### Name of journal: *World Journal of Gastroenterology*

### ESPS Manuscript NO: 8927

### Columns: RESEARCH REPORT

### Association of caveolin-3 and cholecystokinin A receptor with cholesterol gallstone disease in mice

Xu GQ *et al*. Caveolin-3, CCK-AR and cholesterol gallstone disease

Guo-Qiang Xu, Cheng-Fu Xu, Hong-Tan Chen, Shan Liu, Xiao-Dong Teng, Gen-Yun Xu, Chao-Hui Yu

**Guo-Qiang Xu, Cheng-Fu Xu, Hong-Tan Chen, Shan Liu, Chao-Hui Yu,** Department of Gastroenterology, the First Affiliated Hospital, College of Medicine, Zhejiang University, Hangzhou 310003, Zhejiang Province, China

**Xiao-Dong Teng,**Department of Pathology, the First Affiliated Hospital, College of Medicine, Zhejiang University, Hangzhou 310003, Zhejiang Province, China

**Gen-Yun Xu,** Department of Laboratory Medicine, the First Affiliated Hospital, College of Medicine, Zhejiang University, Hangzhou 310003, Zhejiang Province, China

**Author contributions:** Xu GQ, Xu CF, Chen HT and Yu CH designed the research; Xu GQ, Xu CF, Chen HT, Liu S, Teng XD, Xu GY and Yu CH performed the research; Xu GQ, Xu CF, Chen HT and Liu S analyzed the data; and Xu GQ and Xu CF wrote the paper.

**Supported by** National Natural Science Foundation of China, No. 81070366

**Correspondence to: Guo-Qiang Xu, MD,** Department of Gastroenterology, the First Affiliated Hospital, College of Medicine, Zhejiang University, 79 Qingchun Road, Hangzhou 310003, Zhejiang Province, China. zyxgq@sina.cn

**Telephone:** + 86-571-87236518 **Fax:** + 86-571-87236611

**Received:** January 13, 2014 **Revised:** March 19, 2014

**Accepted:** April 21, 2014

**Published online:**

**Abstract**

**AIM**: To investigate the role of caveolin-3 (CAV3) and cholecystokinin A receptor (CCKAR) in cholesterol gallstone disease.

**METHODS**: To establish a mouse model of cholesterol gallstone disease, male C57BL/6 mice were fed with a lithogenic diet containing 1.0% cholic acid, 1.25% cholesterol and 15% fat; a similar control group was given a normal diet. The fresh liver weights and liver-to-body weight ratio were compared between the two groups after one month. Serum lipid profile and bile composition were determined with an autoanalyzer. The *Cav3* and *Cckar* mRNA and CAV3 and CCKAR protein levels in the liver and gallbladder were determined *via* real-time polymerase chain reaction and western blot, respectively.

**RESULTS**: Establishment of the mouse cholesterol gallstone disease model was verified by the presence of cholesterol gallstones in mice fed the lithogenic diet. Compared with mice maintained on a normal diet, those fed the lithogenic diet had significantly higher mean liver-to-body weight ratio (0.067 ± 0.007 *vs* 0.039 ± 0.007, *P* < 0.01), serum total cholesterol (4.22 ± 0.46 mmol/L *vs* 2.21 ± 0.11 mmol/L, *P* < 0.001), bile total cholesterol (1.33 ± 0.33 mmol/L *vs* 0.21 ± 0.11 mmol/L, *P* < 0.001), and bile phospholipid concentrations (3.55 ± 1.40 mmol/L *vs* 1.55 ± 0.63 mmol/L, *P* = 0.04), but lower total bile acid concentrations (726.48 ± 51.83 μmol/L *vs* 839.83 ± 23.74 μmol/L, *P* = 0.007). The lithogenic diet was also associated with significantly lower CAV3 in the liver and lower CAV3 and CCKAR in the gallbladder relative to the control mice (all with *P* < 0.05).

**CONCLUSION**: Our results suggest that CAV3 and CCKAR may be involved in cholesterol gallstone disease.

© 2014 Baishideng Publishing Group Co., Limited. All rights reserved.

**Key words**: Cholesterol gallstone disease; Caveolin-3; Cholecystokinin A receptor; Lithogenic diet; Mechanism

**Core tip:**Cholesterol gallstone disease (CGD) is one of the most common digestive diseases worldwide, while the mechanisms of this disease are not fully understood. In this study, we established a mouse model of CGD and observed that the formation of gallstones was accompanied by increase in serum and bile total cholesterol concentrations, while decrease in total bile acid concentration in bile. The formation of gallstones was also accompanied by downregulation of hepatic caveolin-3 (CAV3) expression, and downregulation of CAV3 and cholecystokinin A receptor (CCKAR) expression in the gallbladder. Our results suggest that CAV3 and CCKAR may be involved in CGD.

Xu GQ, Xu CF, Chen HT, Liu S, Teng XD, Xu GY, Yu CH. Association of caveolin-3 and cholecystokinin A receptor with cholesterol gallstone disease in mice.

*World J Gastroenterol* 2014; In press

**Available from:** URL: http://www.wjgnet.com/esps/

**DOI:** http://dx.doi.org/10.3748/wjg.v20.i0.0000

**INTRODUCTION**

Cholesterol gallstone disease (CGD) accounts for 80%-90% of the gallstones found at cholecystectomy, and is the most common digestive disease requiring hospital admission[1,2]. Epidemiological studies conducted in the United States showed that 10 to 15% of adults are affected by CGD, and more than one million people are newly diagnosed annually[3,4]. As the western lifestyle is adopted in developing countries, CGD prevalence increases accordingly[5,6].

CGD is resulted from bile lipids and bile salts imbalance in the gallbladder[7]. The development of CGD is associated with supersaturation of bile with cholesterol, rapid precipitation of cholesterol crystals in the gallbladder, increased bile salt hydrophobicity, and inflammation of the gallbladder[8]. Of these events, precipitation of excess cholesterol in the bile is a prerequisite for gallstone formation[9]. In general, cholesterol is solubilized in mixed micelles together with bile salts and phospholipids in bile. However, cholesterol precipitation and subsequent cholesterol gallstone formation may occur as a result of cholesterol supersaturation in bile[10,11].

Multifactorial factors are involved in the mechanism of CGD[12]. Impaired gallbladder emptying, which provides sufficient time for cholesterol crystal nucleation, contributes significantly to gallstone formation[13]. Caveolins are a family of small integral membrane proteins consisting of caveolin-1, caveolin-2, and caveolin-3 (CAV3)[14]. Caveolin-1 and caveolin-2 are the major structural proteins of caveolae, and are abundantly coexpressed in endothelial and adipose cells[15], while CAV3 is mainly found in muscle cells[16]. Caveolins are postulated to be mainly involved in modulation of cholesterol movement and storage[14,17]. A recent *in vitro* study observed that CAV3 was involved in the regulation of gallbladder muscle hypomotility[18]. Knockdown of CAV3, mediated *via* small interfering RNA, decreased contraction of gallbladder muscle cells isolated from guinea pigs, and increased cholecystokinin A receptor (CCKAR) in the caveolae[18]. CCKAR is a major physiologic mediator of smooth muscle contraction of the gallbladder. Lack of CCKAR may deteriorate gallbladder contraction and enhance gallstone formation[19]. Polymorphism of *Cckar* gene was associated with gallstone in human patients[20,21].

To date, the *in vivo* association between CAV3 and CGD has not been fully elucidated, and whether CCKAR is differentially expressed during the process of cholesterol gallstone formation remains uncertain. In this study, we established a mouse model of CGD induced by a lithogenic diet, and investigated the association of CAV3 and CCKAR with CGD.

**MATERIALS AND METHODS**

***Animals***

Eight-week-old male C57BL/6 mice were purchased from Shanghai SLAC Laboratory Animals (Shanghai, China) and maintained in a standard facility. After acclimatizing the mice to laboratory conditions for two weeks, the mice were randomly divided into two groups (*n* = 8, each), and fed for one month *ad libitum* either a standard laboratory chow diet (control) or a lithogenic diet containing 1.0% cholic acid, 1.25% cholesterol and 15% fat. All experiments were carried out in accordance with current institutional guidelines for the care and use of experimental animals.

***Serum and bile analysis***

The mice were sacrificed after one month of experimental feeding. Blood, liver, bile, and gallbladder samples were harvested. Serum triglyceride, total cholesterol, high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C) levels were measured in a Hitachi autoanalyzer 7600 (Hitachi, Tokyo, Japan). The bile samples were diluted 6-fold with deionized water, and bile composition was also analyzed using the Hitachi autoanalyzer 7600 in accordance with standard procedures.

***Real-time polymerase chain reaction***

To evaluate the expression levels of *Cav3* and *Cckar* mRNA in the livers and gallbladders, real-time polymerase chain reaction (PCR) was performed using an ABI 7500 Sequence Detection System (Applied Biosystems, Foster City, CA) with a TaKaRa real-time PCR kit in accordance with the operator’s manual. All primers used in this study (Table 1) were designed with Primer Premier 5.0 software (Premier, Canada) and synthesized at Invitrogen (Invitrogen Biotechnology, Shanghai, China). PCR products were quantified by measuring the calculated cycle thresholds (CTs) for individual targets and *β-actin* mRNA. The comparative 2-ΔΔCT method was used for quantification and statistical analysis, and results were expressed as fold changes relative to controls.

***Western blot***

Proteins extracted from mouse livers and gallbladders were run on 12% SDS-PAGE and transferred onto nitrocellulose membranes. The transferred membranes were incubated for 2 h at room temperature with blocking buffer TBST (20 mmol/L Tris-HCl, 140 mmol/L NaCl, 0.05% Tween-20, pH 7.5) containing 5% skim milk, and then incubated overnight at 4°C with anti-CAV3 (1:3000; BD transduction) and anti-CCKAR (1:500; Santa Cruz) antibodies. After five 3-min washes in TBST, the membranes were incubated for 1 h at room temperature with horseradish peroxidase-conjugated secondary antibody (1:1000, Beijing Zhongshan Biotechnology, Beijing, China). The antigens were detected using an EZ-ECL kit (Biological Industries, Israel). The intensities of the separate bands were analyzed using QuantityOne software (Bio-Rad), and normalized ratio to that of glyceraldehyde 3-phosphate dehydrogenase (GAPDH; Cell Signaling Technology) levels.

***Statistical analysis***

Statistical analyses were performed using SPSS software version 13.0 (SPSS, Chicago, IL). Data are expressed as mean ± standard deviation and were compared using Student's *t*-test. *P* < 0.05 (2-sided tests) was considered statistically significant.

**RESULTS**

Mice fed lithogenic diet containing 1.0% cholic acid, 1.25% cholesterol and 15% fat for one month developed gallstones. In addition, the mice fed the lithogenic diet had significantly higher mean liver weight and liver-to-body weight ratio compared with the control mice fed a normal diet (Figure 1). Histological analysis showed significant fatty infiltration of the liver in mice fed a lithogenic diet, while the control mice exhibited normal liver architecture.

Mice fed a lithogenic diet had significantly higher levels of serum total cholesterol, HDL-C, and LDL-C compared with the control mice (Table 2). Bile total cholesterol and phospholipid concentrations were also significantly higher in mice fed a lithogenic diet, while total bile acid levels in bile were significantly lower (Table 2).

To investigate the associations between CAV3 and CCKAR and CGD, the mRNA levels of *Cav3* and *Cckar* in the livers and gallbladders were analyzed. Real-time PCR results showed that mRNA levels of *Cav3* and *Cckar* in the gallbladder of mice fed a lithogenic diet were significantly lower than those of the control mice (Figure 2A). *Cav3* mRNA levels in the liver of mice fed a lithogenic diet was also significantly lower than those of the controls (Figure 2B). *Cckar* mRNA was not detected in the liver.

The protein CAV3 and CCKAR were analyzed in the livers and gallbladders by western blot. In accord with the mRNA expression results, the protein levels for CAV3 and CCKAR in the gallbladder of mice fed a lithogenic diet were significantly lower compared with the controls (Figure 3). The protein levels of CAV3 in the liver of mice fed a lithogenic diet were also significantly lower compared with the controls (Figure 4). These results suggested that the development of CGD was accompanied by downregulation of CAV3 expression in the liver, and downregulation of CAV3 and CCKAR expression in the gallbladder, implying that CAV3 and CCKAR may be involved in CGD.

**DISCUSSION**

In this study, we established a mouse model of CGD and observed that the formation of gallstones was accompanied by increases in serum and bile total cholesterol concentrations, while total bile acid concentrations in bile decreased. Lowe levels of hepatic CAV3 expression, and CAV3 and CCKAR in the gallbladder were also found in the mouse model relative to the normal control mice.

The mechanisms underlying the pathogenesis of CGD are incompletely understood[22]. Epidemiologic studies imply the involvement of multiple environmental factors and genetic elements in cholesterol gallstone formation[23,24]. The former include diet as a risk factor for cholesterol gallstone formation[25]. In the present study, mice developed cholesterol gallstones fed for one month with a lithogenic diet containing 1.0% cholic acid, 1.25% cholesterol and 15% fat. Significant increases in serum total cholesterol and LDL-C levels were observed in mice fed the lithogenic diet. This change mimics the alterations in serum lipid profiles observed in CGD patients[26,27].

Hypersecretion of biliary cholesterol and cholesterol supersaturation of the bile are the most important prerequisites for gallstone formation[11]. Bile acids are the major components of bile, and are involved in cholesterol elimination. Decreases in total bile acid levels may be associated with reduced cholesterol elimination in the bile and subsequent cholesterol supersaturation. In the present study, we observed that bile total cholesterol and phospholipid levels were significantly higher in the CGD mouse model, while total bile acid concentration in bile were lower than in the control mice.

Decreased gallbladder motility is crucial to the formation of cholesterol crystals in bile[13]. The mechanisms associated with decreased gallbladder motility remain unclear. Caveolins are scaffolding proteins that have important roles in cholesterol homeostasis[14,17]. Besides its role in cholesterol metabolism, CAV3 was also observed involved in regulation of gallbladder muscle hypomotility *in vitro*[18]. However, the *in vivo* association between CAV3 and CGD is not fully understood. In this study, we observed that CAV3 expression was lower in the liver and gallbladder of the mouse model of CGD, relative to the normal controls.

CCKAR is a major mediator of smooth muscle contraction of the gallbladder. One-third of CCKAR(-/-) mice spontaneously developed gallstone disease at 12 and 24 months of age[19]. Polymorphism of *Cckar* gene in patients was also an independent risk factor for gallstone disease[21]. However, whether CCKAR is differentially expressed during the process of CGD formation remains unclear. Here, we observed that the expression of CCKAR was significantly lower in the gallbladder of CGD mice compared with these levels in the control group. These observations suggest the involvement of CAV3 and CCKAR in cholesterol gallstones. However, the precise mechanism remains to be determined. Further study is also needed to clarify whether our findings are relevant to humans, and whether there is any therapeutic intervention that can prevent these effects.

In conclusion, our results showed that both mRNA and protein of CAV3 and CCKAR were differentially expressed in the liver and gallbladder of a mouse model of CGD compared with control mice. Further mechanistic investigation may enhance our understanding of the pathogenesis of CGD, and enable exploration of novel therapeutic targets.

**COMMENTS**

***Background***

Cholesterol gallstone disease is a common clinical finding that causes a high disease burden. The pathogenesis of cholesterol gallstones remains unclear, and strategies for prevention and efficient nonsurgical therapies for the disease are missing.

***Research frontiers***

The precise pathogenesis of cholesterol gallstones disease has been extensively investigated during recent years.

***Innovations and breakthroughs***

Authors observed that the formation of gallstones in mice was accompanied by increase in serum and bile total cholesterol concentrations, while decrease in total bile acid concentration in bile. The formation of gallstones was also accompanied by downregulation of hepatic caveolin-3 expression, and downregulation of caveolin-3 and cholecystokinin A receptor expression in the gallbladder.

***Applications***

Their results showed that both mRNA and protein of caveolin-3 (CAV3) and cholecystokinin A receptor (CCKAR) were differentially expressed in the liver and gallbladder of a mouse model of cholesterol gallstone disease (CGD) compared with control mice. Further mechanistic investigation may enhance our understanding of the pathogenesis of CGD, and enable exploration of novel therapeutic targets.

***Terminology***

Caveolins are a family of small integral membrane proteins consisting of caveolin-1, caveolin-2, and caveolin-3. Caveolins are postulated to be mainly involved in modulation of cholesterol movement and storage. Recently, caveolin-3 was observed to be involved in the pathogenesis of cholesterol-induced gallbladder muscle hypomotility. Cholecystokinin A receptor is a major physiologic mediator of smooth muscle contraction of the gallbladder.

***Peer review***

This is an interesting and well written manuscript addressing some known and some postulated causes for the production of cholesterol gallstones in an animal model. This is an interesting piece of work but we wait to see whether this is relevant to humans and secondly whether there is any therapeutic intervention that can prevent these effects.

**REFERENCES**

1 **Diehl AK**. Epidemiology and natural history of gallstone disease. *Gastroenterol Clin North Am* 1991; **20**: 1-19 [PMID: 2022415]

2 **Marschall HU**, Einarsson C. Gallstone disease. *J Intern Med* 2007; **261**: 529-542 [PMID: 17547709 DOI: 10.1111/j.1365-2796.2007.01783.x]

3 **Sandler RS**, Everhart JE, Donowitz M, Adams E, Cronin K, Goodman C, Gemmen E, Shah S, Avdic A, Rubin R. The burden of selected digestive diseases in the United States. *Gastroenterology* 2002; **122**: 1500-1511 [PMID: 11984534]

4 **Stinton LM**, Myers RP, Shaffer EA. Epidemiology of gallstones. *Gastroenterol Clin North Am* 2010; **39**: 157-69, vii [PMID: 20478480 DOI: 10.1016/j.gtc.2010.02.003]

5 **Shaffer EA**. Gallstone disease: Epidemiology of gallbladder stone disease. *Best Pract Res Clin Gastroenterol* 2006; **20**: 981-996 [PMID: 17127183 DOI: 10.1016/j.bpg.2006.05.004]

6 **Yoo EH**, Lee SY. The prevalence and risk factors for gallstone disease. *Clin Chem Lab Med* 2009; **47**: 795-807 [PMID: 19499973 DOI: 10.1515/CCLM.2009.194]

7 **Méndez-Sánchez N**, Cárdenas-Vázquez R, Ponciano-Rodríguez G, Uribe M. Pathophysiology of cholesterol gallstone disease. *Arch Med Res* 1996; **27**: 433-441 [PMID: 8987174]

8 **Hay DW**, Carey MC. Pathophysiology and pathogenesis of cholesterol gallstone formation. *Semin Liver Dis* 1990; **10**: 159-170 [PMID: 2218580 DOI: 10.1055/s-2008-1040470]

9 **Admirand WH**, Small DM. The physicochemical basis of cholesterol gallstone formation in man. *J Clin Invest* 1968; **47**: 1043-1052 [PMID: 5645851 DOI: 10.1172/JCI105794]

10 **Portincasa P**, Moschetta A, Palasciano G. Cholesterol gallstone disease. *Lancet* 2006; **368**: 230-239 [PMID: 16844493 DOI: 10.1016/S0140-6736(06)69044-2]

11 **Wang DQ**, Cohen DE, Carey MC. Biliary lipids and cholesterol gallstone disease. *J Lipid Res* 2009; **50 Suppl**: S406-S411 [PMID: 19017613 DOI: 10.1194/jlr.R800075-JLR200]

12 **Van Erpecum KJ**. Pathogenesis of cholesterol and pigment gallstones: an update. *Clin Res Hepatol Gastroenterol* 2011; **35**: 281-287 [PMID: 21353662 DOI: 10.1016/j.clinre.2011.01.009]

13 **Pauletzki JG**, Xu QW, Shaffer EA. Inhibition of gallbladder emptying decreases cholesterol saturation in bile in the Richardson ground squirrel. *Hepatology* 1995; **22**: 325-331 [PMID: 7601426]

14 **Cohen AW**, Hnasko R, Schubert W, Lisanti MP. Role of caveolae and caveolins in health and disease. *Physiol Rev* 2004; **84**: 1341-1379 [PMID: 15383654 DOI: 10.1152/physrev.00046.2003]

15 **Okamoto T**, Schlegel A, Scherer PE, Lisanti MP. Caveolins, a family of scaffolding proteins for organizing "preassembled signaling complexes" at the plasma membrane. *J Biol Chem* 1998; **273**: 5419-5422 [PMID: 9488658]

16 **Song KS**, Scherer PE, Tang Z, Okamoto T, Li S, Chafel M, Chu C, Kohtz DS, Lisanti MP. Expression of caveolin-3 in skeletal, cardiac, and smooth muscle cells. Caveolin-3 is a component of the sarcolemma and co-fractionates with dystrophin and dystrophin-associated glycoproteins. *J Biol Chem* 1996; **271**: 15160-15165 [PMID: 8663016]

17 **Martin S**, Parton RG. Lipid droplets: a unified view of a dynamic organelle. *Nat Rev Mol Cell Biol* 2006; **7**: 373-378 [PMID: 16550215 DOI: 10.1038/nrm1912]

18 **Cong P**, Pricolo V, Biancani P, Behar J. Effects of cholesterol on CCK-1 receptors and caveolin-3 proteins recycling in human gallbladder muscle. *Am J Physiol Gastrointest Liver Physiol* 2010; **299**: G742-G750 [PMID: 20558763 DOI: 10.1152/ajpgi.00064.2010]

19 **Sato N**, Miyasaka K, Suzuki S, Kanai S, Ohta M, Kawanami T, Yoshida Y, Takiguchi S, Noda T, Takata Y, Funakoshi A. Lack of cholecystokinin-A receptor enhanced gallstone formation: a study in CCK-A receptor gene knockout mice. *Dig Dis Sci* 2003; **48**: 1944-1947 [PMID: 14627338]

20 **Miyasaka K**, Takata Y, Funakoshi A. Association of cholecystokinin A receptor gene polymorphism with cholelithiasis and the molecular mechanisms of this polymorphism. *J Gastroenterol* 2002; **37 Suppl 14**: 102-106 [PMID: 12572876]

21 **Srivastava A**, Pandey SN, Dixit M, Choudhuri G, Mittal B. Cholecystokinin receptor A gene polymorphism in gallstone disease and gallbladder cancer. *J Gastroenterol Hepatol* 2008; **23**: 970-975 [PMID: 17944886 DOI: 10.1111/j.1440-1746.2007.05170.x]

22 **Tsai CJ**, Leitzmann MF, Willett WC, Giovannucci EL. Macronutrients and insulin resistance in cholesterol gallstone disease. *Am J Gastroenterol* 2008; **103**: 2932-2939 [PMID: 18853969 DOI: 10.1111/j.1572-0241.2008.02189.x]

23 **Di Ciaula A**, Wang DQ, Bonfrate L, Portincasa P. Current views on genetics and epigenetics of cholesterol gallstone disease. *Cholesterol* 2013; **2013**: 298421 [PMID: 23691293 DOI: 10.1155/2013/298421]

24 **von Kampen O**, Buch S, Nothnagel M, Azocar L, Molina H, Brosch M, Erhart W, von Schönfels W, Egberts J, Seeger M, Arlt A, Balschun T, Franke A, Lerch MM, Mayerle J, Kratzer W, Boehm BO, Huse K, Schniewind B, Tiemann K, Jiang ZY, Han TQ, Mittal B, Srivastava A, Fenger M, Jørgensen T, Schirin-Sokhan R, Tönjes A, Wittenburg H, Stumvoll M, Kalthoff H, Lammert F, Tepel J, Puschel K, Becker T, Schreiber S, Platzer M, Völzke H, Krawczak M, Miquel JF, Schafmayer C, Hampe J. Genetic and functional identification of the likely causative variant for cholesterol gallstone disease at the ABCG5/8 lithogenic locus. *Hepatology* 2013; **57**: 2407-2417 [PMID: 22898925 DOI: 10.1002/hep.26009]

25 **Méndez-Sánchez N**, Zamora-Valdés D, Chávez-Tapia NC, Uribe M. Role of diet in cholesterol gallstone formation. *Clin Chim Acta* 2007; **376**: 1-8 [PMID: 17055469 DOI: 10.1016/j.cca.2006.08.036]

26 **Kurtul N**, Pençe S, Kocoglu H, Aksoy H, Capan Y. Serum lipid and lipoproteins in gallstone patients. *Acta Medica (Hradec Kralove)* 2002; **45**: 79-81 [PMID: 12325457]

27 **Andreotti G**, Chen J, Gao YT, Rashid A, Chang SC, Shen MC, Wang BS, Han TQ, Zhang BH, Danforth KN, Althuis MD, Hsing AW. Serum lipid levels and the risk of biliary tract cancers and biliary stones: A population-based study in China. *Int J Cancer* 2008; **122**: 2322-2329 [PMID: 18076041 DOI: 10.1002/ijc.23307]

**P-Reviewers:** Bramhall S, Li YY **S-Editor:** Gou SX  **L-Editor: E-Editor:**

**Figure 1 The liver-to-body weight ratio in mice fed a normal or lithogenic diet.**

**Figure 2** ***Cav3* and *Cckar* mRNA in the gallbladder (A) and liver (B) of both control and cholesterol gallstone disease mice.**

**Figure 3 Western blot for caveolin-3 and cholecystokinin A receptor in the gallbladder of both control and cholesterol gallstone disease mice.** CAV3: Caveolin-3; GAPDH: Glyceraldehyde-3-phosphate dehydrogenase.

**Figure 4 Western blot for caveolin-3 in the liver of control and cholesterol gallstone disease mice.** CCKAR: Cholecystokinin A receptor; CAV3: Caveolin-3; GAPDH: Glyceraldehyde-3-phosphate dehydrogenase.

**Table 1 The primers used for real-time reverse-transcription polymerase chain reaction**

|  |  |  |
| --- | --- | --- |
| **Gene** | **Forward primers** | **Reverse primers** |
| *Cav3* | 5'-TGAGGACATTGTGAAGGTAGA-3' | 5'-TACTTGGAGACGGTGAACG-3' |
| *Cckar* | 5'-CTTCCTGTTGCCAAGTGA-3' | 5'-TTAGCCTCTTCTCTTTAGCA-3' |
| *β-actin* | 5'-GAAGATCAAGATCATTGCTCCT-3' | 5'-TGGAAGGTGGACAGTGAG-3' |

**Table 2 Serum lipid profile and bile composition comparison**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  |  | **Control diet** | **Lithogenic diet** | ***P* value** |
| Serum lipid profile | Triglyceride, mmol/L | 0.83 ± 0.15 | 0.75 ± 0.04 | 0.355 |
|  | Total cholesterol, mmol/L | 2.21 ± 0.11 | 4.22 ± 0.46 | < 0.001 |
|  | HDL-C, mmol/L | 1.35 ± 0.11 | 1.86 ±0.10 | < 0.001 |
|  | LDL-C, mmol/L | 0.58 ± 0.12 | 2.19 ± 0.43 | < 0.001 |
| Bile composition | Total cholesterol, mmol/L | 0.21 ± 0.11 | 1.33 ± 0.33 | < 0.001 |
|  | Phospholipids, mmol/L | 1.55 ± 0.63 | 3.55 ± 1.40 | 0.040 |
|  | Total bile acids, μmol/L | 839.83 ± 23.74 | 726.48 ± 51.83 | 0.007 |

HDL-C: High-density lipoprotein cholesterol; LDL-C: Low-density lipoprotein cholesterol.