 ± 1 ^{†Δ}
TC/PL	0.15 ± 0.01	0.19 ± 0.01	0.11 ± 0.01	0.24 ± 0.03 ^Δ
Saturated FFA	4 ± 1	4 ± 1	12 ± 2 ^{*†}	8 ± 1 ^Δ
Myristic (14:00)	0.1 ± 0.1	0.1 ± 0.1	0.4 ± 0.1 ^{*†}	0.3 ± 0.1
Palmitic (16:00)	3.0 ± 1.2	3.5 ± 1.0	9.9 ± 1.8 ^{*†}	6.6 ± 0.6
Unstaurtaed FFA	4 ± 1	6 ± 1	12 ± 2 ^{*†}	11 ± 1 [†]
Palmitoleic (16:01)	0.5 ± 0.2	0.7 ± 0.3	2.1 ± 0.4 ^{*†}	2.0 ± 0.2 [†]
Oleic (18:01)	3.1 ± 1.0	4.6 ± 1.0	7.7 ± 1.3 ^{*†}	7.7 ± 0.6 [†]
Linoleic (18:02)	0.4 ± 0.1	0.5 ± 0.1	1.3 ± 0.3 ^{*†}	1.3 ± 0.0 [†]

Values are the mean ± SEM, CHO=Carbohydrate, FFA=Free Fatty Acids, XOL=Cholesterol, TC=Total Cholesterol, PL=Phospholipid

*
p<0.05 vs Lean 45%,

[†]
p<0.05 vs Lean 75%,

^Δ
p<0.05 vs Obese 45%

Table 3

Serum Data

Lipid Type	Lean 45% CHO	Lean 75% CHO	Obese 45% CHO	Obese 75% CHO
Cholesterol	78 ± 6	108 ± 14	208 ± 17 [*]	304 ± 17 ^{*†}
Triglyceride	74 ± 6	37 ± 2 ^{Δ§}	66 ± 4	52 ± 5 ^{‡Δ}

CHO=Carbohydrate

^{*}
p<0.001 vs. Lean,[†]
p<0.05 vs Obese 45%,^Δ
p<0.02 vs 45% CHO,[§]
p<0.001 vs Lean 45%