[Frontiers in Bioscience 8, s1166-1174, September 1, 2003]

THE MOLECULAR AND METABOLIC BASIS OF BILIARY CHOLESTEROL SECRETION AND GALLSTONE DISEASE

Silvana Zanlungo and Flavio Nervi

Departamento de Gastroenterología, Pontificia Universidad Católica de Chile, Santiago, Chile.

TABLE OF CONTENTS

1. ABSTRACT

This article presents an up to date of selected aspects of the molecular mechanisms of cholesterol metabolism most likely involved in cholesterol gallstones disease, a highly prevalent disease in the Western world. The etiology of cholesterol cholelithiasis is considered to be multifactorial, with interaction of genetic and environmental factors. The production of supersaturated bile by the liver of cholesterol is a key early metabolic event underlying cholesterol lithogenesis. Regulation of hepatic cholesterol trafficking within the hepatocyte appears essential for the production of cholesterol supersaturated bile. Impaired sorting of metabolically active hepatic free cholesterol to the bile acid biosynthetic or lipoprotein production pathways leads to an increased availability of cholesterol for preferential channeling of cholesterol to the canalicular membrane and further secretion into bile. Many of these intrahepatic cholesterol trafficking steps are under genetic control and might be influenced by a variety of environmental factors. This review summarizes recent discoveries related to transhepatic cholesterol flux and biliary lipid secretion, which have provided new insights to the regulation of hepatic cholesterol metabolism as related to gallstone disease.

2. INTRODUCTION

Cholesterol, a key component of cell membranes and precursor of steroid hormones and bile acids, is the major component of gallstones in Western societies. Approximately 10% to 15% of Europeans and North Americans harbour gallstones. The disease is epidemic among Chilean Mestizos, Mapuche Indians and North American Indians, affecting to more than 50% of their adult populations (1-5). The aetiology and pathogenesis of cholesterol gallstone formation are multifactorial, involving complex interactions among multiple genetic and environmental factors. Biliary cholesterol lithogenicity is determined by the relative concentration of the three main lipid components of bile: bile acids, phospholipids and cholesterol. Lithogenic bile usually reveals a disruption of hepatic cholesterol homeostasis, which leads to increased secretion by the liver of biliary cholesterol secretion and subsequent cholesterol supersaturation of gallbladder bile (6-9). Interestingly, supersaturated bile is frequent among individuals of populations where gallstone disease is highly prevalent regardless of the presence of gallstone disease (10, 11), suggesting that bile supersaturated with cholesterol precedes gallstone formation.

Figure 1 schematically shows the sequential mechanisms thought to be involved in the pathogenesis of cholesterol gallstones. These include biliary cholesterol hypersecretion and supersaturation, cholesterol microcrystal formation, stone growth and stasis within the gallbladder. Each of these various steps might be under genetic control and/or influenced through intermediate metabolic pathways linked to a variety of environmental factors. It is likely that biliary cholesterol hypersecretion represents a common pathogenic mechanism for gallstone formation in the majority of patients having this disease. Gallbladder stasis increases the chance of gallstone development from supersaturated bile present in the gallbladder. Gallbladder stasis results when motility of the gallbladder is altered leading to incomplete emptying, increased fasting and residual gallbladder volume, and formation of biliary sludge, which enhances growth and aggregation of cholesterol crystals to form cholesterol gallstones (6-9). This review will be focused on the metabolic factors and their underlying molecular mechanisms responsible for the secretion of lithogenic bile.

3. HEPATIC CHOLESTEROL METABOLISM

The liver plays a critical role in whole body cholesterol homeostasis and lipoprotein cholesterol metabolism by body cholesterol removal through the bile (12-14). Hepatocytes acquire cholesterol by three

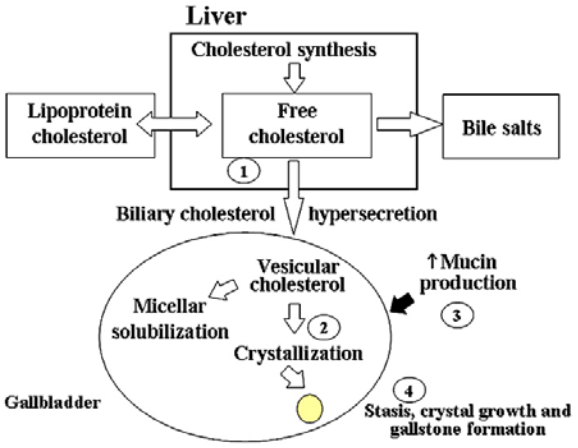

Figure 1. The four pathogenic steps in cholesterol gallstone disease. The first abnormality is the secretion by the liver of higher than normal amounts of biliary cholesterol in the form of unilamellar phosphatidylcholines vesicles. Second, bile salts – dependent micellar solubilization of vesicular cholesterol is incomplete as a function of gallbladder emptying. Third, cholesterol crystal formation is accelerated by the presence of mucin and other still unknown pronucleating factors. Fourth, the majority of subjects with biliary cholesterol supersaturation present a chronic cholecystitis prior to the formation of gallstones. Gallbladder emptying is abnormal in some gallstone patients.

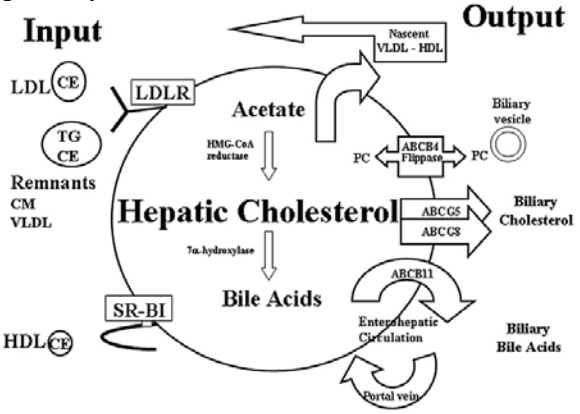

Figure 2. Hepatic cholesterol transport pathways and biliary lipid secretion. Hepatocytes acquire cholesterol by three ways: synthesis in the endoplasmic reticulum (ER), receptor-mediated internalization of chylomicron remnants (CM), VLDL, and LDL, and selective cholesterol uptake from HDL mediated by SR-BI. Particles internalized by LDLR are hydrolyzed in lysosomes releasing free cholesterol, which could be transported with the aid of NPC1 to the plasma membrane and to the interior of the hepatocyte. Cholesterol obtained from HDL is selectively transferred to the hepatocyte and is used as a preferentially source of cholesterol secreted into bile. Cholesterol synthesized in the (ER) and reaching the ER from other sources could be esterified in cholesterol ester-rich droplets, metabolized to bile acids or secreted into bile. SCP2 could delivers cholesterol destined for biliary secretion to the canalicular membrane. Biliary lipid secretion is mediated by different ABC transporters: ABCG5/G8 for cholesterol, ABCB4 for phospholipids (PL) and ABCB11 for bile salts (BS).

pathways: endogenous biosynthesis from acetate in the endoplasmic reticulum (ER) (12), receptor-mediated endocytosis of chylomicron (CM), very low-density lipoproteins (VLDL) and low-density lipoproteins (LDL) (13), and selective cholesterol uptake from high-density lipoproteins (HDL) mediated by the scavenger receptor class B, type I (SR-BI) (15, 16). Cholesterol is highly insoluble in water and, in contrast to fatty acids, cannot be catabolized to simpler carbon molecules. Hepatocytes efficiently eliminate sterols through the bile as cholesterol and newly synthesized bile acids (12-14). Remarkably, biliary cholesterol is mostly derived from preformed cholesterol, which originates from the uptake of plasma lipoprotein cholesterol at the hepatocyte sinusoidal membrane (17, 18).

For biliary secretion, cholesterol must be transported within the hepatocyte towards the canalicular region and secreted into the bile solubilized in phospholipid vesicles (19-22, see Figure 2 for a scheme of hepatic cholesterol transport). Then, it is reasonable to postulate that biliary cholesterol secretion under normal and disease conditions could be controlled by genes encoding proteins responsible for hepatic sterol trafficking, including apolipoproteins, lipoprotein receptors, and enzymes involved in bile acid synthesis and cholesterol esterification, intracellular cholesterol transport proteins, and canalicular lipid transporters. Recent studies using gene targeted mice have provided evidence that indeed various genes involved in hepatic lipid trafficking and metabolism play a key role in biliary lipid secretion (reviewed in 23).

Furthermore, a number of studies have tried to identify specific abnormalities of hepatic cholesterol metabolism in gallstone patients. However, results have not shown a specific pattern of abnormalities. For example, gallstone patients have presented increased or normal activities of hepatic hydroxy-methyl-glutaryl-Co-A reductase (HMG-CoA reductase) (24-27), the rate limiting steps of cholesterol synthesis. Determination of bile salt synthesis or cholesterol 7alpha–hydroxylase (CYP7A1) activity, the principal regulatory enzyme in the synthesis of bile acids, has been found decreased, normal or increased in gallstone patients (24-28).

4. HEPATIC CHOLESTEROL TRAFFICKING AND ITS RELATION TO BILIARY LIPID SECRETION

The receptor-mediated endocytic pathway is one of the major mechanisms for uptake of lipoprotein cholesterol in the liver. In fact, more than 80% of circulating plasma LDL is cleared by endocytosis in this organ (12). Furthermore, the hepatic endocytic pathway is also responsible for metabolism of lipoprotein remnants. We have shown that apoE, a lipoprotein cholesterol transport molecule involved in the uptake of CM remnant by endocytosis, plays a critical role in regulating biliary secretion of dietary cholesterol and diet-induced cholesterol gallstone formation in mice (29). Interestingly, an association between human apoE4 polymorphism and cholesterol gallstone formation has been described (30).

Reverse cholesterol transport is an important pathway that transfers cholesterol carried by HDL from peripheral tissues to the liver (31). For long time, it was known that HDL is utilized as a preferentially source of cholesterol for biliary secretion, either as unesterified cholesterol or as bile acids (17). The molecular mechanisms by which the liver handles HDL cholesterol for biliary secretion have begun to be elucidated. In this context, hepatic overexpression of the HDL receptor SR-BI using adenoviral gene transfer resulted in reduced plasma HDL cholesterol concentrations and increased biliary cholesterol secretion (15). On the other hand, plasma HDL cholesterol concentration was increased and biliary cholesterol secretion was reduced in SR-BI knockout (16). These results suggest that SR-BI is a candidate gene to be involved in gallstone formation due its role in reverse cholesterol transport mediating the transfer of HDL cholesterol from plasma to bile. Consistent with proposal, it has been reported that up-regulation of hepatic SR-BI expression is associated with biliary cholesterol hypersecretion and gallstone formation in gallstone-susceptible C57L mice compared with resistant AKR mice (32). However, the definitive relevance of SR-BI in murine gallstone formation has not been addressed directly and is a matter of some controversy (33).

Under certain conditions, liver cholesterol homeostasis and biliary lipid secretion depend on hepatic cholesterol and/or bile acid synthesis. HMG-CoA reductase is the key enzyme in the novo cholesterol synthesis pathway. In mice, there is an association between HMG-CoA reductase expression/activity and gallstone formation (34). After feeding a lithogenic diet, gallstone-resistant AKR mouse strain down-regulates hepatic HMG-CoA reductase expression, whereas this response was not observed in gallstone susceptible C57L/J strain (34). As mentioned before, in humans, the relation between HMG-CoA reductase expression/activity and gallstone formation is less understood (24-27).

Cholesterol 7alpha-hydroxylase (CYP7A1) is the key regulatory enzyme in the classic bile salt synthesis pathway (35). In rodents, cyp7A1 gene expression is positively regulated by dietary cholesterol, increasing the conversion of cholesterol to bile acids and reducing the cholesterol-induced hypercholesterolemia (35). In addition, cyp7a1 -/- mice had reduced rate of bile acid synthesis and size of the bile salt pool size (36). However, the role of cyp7A1 in cholesterol gallstone formation is not clear yet. In humans, a cholesterol diet apparently does not regulate CYP7A1 (37).

Another key step in liver cholesterol homeostasis is cholesterol storage regulated by acyl-CoA cholesterol acyl transferase (ACAT), which is responsible for esterification of cholesterol with long chain fatty acids to store excess of cholesterol in cholesterol ester-rich droplets. The relevance of ACAT2, the ACAT gene that is expressed in mouse liver and intestine, was demonstrated in ACAT2 knockout mice (38, 39). When fed a high cholesterol diet, these mice exhibited profound effects in cholesterol homeostasis including complete resistance to diet induced cholesterol gallstone formation due to reduced capacity to absorb cholesterol in the intestine (38).

Hepatic cholesterol is transported efficiently through the liver and secreted into bile. Although the detailed molecular and cellular mechanisms involved in this process are not known, it is conceivable that many gene products encoding for carrier proteins and proteins involved in vesicular traffic participate in this complex and regulated hepatocellular system (12, 18, 23). We have provided evidence that hepatic transport of newly synthesized cholesterol from the endoplasmic reticulum to the canalicular membrane for biliary secretion into bile is rapid and microtubule- and Golgi-independent (40). In contrast, hepatic transport of lipoprotein-derived cholesterol for bile secretion seems to be mediated in part by cholesterol-containing vesicles and intracellular cholesterol trafficking proteins (20, 22). Recent attention has been focused towards exploring the potential role of intracellular sterol carrier/transfer proteins as potential mediators of cholesterol movement from intrahepatic compartments to the canalicular membrane. Using antisense techniques and adenovirus-mediated gene transfer, we have established that hepatic sterol carrier protein-2 (SCP-2) regulates biliary secretion of hepatic cholesterol (41-43). Also, it has been reported that disruption of the SCP-2 gene in mice impairs biliary lipid metabolism (44). Consistent with these findings, it has been demonstrated that hepatic SCP-2 levels were elevated in patients with cholesterol gallstones (27) and that hepatic SCP-2 expression levels correlated with biliary cholesterol hypersecretion in mice with genetic predisposition to gallstone disease (45).

We have also demonstrated that the inactivation of the Niemann-Pick type C-1 (NPC1) gene, which encodes an intracellular protein involved in trafficking of endocytosed lipoprotein cholesterol, is associated with abnormal biliary cholesterol secretion in cholesterol-fed mice (46). On the other hand, adenovirus-mediated overexpression of NPC1 in murine liver results in increased biliary cholesterol secretion. Furthermore, NPC1 -/- mice did not increase gallbladder cholesterol concentrations in response to dietary cholesterol (46). This suggests a potential role for the NPC1–dependent endocytic lipoprotein cholesterol uptake pathway in gallstone formation induced by a lithogenic diet. NPC1 has been proposed as a candidate gene in gallbladder lithogenesis (47), but further studies are required to elucidate the direct role of hepatic NPC1 expression on cholesterol gallstone disease. Finally, it has been reported that caveolin-1, the main protein of cholesterol-rich plasma membrane caveolae and found in sinusoidal and canalicular liver membranes, is up-regulated during cholesterol gallstone formation in mice (32). Interestingly, hepatic caveolin-1 overexpression changes plasma HDL cholesterol levels by impairing SR-BI-mediated selective HDL cholesterol uptake (48). Using adenovirus-mediated gene transfer, hepatic caveolin-1 overexpression affected biliary bile salt, rather than cholesterol, secretion in mice (Miquel JF *et al*, unpublished data). Caveolin-1-/- mice have been generated but they have not been characterized with regard to the role of

caveolin-1 in biliary lipid secretion and gallstone formation (49, 50).

Translocation of lipids across the canalicular membrane requires the activity of ATP-binding cassette (ABC) transporters, including the bile salt export pump sister P-glycoprotein ABCB11 (51) and the phospholipid flippase ABCB4 (52). This latter transporter translocates phosphatidylcholines from the inner to the outer leaflet of the canalicular membrane facilitating the formation of canalicular unilamellar vesicles (53), a key cellular mechanism for biliary phospholipid and cholesterol secretion (19-22). Recently, a major hint in the search for the long sought canalicular cholesterol transporter came from the discovery of mutations in the genes encoding human ABCG5 and ABCG8 transporters that cause sitosterolemia (54, 55), a rare disease characterized by increased plasma plant-derived sterols, hypercholesterolemia, premature atherosclerosis, xanthomatosis and impaired biliary cholesterol secretion (56). Using gene manipulated mice, it has been demonstrated that ABCG5 and ABCG8 are essential for determining biliary cholesterol secretion independently of bile acids and phospholipids secretion into bile (57, 58). Overexpression of the human ABCG5 and ABCG8 transporters in mice markedly altered cholesterol transport through the enterohepatic circulation: fractional absorption of dietary cholesterol was decreased, whereas biliary cholesterol secretion and saturation were increased (57). On the other hand, biliary cholesterol secretion was dramatically reduced in ABCG5/G8 knockout mice (58). Despite bile samples of ABCG5/G8 transgenic mice were cholesterol supersaturated, no signs of cholesterol precipitation were observed (57). This may implicate that bile cholesterol supersaturation per se is not sufficient for cholesterol precipitation and gallstone formation and that other factors are involved in this process (see further discussion in 59-61).

5. REGULATORY GENES INVOLVED IN BILIARY LIPID SECRETION

Regulatory genes transcriptionally controlling hepatic lipid metabolism (i.e., sterol regulatory element binding proteins (SREBPs), liver X receptors (LXRs), farnesoid receptor (FXR), and peroxisome proliferator-activated receptors (PPARs)) might also regulate biliary lipid secretion. SREBPs play an important role activating the expression of various genes involved in the synthesis of cholesterol and fatty acids in the liver (62). Various transgenic mice that overexpress the three different SREBPs isoforms (SREBP1a, SREBP1c and SREBP 2) have been generated (62), even though their role in determining cholesterol availability for biliary cholesterol secretion and gallstone formation has not been reported.

LXRalpha has a pivotal role maintaining body cholesterol homeostasis because this nuclear receptor, which is activated by oxysterols, regulates the transcription of key genes involved in cholesterol absorption, transport, storage and catabolism like ABCG5/ABCG8 (63), ABCA1, apoE, SREBP1c, CETP, and cyp7A1 (in rodents, but not apparently in humans) (37, 64). LXRalpha regulation of cyp7A1 is very important in mice: LXRalpha -/- mice exhibit enormous hepatic cholesterol accumulation because they do not up-regulate cyp7A1 under a high-cholesterol diet (65). However, biliary cholesterol or gallstone formation in LXRalpha -/- mice has not been evaluated. Administration of the LXR agonist T0901317 to mice produces multiple effects in cholesterol metabolism such as increases in plasma HDL cholesterol and phospholipid levels as well as biliary cholesterol output (66-69).

FXR is another essential regulator of cholesterol homeostasis (70). This factor regulates expression of key genes involved in bile acid biosynthesis and transport and HDL cholesterol metabolism (71). In FXR -/- mice, increased plasma HDL cholesterol levels and decreased in plasma HDL cholesterol clearance correlated with reduced hepatic SR-BI expression (71). Surprisingly, biliary cholesterol secretion was increased in FXR -/- mice despiteABCG5/G8, SCP2 and SR-BI down-regulation (71). This latter result indicates that SR-BI-independent pathways are actively supplying cholesterol for biliary cholesterol secretion in this mouse model

PPARalpha is a key lipid sensor since this factor regulates the expression of several genes related with fatty acid oxidation and peroxisome proliferation. Recently, regulation of hepatic ABCB4, ABCG5, ABCG8, FXR and LXR by PPARalpha has been established in fasted mice (72). PPARalpha regulation of these genes was associated with increased biliary phospholipid and bile salt secretion. Since PPARalpha is activated by fibrates, these compounds could potentially modulate biliary lipid secretion and gallstone formation. Interestingly, administration of fibrates reduces cyp7a1 expression in mice (73) and increases the risk for gallstone formation in humans (74-76).

6. GALLSTONE GENES

The existence of human gallstone genes is supported by epidemiological (2-5) and family studies (77-79) of gallstone disease In fact, some candidate human genes related to lipoprotein metabolism have been associated to cholesterol gallstone disease. Some specific gene polymorphisms of apo E, apo B, apo A-I, and cholesteryl ester transfer protein were more frequently found in patients with cholelithiasis (30, 80, 81). More recently, mutations of the MDR3 were found associated to mild chronic cholestasis and cholesterol cholelithiasis (82 - 84). Another single gene defect in premature human cholesterol cholelithiasis and hypercholesterolemia was found associated to CYP7A1 deficiency (85)

Similarly, some murine genes have been proposed as candidate genes to be involved in cholesterol gallstone formation based on genetics of experimental cholelithiasis in inbred mice (2, 47). By quantitative trait loci mapping, nine cholesterol gallstone susceptibly loci (Lith alleles) have been identified in mice. Some candidate genes that localize in the Lith loci are HMG-CoA reductase, SCP-2, ABCG5/G8, SCAP (SREBP-cleavage activating protein), FXR and PPARgamma (2). The

localization of PPARgamma gene within a Lith locus is intriguing because this nuclear transcription factor regulates the expression of multiple genes involved in lipid metabolism and its functional activity has been linked to hyperlipidemia, obesity, insulin resistance and type 2 diabetes (86, 87), all well known risk factors associated to cholesterol gallstone disease in humans.

7. METABOLIC RISK FACTORS OF HUMAN CHOLESTEROL CHOLELITHIASIS

A number of epidemiologic studies have linked obesity, diabetes type 2 and hyperlipidemia (high serum triglyceride and low HDL serum cholesterol) to cholesterol gallstone formation (5-8). These conditions are commonly included under the heading of the metabolic syndrome or Syndrome X (88-90). The basic pathophysiological abnormality underlying the metabolic syndrome is insulin resistance, which represents a generalized derangement in metabolic processes (88-90). The major clinical consequences of the metabolic syndrome are coronary heart disease and stroke, type 2 diabetes and its complications (88-90), fatty liver (91,92), and cholesterol gallstones (93, 94).

The mechanistic link between insulin resistance and the metabolic syndrome is complex. Patients with diabetes type 2 that usually have hyperinsulinemia and insulin resistance have been shown to have a higher biliary cholesterol saturation compared to control subjects (95-97). The latter is also a common finding in obese patients (98-101). In addition, insulin treatment of patients with non – insulin dependent diabetes increased biliary cholesterol saturation (96, 97). Whether hyperinsulinemia also affects gallbladder emptying favouring cholesterol crystal formation remains to be elucidated. The role of insulin on the regulatory mechanisms of biliary cholesterol secretion has not been elucidated. It is possible that insulin increases the availability of free cholesterol for secretion into bile since-administration of insulin to increases hepatic cholesterogenesis (102) and decreases bile acid synthesis in the rat (103). In addition, insulin increases LDL receptor activity in human fibroblast suggesting that the hormone might also have the same effect in the liver, increasing the availability of free cholesterol for secretion into bile (104). Recent evidence indicates that leptin, a hormone that promotes satiety, energy metabolism and weight loss, might favour the formation of lithogenic bile and cholesterol gallstones in the genetically obese mouse (105, 106).

Another possible pathogenic metabolic link between hyperinsulinemia and cholelithiasis might be related to the induction of hepatic VLDL production by insulin (107). Insulin resistance might increase sinusoidal secretion of VLDL, which after releasing free fatty acids in muscle and adipose tissue by the action of lipoprotein lipase (107) can return to the liver as VLDL remnants for rapid clearance through lipoprotein receptor–dependent mechanisms. Under these circumstances, the absolute amount of cholesterol returning to the liver as VLDL remnants might be enhanced, increasing the availability of metabolically active free cholesterol for secretion into the bile.

8. PERSPECTIVES

In summary, recent studies have established the relevance of a series of cholesterol transport and cholesterol metabolism-related molecules in controlling the hepatic availability of cholesterol for biliary secretion and the pathogenesis of cholesterol gallstones in mice. Further studies are required to address of the detailed cellular and molecular mechanisms by which these different proteins involved in hepatic cholesterol uptake and transhepatic cholesterol transport determine biliary cholesterol secretion through the canalicular membrane. Because bile is the most important route for elimination of cholesterol from the body, a comprehensive understanding of the mechanisms involved in the modulation of the availability of hepatic cholesterol for biliary cholesterol secretion should facilitate the design of new effective preventive and therapeutical approaches in atherosclerosis and gallstones.

9. ACKNOWLEDGEMENTS

We thank many colleagues that have enthusiastically collaborated with our research in this field. Our work is supported by grants FONDECYT #1030744 to F. Nervi and #1030415 to S. Zanlungo.

10. REFERENTES

1. Nervi F., J.F. Miquel & G. Marshall: The Amerindian epidemics of cholesterol gallstones: The North and South connection. *Hepatology* 37, 947-948 (2003)
2. Carey M.C. & B. Paigen: Epidemiology of the American Indians burden and its likely genetic origin. *Hepatology* 36, 781- 791 (2002)
3. Everhart J.E., F. Yeh, M.C. Hill, R. Fabsitz, B.V. Howard & T.K. Welty: Prevalence of gallbladder disease in American Indian populations: findings from the Strong Heart Study. *Hepatology* 35, 1507- 1512 (2002)
4. Weiss K.M., R.E. Ferrell, C.L. Hanis, & P.N. Styne: Genetics and epidemiology of gallbladder disease in New World Native Peoples. *Am J Hum Genet*. 38, 1259-1287 (1984)
5. Miquel J.F., C. Covarrubias, L. Villarroel, G. Mingrone, A.V. Greco, P. Carvallo, G. Marshall, G. Del Pino & F. Nervi: Genetic epidemiology of cholesterol cholelithiasis among Chilean Hispanics and Maoris. *Gastroenterology* 115, 937-946 (1998)
6. Dowling R.M.: Review: pathogenesis of gallstones. *Alim Pharmacol Therap* 14, 39-52 (2000)
7. Apstein M.D. & M.C. Carey: Pathogenesis of cholesterol gallstones: a parsimonious hypothesis. *Eur J Clin Invest* 26, 343-352 (1996)
8. Lee S.P. & C.W. Ko: Gallstones. In: Textbook of Gastroenterology, 3rd Edition. Eds: Yamada T, Alpers D.H., Laine L, Owyang C, Powell D.W., Lippincott Williams & Wilkins, Philadelphia 2258-2280 (1999)
9. Amigo L., S. Zanlungo, H. Mendoza, J.F. Miquel & F. Nervi: Risk factors and pathogenesis of cholesterol gallstones: state of the art. *Eur Rev Med Pharmacol*. Sci 3, 241-6 (1999)

10. Thistle J. L. & L.J. Schoenfield: Lithogenic bile among young Indian women. *N Eng J Med* 284, 177-181 (1971)
11. Grundy S.M., A.L. Metzger & R.D. Adler: Mechanisms of lithogenic bile formation in American Indian women with cholesterol gallstones. *J Clin Invest* 51, 3026-3043 (1972)
12. Dietschy J.M., S.D. Turley & D.K. Spady: Role of the liver in the maintenance of cholesterol and low density lipoprotein homeostasis in different animal species, including humans. *J Lipid Res* 34, 1637-1659 (1993)
13. Turley S.D. & J.M. Dietschy: The metabolism and excretion of cholesterol by the liver. In: The liver: biology and pathology. Eds: Arias I.M, Jakoby W.B, Popper H, Schachter D, Shafritz D.A., Raven Press, NY 617-641 (1988)
14. Liscum L & N.J. Munn: Intracellular cholesterol transport. *Biochim Biophys Acta* 1438, 19-37 (1999)
15. Kozarsky K.F., M.H. Donahee, A. Rigotti, S.N. Iqbal, E.R. Edelman & M. Krieger: Overexpression of the HDL receptor SR-BI alters plasma HDL and bile cholesterol levels. *Nature* 387, 414-417 (1997)
16. Rigotti A., B.L. Trigatti, M. Penman, H. Rayburn, J. Herz & M. Krieger: A targeted mutation in the murine gene encoding the high density lipoprotein (HDL) receptor scavenger receptor class B type I reveals its key role in HDL metabolism. *Proc Natl Acad Sci USA* 94, 12610-12615 (1997)
17. Bothman K.M. & E. Bravo: The role of lipoprotein cholesterol in biliary steroid secretion. Studies with in vivo experimental models. *Prog Lipid Res* 34, 71-97 (1995)
18. Cohen D.E.: Hepatocellular transport and secretion of biliary lipids. *Curr Opin Lipidol* 10, 295-302 (1999)
19. Cohen D., M. Angelico & M. Carey: Qualisielastic light scattering evidence for vesicular secretion of biliary lipids. *Am J Physiol* 257, G1-G8 (1989)
20. Rigotti A., M.P. Marzolo & F. Nervi: Lipid transport from the hepatocyte into the bile. *Curr Top Membr* 40, 579-615 (1994)
21. Crawford A.R., A.J. Smith, V.C. Hatch, R.P. Oude Elferink, P. Borst & JM Crawford: Hepatic secretion of phospholipid vesicles in the mouse critically depends on mdr2 or MDR3 P-glycoprotein expression. *J Clin Invest* 100, 2562-2567 (1997)
22. Oude Elferink R.P. & A.K. Groen: The mechanism of biliary lipid secretion and its defects. *Gastroenterol Clin North Am* 28, 59-74 (1999)
23. Kosters A., M. Jirsa & A.K. Groen: Genetic background of cholesterol gallstone disease. *Biochim Biophys Acta* 1637, 1-19 (2002)
24. Salen G., G. Nicolau, S. Shefer & E.H. Moscbach: Hepatic cholesterol metabolism in patients with gallstones. *Gastroenterology* 69, 676-684 (1975)
25. Ahlberg J., B. Angelin & K. Einarsson: Hepatic 3-hydroxi-3-methylglutaryl coenzyme A reductase activity and biliary lipid composition in man: relation to cholesterol gallstone disease and effects of cholic and chenodeoxycholic acid treatment. *J Lipid Res* 22, 410-422 (1981)
26. Reihner E., B. Angelin, I. Bjorkhem & K. Einarsson: Hepatic cholesterol metabolism in cholesterol gallstone disease. *J Lipid Res* 32, 469-475 (1991)
27. Ito T., S. Kawata, Y. Imai, H. Kakimoto, J.M. Trzaskos & Y. Matsuzawa: Hepatic cholesterol metabolism in patients with cholesterol gallstones: enhanced intracellular transport of cholesterol. *Gastroenterology* 110, 1619-1627 (1996)
28. Muhrbeck O., F.H. Wang, I. Bjorkhem, M. Axelson & K. Einarsson: Circulating markers for biosynthesis of cholesterol and bile acids are not depressed in asymptomatic gallstone subjects. *J Hepatol* 27, 150-155 (1997)
29. Amigo L., V. Quiñones, P. Mardones, S. Zanlungo, J.F. Miquel, F. Nervi & A. Rigotti: Impaired biliary cholesterol secretion and decreased gallstone formation in apolipoprotein E-deficient mice fed a high-cholesterol diet. *Gastroenterology* 118, 772-779 (2000)
30. Juvonen T., K. Kervinen, M.I. Kairaluoma, L.H. Lajunen & Y.A. Kesaniemi: Gallstone cholesterol content is related to apolipoprotein E polymorphism. *Gastroenterology* 104, 1806-1813 (1993)
31. Tall A. R.: An overview of reverse cholesterol transport. *Eur Heart J* 19, A31-A35 (1998)
32. Fuchs M., B. Ivandic, O. Muller, C. Schalla, J. Scheibner, P. Bartsch & E.F. Stange: Biliary cholesterol hypersecretion in gallstone-susceptible mice is associated with hepatic up-regulation of the high-density lipoprotein receptor SR-BI. *Hepatology* 33, 1451-1459 (2001)
33. Rigotti A., S. Zanlungo, J.F. Miquel & D. Wang: HDL receptor SR-BI and cholesterol gallstones. *Hepatology* 35, 240-241(2002)
34. Khanuja B., Y.C. Cheah, M. Hunt, P.M. Nishina, D.Q. Wang, H.W. Chen, J.T. Billheimer, M.C. Carey & B. Paigen: Lith1, a major gene affecting cholesterol gallstone formation among inbred strains of mice. *Proc Natl Acad Sci USA* 92, 7729-33 (1995)
35. Schwarz M., E.G. Lund & D.W. Russel: Two 7alpha-hydroxylase enzymes in bile acid biosynthesis. *Curr Opin Lipidol* 9, 113-118 (1998)
36. Schwarz M., D.W. Russell, J.M. Dietschy & S.D. Turley: Marked reduction in bile acid synthesis in cholesterol 7alpha-hydroxylase-deficient mice does not lead to diminished tissue cholesterol turnover or to hypercholesterolemia. *J Lipid Res* 39, 1833-1843 (1998)
37. Chen J.Y., B. Levy-Wilson, S. Goodart & A.D. Cooper: Mice expressing the human CYP7A1 gene in the mouse CYP7A1 knock-out background lack induction of CYP7A1 expression by cholesterol feeding and have increased hypercholesterolemia when fed a high fat diet. *J Biol Chem* 77, 42588-42595 (2002)
38. Buchman K.K, M. Accad, S. Novak, R.S. Choi, J.S. Wong, R.L. Hamilton, S. Turley & R.V. Farese Jr.: Resistence to diet-induced hypercholesterlemia and gallstone formation in ACAT2-deficient mice. *Nat Med* 6, 1341-1347 (2000)
39. Zanlungo S. & F. Nervi: The ACAT2 gene encodes a gatekeeper of intestinal cholesterol absorption that regulates cholesterolemia and gallstone disease. *Hepatology* 33, 760-761 (2000)
40. Puglielli L., A. Rigotti, A.V. Greco, M.J. Santos, F. Nervi: Sterol carrier protein-2 is involved in cholesterol transfer from the endoplasmic reticulum to the plasma membrane in human fibroblasts. *J Biol Chem* 32, 18723-18726 (1995)
41. Puglielli L., A. Rigotti, L. Amigo, L. Núñez, A.V. Greco, M.J. Santos & F. Nervi: Modulation of intrahepatic

cholesterol trafficking: evidence by in vivo antisense treatment for the involvement of sterol carrier protein-2 in newly synthesized cholesterol transport into rat bile. *Biochem J* 317, 681-687 (1996)
42. Zanlungo S., L. Amigo, H. Mendoza, J.F. Miquel, C. Vío, J.M. Glick, A. Rodríguez, K. Kozarsky, V. Quiñones, A. Rigotti & F.Nervi: Sterol carrier protein 2 gene transfer changes lipid metabolism and enterohepatic sterol circulation in mice. *Gastroenterology* 119, 1708-1719 (2000)
43. Amigo L., S. Zanlungo, J.F. Miquel, J. Glick, H. Hyogo, D. Cohen & F. Nervi: Overexpression of Sterol Carrier Protein 2 Gene Inhibits VLDL Production and Reciprocally Enhances Biliary Lipid Secretion. *J Lipid Res* 44, 399-407 (2003)
44. Fuchs M., A. Hafer, C. Münch, F. Kannenberg, S. Teichmann, J. Scheibner, E. F. Stange & U. Seedorf: Disruption of the sterol carrier protein 2 gene in mice impairs biliary lipid and hepatic cholesterol metabolism. *J Biol Chem* 276, 48058-48065 (2001)
45. Fuchs M., F. Lammert, D. Q-H. Wang, B. Paigen, M.C. Carey & D.E. Cohen: Sterol carrier protein 2 participates in hypersecretion of biliary cholesterol during gallstone formation in genetically gallstone-susceptible mice. *Biochem J* 336:33-37 (1996)
46. Amigo L., H. Mendoza, V. Quiñones, J.F. Miquel & S. Zanlungo: Relevance of Niemann-Pick Type C1 protein expression in controlling plasma cholesterol and biliary lipid secretion in mice. *Hepatology* 36, 819-828 (2002)
47. Lammert F., M.C. Carey & B. Paigen: Chromosomal organization of candidate genes involved in cholesterol gallstone formation: a murine gallstone map. *Gastroenterology* 120, 221-238 (2001)
48. Frank P.G, A. Pedraza, D.E. Cohen & M.P. Lisanti: Adenovirus-mediated expression of caveolin-1 in mouse liver increases plasma high-density lipoprotein levels. *Biochemistry* 40, 10892-10900 (2001)
49. Drab M., M. Verkade, M. Elger, M. Kasper, M. Lohn, B. Lauetbach, J. Menne, C. Lindschau, F. Mende, F. C. Luft, A. Schedl, H. Haller & T. V. Kurzchalia: Loss of caveolae, vascular dysfunction, and pulmonary defects in caveolin-1 gene-disrupted mice. *Science* 293, 2449-2452 (2001)
50. Razani B., J.A. Engelman, X.B. Wang, W. Schubert, X.L. Zhang, C.B. Marks, F. Macaluso, R.G. Russell, M. Li, R.G. Pestell, D. Di Vizio, H. Hou Jr., B. Kneitz, G. Lagaud, G.J. Christ, W. Edelmann & M.P. Lisanti: Caveolin-1 null mice are viable but show evidence of hyperproliferative and vascular abnormalities. *J Biol Chem* 276, 38121-38138 (2001)
51. Wang R., M. Salem, I.M. Yousef, B. Tuchweber, P. Lam P, S.J. Childs, C.D. Helgason, C. Ackerley, M.J. Phillips & V. Ling: Targeted inactivation of sister of P-glycoprotein gene (spgp) in mice results in non progressive but persistent intrahepatic cholestasis. *Proc Natl Acad Sci USA* 98, 2011-2016 (2001)
52. Smit J.J., A.H. Schinkel, R.P. Oude Elferink, A.K. Groen, E. Wagenaar, L. van Deemter & C.A. Mol: Homozygous disruption of the murine Mdr2 P-glycoprotein gene leads to a complete absence of phospholipid from bile and to liver disease. *Cell* 75, 451-462 (1993)
53. Ulloa N., J. Garrido & F. Nervi: Ultracentrifugal isolation of vesicular carriers of biliary cholesterol in native human and rat bile. *Hepatology* 7, 235-244 (1987)
54. Berge K.E., H. Tian , G.A. Graf, L. Yu, N.V. Grishin, J. Schultz, P. Kwiterovich, B. Sahn, R. Barnes & H.H. Hobbs: Accumulation of dietary cholesterol in sitosterolemia caused by mutations in adjacent ABC transporters. *Science* 290, 1771-1775 (2000)
55. Lee M.H., K. Lu, S. Hazard, H. Yu, S. Shulenin, H. Hidaka, H. Kojima, R. Allikmets, N. Sakuna, R. Pegoraro, A.K. Srivastava, G. Salen, M. Dean & S.B. Patel: Identification of a gene, ABCG5, important in the regulation of dietary cholesterol absorption. *Nat Genet* 27, 79-83 (2001)
56. Bjorkhem I., K. Boberg & E. Leitersdorf: Inborn errors in bile acid biosynthesis and storage of sterols other than cholesterol. In: The metabolic and molecular bases of inherited disease, Volume II. Eds: Scriver C., Beaudet A., Sly W., Valle D, McGraw-Hill, NY 2961–2988 (1995)
57. Yu L., J. Li-Hawkins, R. E. Hammer, E. Berge, J.D. Horton, J.C Cohen & H. H. Hobbs: Overexpression of ABCG5 and ABCG8 promotes biliary cholesterol secretion and reduces fractional absorption of dietary cholesterol. *J Clin Invest* 110, 671-680 (2002)
58. Yu L., R.E. Hammer, J. Li-Hawkins, K. von Bergmann, D. Lutjohann, J.C. Cohen & H.H. Hobbs: Disruption of Abcg5 and Abcg8 in mice reveals their crucial role in biliary cholesterol secretion. *Proc Natl Acad Sci USA* 99, 16237-42 (2002)
59. Wittenburg H. & M.C. Carey: Biliary cholesterol secretion by the twinned sterol half-transporters ABCG5 and ABCG8. *J Clin Invest* 110, 605-609 (2002)
60. Small D.M.: Role of ABC transporters in secretion of cholesterol from liver into bile. *Proc Natl Acad Sci USA* 100, 1-6 (2003)
61. Zanlungo S., J.F. Miquel, A. Rigotti & F. Nervi: The ABCs of biliary cholesterol secretion and their implication for gallstone disease. *Hepatology* 37, 940 -942 (2003)
62. Horton J.D., J.L. Goldstein & M.S. Brown: SREBPs: activators of the complete program of cholesterol and fatty acid synthesis in the liver. *J Clin Invest* 109, 1125-1131 (2002)
63. Repa J.J., K.E. Berge, C. Pomajzl, J.A. Richardson, H. Hobbs & D.J. Mangelsdorf: Regulation of ATP-binding cassette sterol transporters ABCG5 and ABCG8 by the liver X receptors alpha and beta. *J Biol Chem* 277, 18793-12800 (2002)
64. Edwards P.A., H.R. Kast & A.M. Anisfeld: BAREing it all: the adoption of LXR and FXR and their roles in lipid homeostasis. *J Lipid Res* 43, 2-12 (2002)
65. Peet D.J., S.D. Turley, W. Ma, B.A. Janowski, J.M. Lobbacaro, R.E. Hammer & D.J. Mangelsdorf: Cholesterol and bile acid metabolism are impaired in mice lacking the nuclear oxysterol receptor LXRalpha. *Cell* 93, 963-704 (1998)
66. Schultz J.R., H. Tu, J.J. Repa, J.C. Medina, L. Li, S. Schwendner & S. Wang: Role of LXRs in control of lipogenesis. Genes Dev 14, 2831-2838 (2000)
67. Repa J.J., G. Liang, J. Ou, Y. Bashmakov, J.M. Lobaccaro, I. Shimomura, & B. Shan: Regulation of mouse sterol regulatory element-binding protein-1c gene (SREBP-

1c) by oxysterol receptors, LXRalpha and LXRbeta. *Genes Dev* 14, 2819-2830 (2000)
68. Plosch T., T. Kok, V.W. Blocks, M.J. Smith, R. Havinga, G. Chimini, A.K. Groen & F. Kuipers: Increased hepatobiliary and fecal cholesterol excretion upon activation of the liver X receptor is independent of ABCA1. *J Biol Chem* 277, 33870-33877 (2002)
69. Yu L., J. York, K. von Bergmann, D. Lutjohann, J.C. Cohen, & H. H. Hobbs: Stimulation of cholesterol excretion by the liver X receptor agonist requires ATP-binding cassette transporters G5 and G8. *J Biol Chem* 278, 15565-15570 (2003)
70. Sinal C.J., M. Tohkin, M. Miyata, J.M. Ward, G. Lambert, F.J. Gonzalez: Targeted disruption of the nuclear receptor FXR/BAR impairs bile acid and lipid homeostasis. *Cell* 102, 731-744 (2000)
71. Lambert G., M.J.A. Amar, G. Guo, H.B. Brewer Jr, F.J. Gonzalez & C.J. Sinal: The farnesoid X-receptor is an essential regulator of cholesterol homeostasis. *J Biol Chem* 278, 2563-2570 (2002)
72. Kok T., H. Walters, W.W. Blocks, R. Havinga, P.L.M. Jansen, B. Stales & F. Kuipers: Induction of hepatic ABC transport is part of the PPARalpha-mediated fasting response in the mouse. *Gastroenterology* 124, 160-171 (2003)
73. Post S.M., H. Duez, P.P. Gervois, B. Staels, F. Kuipers & H.M. Princen: Fibrates suppress bile acid synthesis via peroxisome proliferator-activated receptor-alpha-mediated downregulation of cholesterol 7alpha-hydroxylase and sterol 27-hydroxylase expression. *Arterioscler Thromb Vasc Biol* 21, 1840-1845 (2001)
74. Stahlberg D., E. Reihner, M. Rudling, L. Berglund, K. Einarsson & B. Angelin: Influence of bezafibrate on hepatic cholesterol metabolism in gallstone patients: reduced activity of cholesterol 7alpha-hydroxylase. *Hepatology* 21, 1025-1030 (1995)
75. Grundy S.M., E.H. Ahrens Jr, G. Salen, P.H. Schreibman & P.J. Nestel: Mechanisms of action of clofibrate on cholesterol metabolism in patients with hyperlipidemia. *J Lipid Res* 13, 531-551 (1972)
76. Kesaniemi Y.A. & S.M. Grundy: Influence of gemfibrozil and clofibrate on metabolism of cholesterol and plasma triglycerides in man. *JAMA* 251, 2241-2246 (1984)
77. Gilat T., C. Feldman, Z. Halpern, M. Dan & S. Bar-Meir: An increased familial frequency of gallstone disease. *Gastroenterology* 84, 242-246 (1983)
78. Sarin S.K., V.S. Negi, R. Dewan, S. Sasan & A. Saraya: High familial prevalence of gallstones in the first-degree relatives of gallstone patients. *Hepatology* 22, 138-141 (1995)
79. Van der Linden W.: Genetic factors in gallstone disease. *Clin Gastroent* 2, 603-614 (1973)
80. Bertomeau A., E. Ros, E. Zambon, M. Vela, R.M. Pérez-Ayuso, E. Targarona, M. Trías, C. Sanllehy E. Casals & J.M. Ribo: Apolipoprotein E polymorphism and gallstones. *Gastroenterology* 111, 1603-1610 (1996)
81. Juvonen T., M.J. Savolainen, M.I. Kairaluoma, L.H.J. Lajunen, S. E. Humphries & Y.A. Kesäniemi: Polymorphisms at the apoB, apoA-I, and cholesteryl ester transfer protein gene loci in patients with gallbladder disease. *J Lipid Res* 36, 804-812 (1995)
82. Marleen J., L. de Vree, E. Jacquemin, E. Sturm, D. Cresteil, P. J. Bosma, J. Aten, J. Deleuze, M. Desrochers, M. Burdelski, O. Bernard, R. P. J. Oude Elferink & M. Hadchouel: Mutations in the MDR3 gene cause progressive familial intrahepatic cholestasis. *Proc Natl.Acad.Sci USA* 95, 282–287 (1998)
83. Rosmorduc O., B. Hermelin & R. Poupon: MDR3 gene defect in adults with symptomatic intrahepatic and gallbladder cholesterol cholelithiasis. *Gastroenterology* 120,1459-1467 (2001)
84. Jacquemin, E.: Role of multidrug resistance 3 deficiency in pediatric and adult liver disease: one gene for three diseases: *Semin Liver Dis* 21, 551 - 562 (2001)
85. Pullinger C. R., C. Eng, G. Salen, S. Shefer, A. K. Batta, S. K. Erickson, A. Verhagen, C. R. Rivera, S. J. Mulvihill, M. J. Malloy & J. P. Kane: Human cholesterol 7alpha -hydroxylase (CYP7A1) deficiency has a hypercholesterolemic phenotype. *J Clin Invest* 110, 109-117 (2002)
86. Kersten S., B. Desvergne & W. Wahli: Roles of PPARs in health and disease. *Nature* 405, 421– 24 (2000)
87. Bocher V., I. Pineda– Torra, J.C. Fouchart & B. Staels: PPARs: transcription factors controlling lipid and lipoprotein metabolism. *Ann NY Acad Sci* 967, 7–8 (2002)
88. Hansen B.C.: The metabolic Syndrome X. *Ann NY Acad Sci* 892, 1- 4 (1999)
89. Folson A.R.: Insulin resistance and cardiovascular disease. In: Insulin Resistance: The Metabolic Syndrome X. Eds: GM Reaven G.M., Laws A., Humana Press, NJ 333-346 (1999)
90. Stern M.P. & B.D. Mitchell: Genetics of insulin resistance. In: Insulin Resistance: The Metabolic Syndrome X. Eds: GM Reaven G.M., Laws A., Humana Press, NJ 3-18 (1999)
91. Pagano G., G. Pacini, G. Musso, R. Gambino, F. Mecca, Nadia Depetris, M. Cassader, E. David, P. Cavallo-Perin & M. Rizzetto: Nonalcoholic steatohepatitis, insulin resistance, and metabolic syndrome: Further evidence for an etiologic association. *Hepatology* 35:367-372 (2002)
92. Marchesini G. & G. Forlani: NASH: From liver diseases to metabolic disorders and back to clinical hepatology. *Hepatology* 35: 497-499 (2002)
93. Ruhl C. & J. Everhart: Association of diabetes, serum insulin, and C-peptide with gallbladder disease. *Hepatology* 31, 299-303 (2000)
94. Diehl A.K.: Cholelithiasis and the insulin resistance syndrome. *Hepatology* 31, 528– 29 (2000)
95. de Leon M.P., R. Ferenderes & N. Carulli: Bile lipid composition and bile acid pool size in diabetes. *Am J Dig Dis* 23, 710-716 (1978)
96. Kajiyama G., K. Oyamada, S. Nakao & A. Miyoshi: The effect of diabetes mellitus and its treatment on the lithogenicity of bile in man. *Hiroshima J Med Sci* 30:221-227 (1981)
97. Bennion L.J. & S.M. Grundy: Effects of diabetes mellitus on cholesterol metabolism in man. *N Engl J Med* 296, 1365-1371 (1977)
98. Haber G.B. & K.W. Heaton: Lipid composition of bile in diabetics and obesity-matched controls. *Gut* 20, 518-522 (1979)
99. Bennion L.J. & S. M. Grundy: Effects of obesity and caloric intake on biliary lipid metabolism in man. *J Clin Invest* 56, 996–1011 (1975)

100. Shaffer E.A. & D. Small: Biliary lipid secretion in cholesterol gallstone disease. The effect of cholecystectomy and obesity. *J Clin Invest* 58, 828-840 (1977)
101. Reuben A., P.N. Maton, G. Murphy & H. Dowling: Bile lipid secretion in obese and non-obese individuals with and without gallstones. *Clin Sc* 69, 71– 79 (1985)
102. Nepokroeff C.M., M.R. Lakshmanan, G.C. Ness, R.E. Dugan & J.W. Porter: Regulation of the diurnal rhytm of rat liver beta-hydroxy-beta-methylglutaryl coenzyme A reductase activity by insulin, glucagons, cyclic AMP and hydrocortisone. *Arch Biochem Biophys* 160, 387–396 (1974)
103. Nervi F., C. Severin & V. Valdivieso: Bile acid pool changes and regulation of cholate synthesis in experimental diabetes. *Biochim Biophys Acta* 529, 212-223 (1978)
104. Chait A., E.L. Bierman & J.J. Albers: Low-density lipoprotein receptor activity in cultured human skin fibroblasts. Mechanism of insulin-induced stimulation. *J Clin Invest* 64, 1309-1319 (1979)
105. VanPatten S., N. Ranginani, S. Shefer, L.B. Nguyen, L. Rossetti & D.E. Cohen: Impaired biliary lipid secretion in obese Zucker rats: leptin promotes hepatic cholesterol clearance. *Am J Physiol Gastrointest Liver Physiol* 281, G393-404 (2001)
106. Hyogo H., S. Tazuma & D. E. Cohen: Cholesterol gallstones. *Curr Opin Gastroenterol* 18, 336–371 (2002)
107. Zammit V.A.: Insulin stimulation of hepatic triacylglycerol secretion in the insulin – replete state. Implications for the etiology of peripheral insulin resistance. *Ann NY Acad Sci* 967, 52– 65 (2002)

Key Words: Gall bladder, Stone, Cholesterol Gallstones, Pathogenesis, Biliary Lipids, Hepatic Cholesterol, Metabolism, ABC Canalicular Transporters, Review

Send correspondence to: Flavio Nervi, M.D., Pontificia Universidad Católica de Chile, Departamento de Gastroenterología, 367 Marcoleta, Santiago, Casilla 114 – D, Chile, Tel: 562-686-3822, Fax: 562-639-7780, E-mail: fnervi@med.puc.cl