ANNALS of Hepatology

CONCISE REVIEW

November-December, Vol. 13 No. 6, 2014: 728-745

Cholesterol cholelithiasis in pregnant women: pathogenesis, prevention and treatment

Ornella de Bari,* Tony Y. Wang,*,** Min Liu,*** Chang-Nyol Paik,* Piero Portincasa,**** David Q.-H. Wang*

* Department of Internal Medicine, Division of Gastroenterology and Hepatology, Saint Louis University School of Medicine, St. Louis, USA.
** Department of Biomedical Engineering, Washington University, St. Louis, USA.
*** Department of Pathology and Laboratory Medicine, University of Cincinnati College of Medicine, Cincinnati, USA.
**** Clinica Medica "A. Murri", Department of Biomedical Sciences and Human Oncology, University of Bari Medical School, Bari, Italy.

ABSTRACT

Epidemiological and clinical studies have found that gallstone prevalence is twice as high in women as in men at all ages in every population studied. Hormonal changes occurring during pregnancy put women at higher risk. The incidence rates of biliary sludge (a precursor to gallstones) and gallstones are up to 30 and 12%, respectively, during pregnancy and postpartum, and 1-3% of pregnant women undergo cholecystectomy due to clinical symptoms or complications within the first year postpartum. Increased estrogen levels during pregnancy induce significant metabolic changes in the hepatobiliary system, including the formation of cholesterol-supersaturated bile and sluggish gallbladder motility, two factors enhancing cholelithogenesis. The therapeutic approaches are conservative during pregnancy because of the controversial frequency of biliary disorders. In the majority of pregnant women, biliary sludge and gallstones tend to dissolve spontaneously after parturition. In some situations, however, the conditions persist and require costly therapeutic interventions. When necessary, invasive procedures such as laparoscopic cholecystectomy are relatively well tolerated, preferably during the second trimester of pregnancy or postpartum. Although laparoscopic operation is recommended for its safety, the use of drugs such as ursodeoxycholic acid (UDCA) and the novel lipid-lowering compound, ezetimibe would also be considered. In this paper, we systematically review the incidence and natural history of pregnancy-related biliary sludge and gallstone formation and carefully discuss the molecular mechanisms underlying the lithogenic effect of estrogen on gallstone formation during pregnancy. We also summarize recent progress in the necessary strategies recommended for the prevention and the treatment of gallstones in pregnant women.

Key words. Bile acids. Biliary lipids. Biliary sludge. Estrogen. Ezetimibe. Gallstones. Progesterone.

INTRODUCTION

Epidemiological and clinical studies showed that at all ages, women are twice as likely as men to form cholesterol gallstones. This gender difference begins since puberty and continues through the childbearing years. Most, but not all, studies have found that the risk of developing cholesterol gallstones is markedly increased by oral contraceptive steroids and conjugated estrogens in premenopausal women.[1-7] Estrogen therapy to postmenopausal women and to men with prostatic carcinoma also induces similar lithogenic effects.[8-13] These findings underscore the importance of female sex hormones on the pathogenesis of gallstones. Furthermore, hormonal changes that occur during pregnancy put women at even higher risk for gallstone formation.[14-17] Biliary cholesterol concentrations in gallbladder bile increase gradually from the first to the third trimester of pregnancy, along with a progressive increase in the incidence of biliary sludge (a precursor to gallstones) and gallstones.[18-20] Clinical studies in the USA and Europe have found that the incidence rates of biliary sludge and gallstones are up to 30 and 12%, respectively, during pregnancy and postpartum.[20-28] Although most women remain asymptomatic, 1-3% of pregnant women undergo cholecystectomy due to clinical symptoms or complications within the first year postpartum.[20-28] Because more than 4 million women give birth

Correspondence and reprint request: David Q.-H. Wang, M.D., Ph.D.
Department of Internal Medicine, Division of Gastroenterology and Hepatology, Saint Louis University School of Medicine, St. Louis, MO 63104, USA.
Ph.: (314) 977-8737, Fax: (314) 977-9909
E-mail: dwang15@slu.edu

Manuscript received: March 30, 2014.
Manuscript accepted: July 22, 2014.

annually in the USA, it is estimated that at least 40,000 young healthy women require postpartum cholecystectomy each year. Thus, pregnancy-associated gallbladder disease is a significant cause of morbidity in young healthy women.

Parity and length of the fertility period increase the incidence of gallstones,[29] as well as both the frequency and number of pregnancy are important risk factors for gallstone formation.[14,16,30-34] Of note, gallbladder disease is the most common non-obstetrical cause of maternal hospitalization in the first year postpartum,[35,36] with 30% attacks of biliary colic in women with gallstones.[23] Following acute appendicitis, acute cholecystitis is the second most common indication for non-obstetric surgery in pregnant women.[37] Again, it indicates that gallbladder disease is a significant cause of morbidity for pregnant women.[27] Although pregnancy constitutes a defined period of metabolic stress, a subclinical trend to form gallstones clearly exists.[27] Pregnant women with gestational diabetes or high blood pressure are at risk of later developing diabetes mellitus or hypertension.[38,39] A similar phenomenon could happen with biliary sludge and gallstones. Thus, elevated estrogen levels are a critical risk factor for developing gallbladder disease during pregnancy. Moreover, biliary sludge and gallstones can spontaneously disappear after parturition in approximately 60% of cases mostly due to a sharp decrement in estrogen levels.[20] It is important in clinical practice, therefore, to find a way to reduce the risk of cholelithiasis in pregnant women.

Human and animal studies have found that estrogen increases susceptibility to cholesterol cholelithiasis by promoting hepatic secretion of biliary cholesterol,[18,19,40-47] enhancing bile lithogenicity and impairing gallbladder motility function.[10,48,49] Such alterations, in turn, lead to a dramatic increase in cholesterol saturation of bile and gallbladder stasis, thereby enhancing cholelithogenesis.[1,50-52] We have found that the hepatic estrogen receptor α (ERα), but not ERβ, plays a major role in cholesterol gallstone formation in mice exposed to high doses of 17β-estradiol.[53] A novel concept has been proposed that high levels of estrogen promote the formation of cholesterol gallstones through the ERα signaling cascade in the liver and higher risks for gallstone formation in women than in men are related to differences in how the liver handles cholesterol in response to estrogen.[54]

In this paper, we systematically review the incidence and natural history of pregnancy-related biliary sludge and gallstones and carefully discuss the molecular mechanisms underlying the lithogenic effect of estrogen on gallstone formation during pregnancy. We also summarize recent advances in the prevention and the treatment of cholesterol gallstones in pregnant women.

CHANGES IN PLASMA ESTROGEN LEVELS DURING THE MENSTRUAL CYCLE AND DURING PREGNANCY

The menstrual cycle is essential for the production of eggs and for the preparation of the uterus for pregnancy.[55] It consists of:

- The ovarian cycle that describes changes in the follicles of the ovary and
- The uterine cycle that outlines changes in the endometrial lining of the uterus.

Both the ovarian and the uterine cycles are divided into three phases. The ovarian cycle is composed of:

- Follicular phase,
- Ovulation, and
- Luteal phase.

Whereas the uterine cycle is divided into:

- Menstruation,
- Proliferative phase, and
- Secretory phase.[56]

The stages of the menstrual cycle are characterized by fluctuations of the four major circulating hormones: follicle-stimulating hormone (FSH), luteinizing hormone (LH), estradiol and progesterone.[57,58] Although plasma concentrations of these hormones vary substantially on an hourly basis, their daily profiles provide characteristic changes during the menstrual cycle.[57] The follicular phase is defined by a preovulatory estrogen peak in the absence of progesterone.[8]

During the luteal phase, following ovulation, a simultaneous increase of both estradiol and progesterone occurs.[56,58-60]

Plasma concentrations of unconjugated estrone (E_1), estradiol (E_2) and estriol (E_3) increase steadily as pregnancy progresses.[3,4,19] Estradiol levels in plasma are the highest, followed by estriol and estrone. Only during pregnancy, fetal liver produces some amounts of estetrol (E_4); however, its plasma concentration is still very low in pregnant women.[4,20] In pregnant women, plasma estradiol concen-

trations range from 6 to 30 ng/mL.[3,29] At 9 weeks of pregnancy, i.e., 23% of gestational length, the placenta becomes the primary source of estrogen.[61] In the late stage of pregnancy, plasma concentrations of estradiol and estrone are approximately 100-fold higher than the respective average values in the menstrual cycle.[4,23] These alterations greatly enhance susceptibility to cholesterol gallstones in pregnant women.

GALLSTONE PREVALENCE IN PREGNANT WOMEN

During pregnancy, elevated estrogen levels are often associated with a significant increase in hepatic secretion of biliary cholesterol. As a result, bile becomes supersaturated with cholesterol and is more lithogenic. Additionally, high levels of estrogen and progesterone could impair gallbladder motility function by inhibiting gallbladder smooth muscle contractile function, thus leading to gallbladder stasis.[10,49] Such abnormalities greatly promote the formation of biliary sludge and gallstones in pregnant women. The incidence of disease appears to be increased in the last two trimesters of pregnancy. However, approximately one third of pregnant women with gallstones is asymptomatic.[21,23,24] When symptoms do occur in pregnant women, however, the most common clinical presentations are biliary colic, acute cholecystitis, gallstone pancreatitis, and jaundice.[62]

As listed in table 1, the incidence rates of gallstones range from 1.2 to 6.3% during pregnancy.[21-25] Since the concentration of plasma female sex hormones increases proportionally with duration of gestation, the risk of gallstone formation is especially hazardous in the third trimester of pregnancy. Furthermore, increasing parity could be a significant risk factor for gallstones, especially in younger women.[61,63,64] Clinical studies have observed that approximately 5.1% of women developed gallbladder disease after one pregnancy, 7.6% after two pregnancies, and 12.3% after 3 or more pregnancies.[14,27,30-32] A study in Chile reported that the incidence of gallstones was 12.2% of multiparous women compared to 1.3% of nulliparous women within the same age.[65] Another study has reported that women under the age of 25 years with ≥ 4 pregnancies were 4 to 12 times more likely to develop cholesterol gallstones compared to nulliparous women of the same age and body weight.[66]

It has been shown that the prevalence of gallstones in women who had two to three or more pregnancies was 2- to 3-fold higher compared with nulliparous women.[67] In the Italian GREPCO study, the prevalence of gallstones positively correlated with the number of pregnancies and age.[68] The frequency appears to increase after two, three or more pregnancies, and this trend was more marked in younger women at the ages of 25 to 30 years *vs.* 35 to 40 years.[61,64] Several population studies attempted to quantify the risk of gallstones caused by multiple pregnancies, adjusting for at least some known confounding factors. However, the number of multiple pregnancies and the adjustment for confounding factors were not uniform. The results from the Sirmi-

Table 1. Gallstones formation during pregnancy and after delivery.

Authors	Subjects (n)	Incidence of gallstones				
		Nulliparous	First trimester	Second trimester	Third trimester	Postpartum (weeks)
Maringhini, *et al.* (1993)[20]	272	-	6.0	2.0[a]	-	2.4[b] (2-4)
Bolukbas, *et al.* (2006)[21]	97	-	6.3	-	-	-
Maringhini, *et al.* (1987)[22]	298	-	-	-	-	5.2 (1)
Valdivieso, *et al.* (1993)[23]	980	1.3	-	-	-	12.2 (1)
Basso, *et al.* (1992)[24]	521	-	-	2.7	-	-
Stauffer, *et al.* (1982)[25]	313	-	-	3.5[c]	-	-
Tsimoyiannis, et a. (1994)[61]	669	-	-	1.2	1.9	2.1 (24)
Ko, *et al.* (2005)[27]	3,254	-	-	1.9	1.8[d]	2.8[e] (4-6)
Glasinovic, *et al.* (1989)[28]	259	-	3.1	-	-	11.2

[a] The value shows the incidence of gallstones that were found after the first trimester. [b] The value represents the incidence of gallstones that were detected at 2 to 4 weeks after delivery from the first trimester. [c] The value indicates the incidence of gallstones that were observed during gestation. [d] The value shows the cumulative incidence of gallstones by the third trimester. [e] The values indicates the incidence of new gallstones that were detected during the 4-6 weeks of the first post-partum examination after childbirth.

one study,[14] the Framingham study[16] and the MICOL study[69] were surprisingly similar, and the relative risks were 1.7, 1.6 and 1.7, respectively. These and other studies provided definitive evidence that multiple pregnancies are a critical risk factor for the development of gallstones.

Biliary sludge in pregnancy is attributed to two major mechanisms: estrogen-induced metabolic abnormalities in the formation of lithogenic bile and progesterone-induced smooth muscle relaxation with subsequent gallbladder hypomotility.[51,52,70-72] As shown in tables 1 and 2, the frequency of biliary sludge has been observed to be higher than that of gallstones (~15.0% *vs.* ~6.0%) during pregnancy.[23,73] Although not all cases of biliary sludge evolve to gallstones, the presence of sludge is a necessary precursor involved in the formation of gallstones.[27,51,52,74] Clinical studies have reported that biliary sludge can spontaneously disappear and recur over time, or it can proceed to gallstones. Biliary sludge is mostly asymptomatic and disappears when the primary condition resolves; however, in some cases it can progress into gallstones or migrate to the biliary tract, occluding the cystic duct or the common bile duct and causing biliary colic, cholangitis or pancreatitis. It should be noted that biliary sludge in the majority of women usually disappears a few weeks after the pregnancy concludes/terminates/ends.[66] However, only a fraction of pregnant women with sludge develop gallstones.[72] Gallstones and biliary sludge can spontaneously resolve in most women during the first year after delivery. However, it is considered that women with multiple or closely spaced pregnancies may form gallstones as sludge recurs or persists.[75]

Based on ultrasonographic assessment of the gallbladder in 298 women in the immediate postpartum period, the incidence of biliary sludge was found to be 26.2% and the incidence of gallstones was 5.2%. After 1 year of follow-up, only 2 out of 45 patients with biliary sludge, but 13 out of 15 patients with gallstones still had abnormal ultrasonographic findings.[22] In another report, the prevalence of biliary sludge in pregnant women ranged from 5 to 36% and the prevalence of gallstones from 2 to 11%.[63] A prospective study of 272 women recruited in the first trimester of pregnancy and followed for almost one year postpartum found that new biliary sludge developed in 67 women and gallstones in 6 women in pregnancy. Whereas, women with biliary sludge remained generally asymptomatic, 28% of women with gallstones developed biliary pain during pregnancy. About half of the study population (115 women) was reexamined in the postpartum period. Of these, 92 had biliary sludge and 23 had gallstones. Biliary sludge disappeared in 56 affected women (61%) and gallstones were dissolved in 6 women (28%) by several months post-partum.[20]

It has been observed that over a 7- to 8-month period from the first trimester to the early postpartum, the cumulative incidence of biliary sludge was 5%, whereas an additional 5% developed incident gallstones or progressed from baseline sludge to gallstones. Overall, 4.2% of women had newly formed biliary sludge or gallstones that were found by ultrasonography in the early postpartum.[27] Several groups performed abdominal ultrasound examinations in women in the immediate postpartum period comparing the same examinations performed in the same women at the beginning of pregnancy or in age matched nulliparous women. In two large Chilean studies, approximately 12.2% of women were found to have gallstones in the postpartum period compared with 3.1% in the same women at the beginning of pregnancy and 1.3% in nulliparous women.[23,72]

When gallbladder motility is restored in the postpartum period, biliary sludge and gallstones could

Table 2. Biliary sludge formation during pregnancy and after delivery.

Authors	Subjects (n)	Incidence of biliary sludge				
		Nulliparous	First trimester	Second trimester	Third trimester	Postpartum (weeks)
Maringhini, *et al.* (1993)[20]	272	-	15.0	14.0[a]	-	29.6[b] (2-4)
Bolukbas, *et al.* (2006)[21]	97	-	10.9	-	-	
Maringhini, *et al.* (1987)[22]	298	-	-	-	-	26.2 (1)
Ko, *et al.* (2005)[27]	3,254	-	-	3.2	4.5[c]	5.1[d] (4-6)

[a] The value shows the incidence of biliary sludge that was found after the first trimester. [b] The value represents the incidence of biliary sludge that were detected at 2 to 4 weeks after delivery from the first trimester. [c] The value shows the cumulative incidence of biliary sludge by the third trimester. [d] The values indicates the incidence of new formed biliary sludge that was detected during the 4-6 weeks of the first postpartum examination after childbirth.

pass into the cystic duct or the common bile duct, and this is a condition at risk of biliary colic or other complications such as obstructive jaundice or pancreatitis. Alternatively, because of a sharp reduction in estrogen concentrations after childbirth, postpartum changes in lipid composition of bile could favor dissolution of biliary sludge and gallstones. Maringhini, *et al.* reported that both biliary sludge and gallstones disappeared in 61 and 28%, respectively, during the first year after delivery.[20] This suggests that pregnancy-associated biliary lithogenicity and gallbladder hypomotility may be a crucial factor in the formation of biliary sludge and gallstones during gestation.[51]

Ko, *et al.* evaluated the incidence and natural history of pregnancy-related biliary sludge and gallstones and reported an incidence of biliary sludge or gallstones of 5.1% by the second trimester, 7.9% by the third trimester, and 10.2% by 4 to 6 weeks postpartum.[27] Twenty-eight women (0.8%) underwent cholecystectomy within the first year postpartum.[27]

Biliary diseases at delivery or hospitalization within 1 year postpartum were documented in approximately 0.5% of all births in Washington State between 1987 and 2001, with cholecystectomy performed within 1 year postpartum for approximately 0.4% of births during this time period. A population-based study found that 2.1% of women aged 20-29 year and 4.9% of women aged 30-39 year have undergone cholecystectomy.[30,35] Because many of these women would have undergone cholecystectomy in the first postpartum year, these findings suggest that the postpartum period is a time of high risk for symptomatic gallstone disease requiring further treatment. Notably, hospitalizations resulting from gallstone-related disease in the first year postpartum period incur median charges of $5,759 in the mid-1990s, with a median length of stay of 3 days.[35]

MOLECULAR MECHANISMS UNDERLYING THE LITHOGENIC EFFECT OF ESTROGEN ON THE FORMATION OF CHOLESTEROL GALLSTONES IN PREGNANT WOMEN

Pregnancy and parity are two important risk factors for the formation of cholesterol gallstones in women.[23,29,31,62,76] As shown in figure 1, increased levels of female sex hormones during pregnancy induce a variety of metabolic changes in the hepatobiliary system, which ultimately cause bile to become supersaturated with cholesterol and enhance cholelithogenesis.[10] Moreover, biliary lithogenicity occurs as a result of estrogen-induced increase in cholesterol biosynthesis, providing excess amounts of cholesterol for biliary hypersecretion during pregnancy.[10] So, cholesterol saturation index of bile increases especially during the second and third trimesters of pregnancy. The secretion rate of cholesterol relative to that of bile acids and phospholipids is increased markedly.[77] Moreover, hepatic secretion of biliary cholesterol has been found to be increased by 40% in nonpregnant women treated with estrogen.[8] In addition, the gallbladder becomes sluggish and enlarged and empties incompletely, which further increases the risk of gallstone formation.[77]

Estrogen could promote cholesterol cholelithogenesis by enhancing functions of estrogen receptors in the liver and gallbladder.[54] ERα and ERβ are both expressed in the hepatocytes although they have overlapping but not identical tissue expression patterns.[53,78-80] However, gene expression levels of ERα are ~50-fold higher compared with ERβ. These findings imply that ERα is a major steroid hormone receptor producing the biological effects of estrogen in the liver.[10] We investigated the molecular mechanisms of how estrogen influences cholesterol gallstones formation in a mouse model of gonadectomized gallstone-resistant AKR mice.[53] Expression levels of ERα were significantly increased after its activation by 17β-estradiol (E_2), the ERα-selective agonist propylpyrazole (PPT), and tamoxifen. However, the E_2 effects on expression of the hepatic ERα mRNA was blocked by the antiestrogenic ICI 182,780. The ERβ-selective agonist diarylpropionitrile (DPN) did not influence gene expression of ERα, but up-regulates the expression of ERβ in the liver.[53] Although the prevalence of gallstones displayed a dose-dependent increase in gonadectomized mice treated with various doses of exogenous E_2, all mice treated with E_2 at 6 μg/day plus ICI 182,780 were gallstones free. These findings indicate that the lithogenic effects of E_2 are blocked by this antiestrogenic agent. Moreover, 75% of the mice treated with PPT formed gallstones, suggesting its strong lithogenic effect. Tamoxifen promoted gallstone formation with a prevalence rate of 50%, indicating its estrogen-like effects on biliary lipid metabolism. By contrast, DPN did not influence gallstone formation. Thus, the receptor-dependent effects of E_2 contribute to biliary cholesterol hypersecretion and lithogenicity of bile, promoting gallstone formation through the hepatic ERα, but not ERβ pathway.

One mechanism of estrogen-mediated hepatic secretion of biliary cholesterol is that the activity of 3-

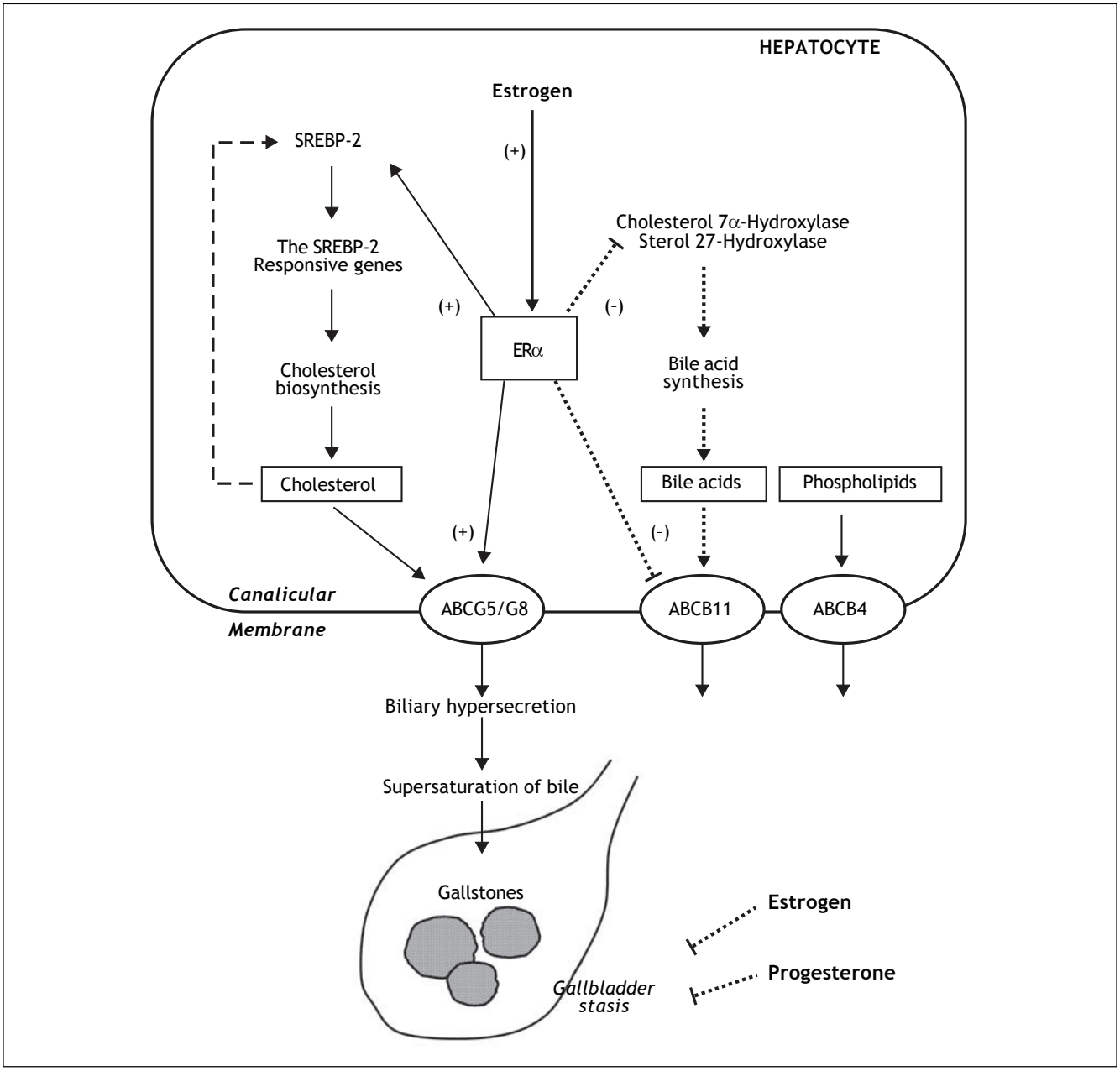

***Figure 1**. The proposed working model underlying the potential lithogenic mechanisms of estrogen through the estrogen receptor a (ERα) pathway in the liver during pregnancy. In the liver, there is an "estrogen-ERα-SREBP-2" pathway promoting cholesterol biosynthesis and hepatic hypersecretion of biliary cholesterol in response to estrogen. The negative feedback regulation of cholesterol biosynthesis (as shown in a dashed line) is inhibited by ERα that is activated by estrogen, mostly through stimulating the activity of sterol regulatory element-binding protein-2 (SREBP-2) with the resulting activation of the SREBP-2 responsive genes for the cholesterol biosynthetic pathway. Consequently, these alterations induce excess secretion of newly synthesized cholesterol and supersaturation of bile that predisposes to cholesterol precipitation and gallstone formation. By contrast, estrogen could reduce bile acid biosynthesis by inhibiting cholesterol 7α-hydroxylase in the classical pathway and sterol 27-hydroxylase in the alternative pathway through the ERα signaling cascade (as shown in a dotted line). Moreover, the hepatic ERα activated by estrogen could stimulate the activity of ATP-binding cassette (ABC) transporters ABCG5/G8 on the canalicular membrane of the hepatocyte and promote biliary cholesterol hypersecretion. It is likely that expression levels of ABCB11 are inhibited by estrogen through the ERα pathway, inducing biliary bile acid hyposecretion (as shown in a dotted line). In addition, high levels of estrogen and progesterone induce smooth muscle relaxation with subsequent impaired gallbladder motility function, leading to gallbladder stasis (as shown in dotted lines). All of these alterations promote the formation of biliary sludge and gallstones in pregnant women exposed to high levels of estrogen.[10]*

hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, the rate-limiting enzyme in hepatic cholesterol biosynthesis, is enhanced, followed by increased delivery of cholesterol from hepatic *de novo* synthesis to bile.[1,53,81-83] Estrogen could increase the capacity of dietary cholesterol to induce cholesterol supersaturation of bile.[1,53,81,82,84] To elucidate the molecular mechanism by which E2 increases hepatic output of biliary cholesterol, we quantitated the contribution of newly synthesized cholesterol to biliary output in gonadectomized AKR mouse treated with high doses of estrogen and fed a high-cholesterol diet.[83] Compared with control mice (i.e., female AKR mice with intact ovaries), E_2-treated mice with gonadectomy displayed a significantly higher hepatic output of biliary total and newly synthesized cholesterol, regardless of whether chow or the high-cholesterol diet was fed. These biological effects of E_2 were abolished by ICI 182,780. Thus, the origin of biliary cholesterol possibly comes mostly from the high cholesterol diet and partly from lipoproteins such as HDL carrying cholesterol from extrahepatic tissues via a reverse cholesterol transport pathway. To regulate the hepatic cholesterol biosynthesis, E_2 could activate ERα which, in turn stimulates mRNA expression of sterol regulatory element binding proteins-2 (SREBP-2) and five major SREBP-2-responsive genes (HMG-CoA synthase, HMG-CoA reductase, farnesyl diphosphate synthase, squalene synthase, and lathosterol synthase) in the liver.[83] Compared to the chow diet in control mice, expression levels of SREBP-2 were significantly reduced by the high dietary cholesterol. These findings suggest that cholesterol biosynthesis may be inhibited by a negative feedback regulation through the SREBP-2 pathway in response to high dietary cholesterol. By contrast, E_2-treated mice still showed significantly higher expression levels of SREBP-2 and the SREBP-2-responsive genes, even under high dietary cholesterol loads. These results indicate that with high levels of E_2, there is a continuous cholesterol synthesis in the liver because the negative feedback regulation of its synthesis by the SREBP-2 pathway may be inhibited by E_2 through the hepatic ERα. Likely, under the normal physiological conditions, there is a negative feedback regulation of cholesterol biosynthesis by cholesterol. With increased E_2 levels, however, there is a possible "estrogen-ERα-SREBP-2" pathway for the regulation of hepatic cholesterol biosynthesis. This suggests a loss in the negative feedback regulation of cholesterol biosynthesis, which, in turn, results in hepatic secretion of excess amounts of newly synthesized cholesterol and supersaturation of bile that predisposes to the precipitation of solid cholesterol crystals and the formation of gallstones.

It is well known that progesterone is a potent inhibitor of hepatic acyl-coenzyme A:cholesterol acyltransferase (ACAT), thereby decreasing hepatic synthesis of cholesteryl esters and presumably allowing more free cholesterol to enter an intrahepatic pool for biliary secretion.[85]

Pregnancy also induces changes in bile acid synthesis characterized by a decreased proportion of chenodeoxycholic acid (CDCA), thereby reducing the ability to solubilize cholesterol and favoring the precipitation of solid cholesterol crystals.[77] These findings could be explained by changes in function of the pumps of the enterohepatic circulation due to gallbladder stasis induced by impaired motility function by estrogen and progesterone. Female sex hormones in pregnancy may directly regulate hepatic synthesis of individual bile acids. This interpretation is based on the studies of individual hormones. Progestins, when administrated in the absence of estrogen, exert little or no effect on bile acid composition or bile acid kinetics.[50,86] By contrast, estrogen decreases the synthesis of CDCA but have little or no effect on cholic acid (CA) synthesis.[1] These results imply the presence of an alteration in coupling of biliary lipids, consistent with the secretion of more lithogenic bile. In addition, alteration of bile acid pool may influence the physical-chemical properties of the bile acid pool and alter cholesterol absorption, cholesterol synthesis and bile acid synthesis.[87-90] The cholesterol saturation index of fasting hepatic and gallbladder bile is increased during the second and the third trimesters of pregnancy.[77] Hepatic hypersecretion of cholesterol and hydrophobic bile acids also favor cholesterol crystallization.[91,92]

After the first trimester of pregnancy, real-time ultrasonography shows that fasting gallbladder volume and postprandial residual volume are twice as large as in control healthy subjects, a condition pointing to gallbladder stasis. In early pregnancy, there is a 30% decrease in emptying rate, and in late pregnancy, incomplete gallbladder emptying leads to a large residual volume that causes biliary sludge and the retention of solid cholesterol crystals.[52] Such abnormal motility patterns of the gallbladder also contribute to the increased risk of cholesterol gallstones. In addition, the lithogenic effects of supersaturated bile induced mainly by estrogen and dysfunctional gallbladder motility induced mainly by progesterone during pregnancy are hallmarked by the early appearance of biliary sludge, implying

trapped cholesterol crystals and microlithiasis within the gallbladder mucin gel (Figure 1).[93] Progesterone also causes a sluggish enterohepatic circulation of bile acids with secondary hyposecretion.[77] In addition, gallbladder stasis progresses during the first 20 weeks of pregnancy and an increase in fasting gallbladder and postprandial residual volumes is directly related to duration of gestation and circulating progesterone concentrations. Gallbladder volumes rapidly return to control values after delivery.[8] Lastly, studies of muscle strips isolated from the gallbladders of a variety of species found that progesterone inhibits smooth muscle contraction.[94-96] Increased levels of progesterone block the G-protein function in the gallbladder smooth muscle cells. In pregnancy, this effect is responsible for signal-transduction decoupling and impaired gallbladder contractility to cholecystokinin.[97] Inhibition of gallbladder contraction could be due to the specific effect of progesterone on the availability of calcium for excitation-coupled contraction.

Although a critical role for estrogen in enhancing cholelithogenesis by activating classical ERα, but not ERβ in the liver has been established, the mechanisms mediating estrogen's lithogenic actions on gallstone formation have become more complicated with the identification of a novel estrogen receptor, the G protein-coupled receptor 30 (GPR30). Furthermore, *GPR30* has been mapped to mouse chromosome 5 and is co-localized with a new gallstone gene *Lith*.[18] However, identifying the lithogenic mechanisms of *GPR30* has been a focal point of interest because it remains unknown whether *GPR30* plays a major role in estrogen-induced gallstones and whether it acts independently of or in conjunction with ERα on inducing gallstone formation. Obviously, more studies are needed to distinguish the lithogenic actions of *GPR30* from those of ERα in the future.

EFFECT OF OTHER RISK FACTORS ON THE FORMATION OF CHOLESTEROL GALLSTONES IN PREGNANT WOMEN

During the period of a normal, healthy pregnancy, besides elevated levels of estrogen, the body undergoes substantial hormonal, immunological, and metabolic changes.[98-101] Some of these alterations could become risk factors contributing to the formation of cholesterol gallstones in pregnant women, which include a high-cholesterol and high-fat diet, weight gain, and insulin resistance, as well as altered gut microbiota and immune function.[102-108] Because many excellent review articles have summarized these topics in detail, we will briefly discuss it here. Obviously, to keep the fetus growing, pregnant women usually consume large amounts of nutrient food containing high calorie and high dietary fats, cholesterol, carbohydrates and proteins, thereby leading to a marked increase in body fat and a reduction in insulin sensitivity. Of special note is that in contrast to the obese state where they are detrimental to long-term health, excess adiposity and loss of insulin sensitivity are beneficial in the context of a normal pregnancy, as they support growth of the fetus and prepare the body for the energetic demands of lactation.[109-111] Although reduced insulin sensitivity in pregnancy is still unknown, it has been correlated with changes in immune status in pregnancy, including elevated levels of circulating cytokines (e.g., TNFα and IL-6) that are thought to drive obesity-associated metabolic inflammation.[112,113] In pregnancy, immunological changes occur at the placental interface to inhibit rejection of the fetus, while at the mother's mucosal surfaces, elevated inflammatory responses often result in exacerbated bacterially mediated infectious diseases. These may increase a risk for biliary infection, gallbladder sludge, and cholesterol crystallization. Furthermore, bacterial load in the intestine is reported to increase over the course of gestation. Although these alterations may be beneficial in pregnancy, they could further enhance adiposity and worsen insulin insensitivity.[114-118] Insulin resistance is a risk factor for incident gallbladder sludge and stones during pregnancy, even after adjustment for body mass index.[106,119-121] It could promote hepatic cholesterol secretion, increase degrees of biliary cholesterol saturation, and impair cholecystokinin-stimulated gallbladder motility. Moreover, a recent large-scale human study has found that gut microbiota dysbiosis and bacterial community assembly are associated with the formation of cholesterol gallstones.[122] Gut microbiota may have a role in the regulation of bile acid metabolism by reducing bile acid pool size and composition.[123] Furthermore, human-associated microbial communities are linked with a variety of diseases, e.g., obesity, diabetes, and nonalcoholic fatty liver disease,[124,125] all of which are risk factors for gallstone formation. It is very likely that bacteria colonized in the biliary tract may work as a nucleus for cholesterol crystallization. Therefore, changes in gut microbiota may be involved in gallstone formation, especially in pregnant women.[126-129]

DIAGNOSIS OF BILIARY SLUDGE AND GALLSTONES IN PREGNANT WOMEN

While pregnant women display a high incidence of biliary sludge, many of them are asymptomatic throughout the pregnancy and postpartum period.[23,24,27] Because biliary sludge is often diagnosed incidentally by antenatal ultrasonography conducted for other reasons, regularly monitoring the development of gallstones in asymptomatic patients is worthwhile be performed, especially in high-risk women with parity. If biliary sludge is suspected in a pregnant woman, transabdominal ultrasonography is the first diagnostic test.[130] Real-time ultrasonography can identify gallstones as small as 2 mm, with sensitivity > 95%. This technique is rapid, noninvasive and relatively low cost, as well as can be performed at the bedside and does not involve ionizing radiations. As shown in figure 2, biliary sludge is characterized by low amplitude, gravity-dependent sonographic echoes seen in the gallbladder, which does not cast an acoustic shadow.[72,131] By contrast, ultrasound image can show a gallstone(s) in the gallbladder with typical acoustic shadow. As seen by polarizing light microscopy, biliary sludge consists mainly of plate-like cholesterol monohydrate crystals and calcium bilirubinate granules embedded in strands of mucin gel.[75] However, because the sensitivity of ultrasonography for biliary sludge alone is only about 60%, further imaging testing and bile examination should be considered if the result of ultrasonography is negative and clinical suspicion remains high, for example, in a pregnant woman with recurring attacks of biliary colic.[75]

For a pregnant woman with suspected biliary sludge and/or gallstones who have a negative result from transabdominal ultrasonography, further diagnosing depends on the clinical symptoms, i.e., the presence or the absence of biliary colic. It is recommended examining gallbladder bile samples by polarizing light microscopy for pregnant women in whom the clinical suspicion of biliary sludge is high and in whom further treatment such as laparoscopic or elective cholecystectomy would be considered.[132-134] If an upper gastrointestinal endoscopy is required, gallbladder bile could be harvested during this procedure and analyzed by polarizing light microscopy. If an upper gastrointestinal endoscopy is not required, a gallbladder bile sample could be obtained through duodenal intubation.[75] Pregnant women with recurrent episodes of idiopathic acute pancreatitis generally undergo endoscopic retrograde cholangiopancreatography (ERCP) (with shielding of the abdomen), and gallbladder bile can be collected from the duodenum or the common bile duct during this procedure.[72,135,136] Pregnant women who have an indication for endoscopic ultrasonography, such as evaluation of abnormalities seen on previous imaging studies, should undergo this procedure first. Endoscopic ultrasonography is not recommended for pregnant women without another indica-

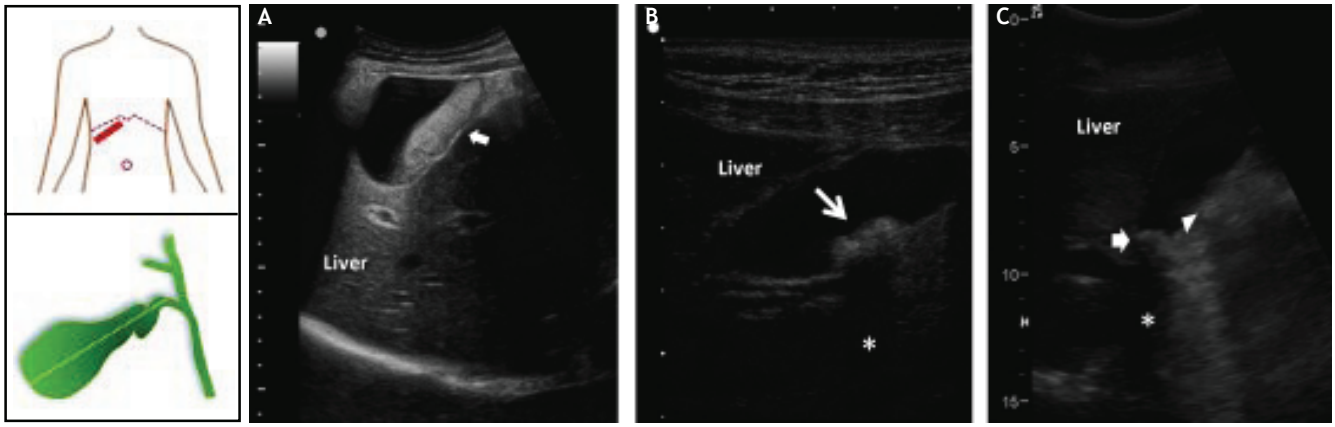

Figure 2. *Ultrasonographic appearance of biliary sludge, gallstones, and gallstone plus biliary sludge in the gallbladder. The left cartoons depict the site of the oblique ultrasonographic scan at the right hypochondrium (top) and the resulting longitudinal section of gallbladder.* ***A.*** *A finely echogenic, dense, gravity-dependent, slowly mobile biliary sludge is seen occupying about 40% of the gallbladder lumen (thick arrows). The thickness of the gallbladder wall is not increased.* ***B.*** *Two mobile echogenic gallstones are indicated within the gallbladder lumen, layering on the distal wall. Each stone size is about 0.5 cm in diameter.* ***C.*** *A 1.5 cm gallstone in diameter (arrow) is detected in the gallbladder infundibulum and is surrounded by biliary sludge (triangle). The asterisk indicates the posterior acoustic shadowing typical of gallstones.*

tion for the diagnosis. If biliary sludge is not identified on these imaging examinations, gallbladder bile samples can be collected for polarizing light microscopy. Further imaging examination such as computerized tomographic (CT) scanning is relatively high cost, low accurateness and availability.

During pregnancy, when common bile duct stones are suspected, radiographic imaging of the bile duct can be performed as long as the mother's pelvis is shielded, the fetus is monitored, and the fetal dose of radiation is less than 5 radiation-absorbed doses.[137,138] However, the safety of radiographic imaging is limited to women in their second and third trimesters. Endoscopic retrograde cholangiography could be safely performed if necessary during pregnancy, with a premature delivery rate of less than 5%.[139-141] Indications for this procedure include acute cholangitis, persistent jaundice, or severe pancreatitis with suspected choledocholithiasis. Contraindications include uncontrolled bleeding tendencies or inability to tolerate sedation or anesthesia.[35] In addition to fetal risks, potential maternal complications include pancreatitis, biliary tract infection, and gastrointestinal bleeding and perforation. The fetus should be shielded from radiation and fluoroscopy time minimized. In addition, fetal monitoring during any invasive procedures should be considered.[142]

PREVENTION AND TREATMENT OF BILIARY SLUDGE AND GALLSTONES IN PREGNANT WOMEN

Following acute appendicitis, gallbladder disease is the second most common indication for non-obstetric surgical intervention during pregnancy.[63,143] Gallstones and biliary sludge are the most common causes of gallbladder disease in pregnant women. Pregnant women should be evaluated specifically for biliary sludge, microlithiasis, or gallstones only after they develop symptoms (Figure 3). If a specific cause leading to biliary sludge is detected, attempts should be made to eliminate it. Biliary sludge and gallstones should be considered similar in almost all respects.

Prevention

Prevention of biliary sludge and gallstones in high-risk pregnant women has a rationale *per se,* since preventive measures may dramatically reduce the risk of cholecystectomy in pregnant and postpartum women.[128] Potentially useful general measures might include constant physical activity although the ultimate beneficial role during pregnancy is controversial. Thus, asymptomatic pregnant women with biliary sludge should undergo careful follow-up and managed expectantly.[75]

No indication exists for drug prescription as preventive measure of biliary sludge and gallstones in pregnancy. This caution includes also cholecystokinin octapeptide (CCK-8) which has been used as prokinetic agent on the gallbladder and prevention of biliary sludge and gallstones in patients with gallbladder stasis because of prolonged total parenteral nutrition.[70,144,145]

Treatment during pregnancy

Guidelines should be applied for optimal management of biliary sludge and gallstones in the pregnant woman. The Food and Drug Administration (FDA) has developed a general rating system to provide therapeutic guidance based on potential benefits and fetal risks, and drugs have been classified into categories A, B, C, D and X based on this system of classification.[146]

In asymptomatic pregnant women with biliary sludge and gallstones, expectant management is the general rule and litholysis is not indicated. However, in symptomatic pregnant women, there is a consensus concerning medical management of gallstones during pregnancy.[72,128,135,147,148] Pain control is mandatory during pregnancy.

In a pregnant woman presenting with abdominal pain, true biliary colic should be distinguished from nonspecific abdominal discomfort. A laparoscopic or open cholecystectomy performed for true biliary colic is usually curative, but symptoms often persist if the procedure is performed in pregnant women with nonspecific dyspepsia and gallstones. Nevertheless, despite the availability of many imaging techniques that can be used to detect biliary sludge, microlithiasis, and gallstones in the gallbladder and the common bile duct, the diagnosis of biliary colic is ultimately based on clinical judgment.

Supportive management is highly recommended if possible, reserving definitive treatment after delivery. Women with uncomplicated biliary colic can be managed with intravenous hydration and narcotic pain control.[149,150] Nevertheless, women who receive supportive treatment are prone to symptomatic relapses, which might increase the likelihood of premature delivery.[149,150] No prospective data exist, moreover, comparing medical *vs.* surgical management with respect to symptomatic relapse, total number of hospital days, or rate of premature labor. With recurrent

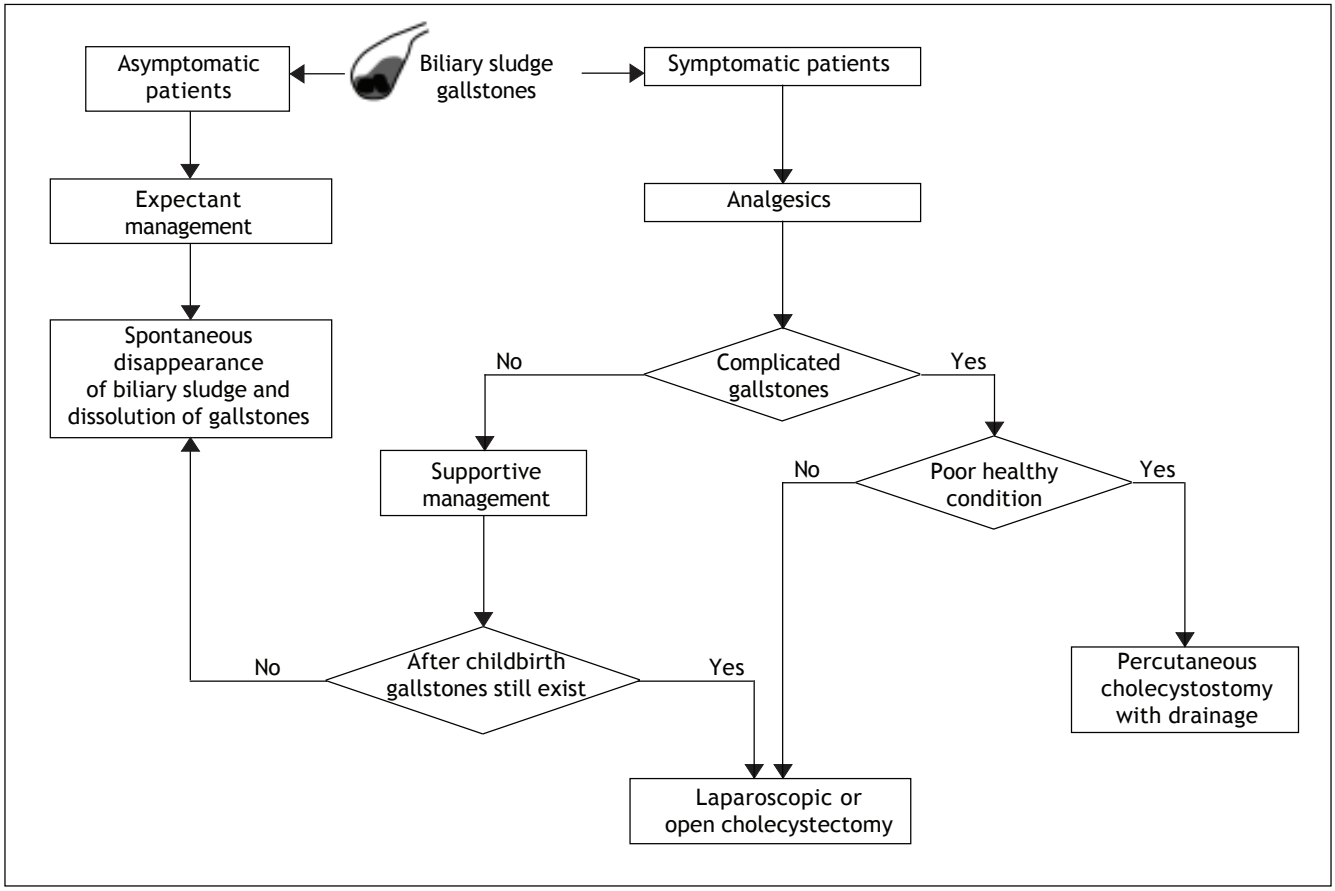

Figure 3. *Flow-chart depicting the standard therapy of biliary sludge and gallstones during pregnancy. For management purposes, biliary sludge and gallstones should be considered similar in almost all respects. Surgery generally is reserved for pregnant women with recurrent or unrelenting biliary pain refractory to medical management or with complications related to gallstones, including obstructive jaundice, acute cholecystitis, gallstone pancreatitis, or suspected peritonitis. For its safety, laparoscopic or elective cholecystectomy is one of the most common treatments for gallbladder gallstones in pregnant women, but it is recommended after the second trimester in order to reduce the rate of spontaneous abortion and preterm labor. If cholecystectomy is required during pregnancy, laparoscopic surgery is first recommended because of its relative safety. Of special note is that the timing of surgery is important. The effect of laparoscopic surgery on a developing fetus in the first trimester is unknown and surgery is more difficult in the third trimester with uterine enlargement. The second trimester, therefore, is believed to be the optimal time for cholecystectomy. A supportive management is highly recommended if possible, delaying more definitive treatment until after childbirth. See text for details.*

or complicated biliary tract disease, management becomes more controversial.

Lu, *et al*. have studied 76 women with 78 pregnancies admitted with biliary tract disease.[149] Of the 63 women who presented with symptomatic cholelithiasis, 10 underwent surgery while pregnant. There were no deaths, preterm deliveries, or intensive care unit admission. Fifty-three patients were treated medically and their clinical courses were complicated by symptomatic relapse in 20 patients (38%), by labor induction to control biliary colic (8 patients), and by premature delivery in 2 patients. Each relapse in the medically managed group led to an additional five days stay in hospital. These findings suggest that surgical management of gallstones in pregnancy is safe and decreases days in hospital, as well as reduces the rate of labor induction and preterm delivery.[149]

The use of analgesics has successfully ameliorated biliary symptoms in 64% of symptomatic pregnant women.[147] Surgery is generally reserved for pregnant women with recurrent or unrelenting biliary pain refractory to medical management or with gallstone-related complications.[151] Elective laparoscopic cholecystectomy is relatively safe and is the first-line option, but it is recommended after the second trimester in order to reduce the rates of spontaneous abortion and preterm labor.[82,147,152-155] Papillotomy for choledocholithiasis yields relatively good results

for mother and fetus.[140] Selection criteria for surgery need to be carefully considered in pregnant women, as illustrated in figure 3.

These with only one episode of uncomplicated biliary colic are at moderate risk for future recurrence of pain and more serious complications, and up to 30% of women will not develop further clinical symptoms.[75] Expectant management is still an option; in principle, oral litholysis is contraindicated in pregnancy. If symptoms or complications of biliary sludge or gallstones do recur, however, surgery should be considered. The indication for surgery is even stronger if more serious complications develop, such as acute cholecystitis, sepsis, gangrene, obstructive jaundice and suspected peritonitis, as well as acute pancreatitis induced by biliary sludge or microlithiasis.[149,156-158] Surgery should be considered during initial hospitalization.

During pregnancy and when required, the first choice remains the laparoscopic, rather than the laparotomic cholecystectomy. Timing should be carefully considered as well.[75] The effect of laparoscopic surgery on a developing fetus in the first trimester of pregnancy is unknown and surgery is more difficult in the third trimester with uterine enlargement. The second trimester, therefore, is believed to be the optimal time for cholecystectomy.[154,159-162] Laparoscopic cholecystectomy is performed safely with minimal fetal morbidity during this period.[147,149,152,153] The risks of preterm labor or premature delivery in each trimester of pregnancy, however, are not clearly defined in the literature. If acute cholecystitis or cholangitis develops, earlier cholecystectomy should be considered.[163]

However, if a pregnant woman is under a poor healthy condition for surgery, percutaneous cholecystostomy with drainage should be considered.[75,164-166] The long-term efficacy of these methods has not been proven by clinical trials and these approaches should be used only in pregnant women who require emergency therapy but are not good candidates for cholecystectomy. The efficacy of percutaneous cholecystostomy with drainage in the treatment of biliary sludge has not been well established.[75]

Ursodeoxycholic acid (UDCA) is a litholytic bile salt (see below) and is used in a subgroup of symptomatic gallstone patients. Although UDCA is the treatment of choice for intrahepatic cholestasis of pregnancy[167,168] and is classified as B drug by FDA (i.e., no evidence of risk in studies), it is not used during pregnancy for biliary sludge and gallstone dissolution. Same would apply to ezetimibe, another agent acting on gallstone dissolution (see below).

Management post pregnancy

Therapeutic options of biliary sludge and gallstones after pregnancy should follow the general guidelines, including expectant management in asymptomatic patients (no consensus about the need for prophylactic cholecystectomy), and cholecystectomy in previously symptomatic patients or those who will become symptomatic after pregnancy. In a small subgroup of women, oral litholysis might have a role, and might include:

- Administration of the cholelitholytic agent UDCA;[75] and
- Administration of the potent intestinal cholesterol absorption inhibitor ezetimibe.[128,169,170]

The effect of UDCA, a hydrophilic bile acid, has been extensively studied for the dissolution of cholesterol gallstones in patients.[171] UDCA has been recommended as first-line pharmacological therapy in a subgroup of symptomatic patients with small, radiolucent cholesterol gallstones, and its long-term administration has been shown to promote the dissolution of cholesterol gallstones and to prevent the recurrence of gallstones.[92,172] The potential cholelitholytic mechanisms of UDCA involve the formation of a liquid crystalline mesophase.[173] It favors the formation of numerous vesicles that are composed predominantly of phospholipids in bile so that the growth of liquid crystals on the cholesterol monohydrate surface and their subsequent dispersions might occur during gallstone dissolution. Consequently, liquid crystalline dissolution allows the transport of a great amount of cholesterol from stones, which is excreted into the duodenum and eventually in the faces.[173] In patients with a rapid loss of body weight, UDCA reduces the incidence of gallstones by 50 to 100%.[174-176] In addition, in patients with biliary sludge and idiopathic pancreatitis, after initial treatment with UDCA to dissolve cholesterol monohydrate crystals, ongoing maintenance therapy successfully prevents the recurrence of biliary sludge and pancreatitis.[26]

Ezetimibe, 1-(4-fluorophenyl)-(3*R*)-[3-(4-fluorophenyl)-(3*S*)-hydroxypropyl]-(4*S*)-(4-hydroxyphenyl)-2-azetidinone, is a highly selective intestinal cholesterol absorption inhibitor that effectively and potently prevents the absorption of cholesterol by inhibiting the uptake of dietary and biliary cholesterol across the brush border membrane of the enterocyte through the Niemann-Pick C1-like 1

(NPC1L1) pathway, possibly a transporter-facilitated mechanism.[169] It could reduce cholesterol concentrations of the liver, which in turn diminish the bioavailability of cholesterol for biliary secretion.[169] Ezetimibe has been found to induce a striking dose-dependent decrease in intestinal cholesterol absorption efficiency, coupled with a significant dose-dependent reduction in biliary cholesterol output and gallstone prevalence rate in gallstone-susceptible mice, even under high dietary cholesterol loads. Of note is that ezetimibe promotes the dissolution of cholesterol gallstones by forming an abundance of unsaturated micelles. Ezetimibe could protect gallbladder motility function by desaturating bile.[177] Furthermore, in a small number of patients with gallstones, ezetimibe has been revealed to significantly reduce biliary cholesterol saturation and retard cholesterol crystallization in bile.[177] These observations clearly demonstrate that ezetimibe is a novel and potential cholelitholytic agent for preventing or treating cholesterol gallstone disease not only in mice but also in humans. To evaluate treatment time, response rate and overall cost-benefit analysis, a more detailed, long-term human study needs to be performed.

More recently, we found from a preliminary study that ezetimibe prevents the formation of E_2-induced cholesterol gallstones by inhibiting intestinal cholesterol absorption. The bioavailability of intestinal source of cholesterol for biliary secretion is markedly reduced and bile is desaturated in mice even on the lithogenic diet. Also, ezetimibe does not influence mRNA levels of the *Er*α*, Er*β and *Gpr30* genes in the liver. Therefore, these results show that ezetimibe is a potential cholelitholytic agent for preventing or treating E_2-induced gallstones. These findings may provide an effective novel strategy for the prevention of cholesterol gallstones, particularly in women and patients exposed to high levels of estrogen. Further clinical studies are warranted in this respect.

Because ezetimibe and UDCA promote the dissolution of cholesterol gallstones by two distinct mechanisms via the formation of an unsaturated micelle and a liquid crystalline mesophase, respectively, it is highly likely that biliary sludge could be prevented and cholesterol gallstones could be dissolved faster by a combination therapy of ezetimibe and UDCA even in pregnant in postpartum women. A major limitation of oral litholysis, however, is the high recurrence rate of gallstones (10% per year and up to 45-50% by 5 years).[178,179]

CONCLUSIONS AND FUTURE DIRECTIONS

Obviously, during pregnancy, bile becomes lithogenic because of a significant increase in estrogen levels, which lead to hepatic cholesterol hypersecretion and biliary lithogenicity. In addition, increased progesterone concentrations impair gallbladder motility function, with the resulting increase in fasting gallbladder volume and bile stasis. Such abnormalities greatly promote the formation of biliary sludge and gallstones. Because plasma concentrations of female sex hormones, especially estrogen, increase linearly with the duration of gestation, the risk of gallstone formation becomes higher in the third trimester of pregnancy. Increasing parity is also a risk factor for gallstones, especially in younger women. Similarly to non-pregnant women, pregnant women are indeed exposed to the whole spectrum of the natural history of biliary sludge and gallstones. Clinical features include no symptoms, one or more episodes of biliary colicky pain and sludge/gallstone-related complications. Due to its noninvasive features, transabdominal ultrasonography is the first-line diagnostic approach in asymptomatic and symptomatic pregnant patients. All available therapeutic options (apart from oral litholysis with UDCA) must be kept accessible in symptomatic patients (i.e. supportive care in patients with only one episode of biliary colic, laparoscopic cholecystectomy followed by open cholecystectomy in high-risk cases, and ERCP if biliary pancreatitis, choledocholithiasis, and cholangitis develop). Open issues for future research agenda must include ways to prevent the formation of biliary sludge and gallstones before and during pregnancy, the role of lifestyles, novel therapeutic medical agents to be tested in larger groups of patients (e.g., ezetimibe), and safe cholelitholytic agents during pregnancy, as well as ways to better prevent and treat gallstone complications.

ACKNOWLEDGMENT

This work was supported in part by research grants DK73917 and DK101793 (to D.Q.-H.W.) and DK92779 (to M.L.) from the National Institutes of Health (US Public Health Service) and research grant MRAR08P011-2012 (to P.P.) from Italian Agency of Drug (AIFA).

CONFLICT OF INTEREST

None of the authors has any financial or any conflict of interest related to the content of this manuscript.

REFERENCES

1. Everson GT, McKinley C, Kern F, Jr. Mechanisms of gallstone formation in women. Effects of exogenous estrogen (Premarin) and dietary cholesterol on hepatic lipid metabolism. *J Clin Invest* 1991; 87: 237-46.
2. Boston Collaborative Drug Surveillance Programme. Oral contraceptives and venous thromboembolic disease, surgically confirmed gallbladder disease, and breast tumours. *Lancet* 1973; 1: 1399-404.
3. The Boston Collaborative Drug Surveillance Program, Boston University Medical Center. Surgically confirmed gallbladder disease, venous thromboembolism, and breast tumors in relation to postmenopausal estrogen therapy. *N Engl J Med* 1974; 290: 15-9.
4. The Coronary Drug Project Research Group. Gallbladder disease as a side effect of drugs influencing lipid metabolism. Experience in the Coronary Drug Project. *N Engl J Med* 1977; 296: 1185-90.
5. Cirillo DJ, Wallace RB, Rodabough RJ, Greenland P, LaCroix AZ, Limacher MC, Larson JC. Effect of estrogen therapy on gallbladder disease. *JAMA* 2005; 293: 330-9.
6. Honore LH. Increased incidence of symptomatic cholesterol cholelithiasis in perimenopausal women receiving estrogen replacement therapy: a retrospective study. *J Reprod Med* 1980; 25: 187-90.
7. Thijs C, Knipschild P. Oral contraceptives and the risk of gallbladder disease: a meta-analysis. *Am J Public Health* 1993; 83: 1113-20.
8. Henriksson P, Einarsson K, Eriksson A, Kelter U, Angelin B. Estrogen-induced gallstone formation in males. Relation to changes in serum and biliary lipids during hormonal treatment of prostatic carcinoma. *J Clin Invest* 1989; 84: 811-6.
9. Grodstein F, Colditz GA, Stampfer MJ. Postmenopausal hormone use and cholecystectomy in a large prospective study. *Obstet Gynecol* 1994; 83: 5-11.
10. Wang HH, Liu M, Clegg DJ, Portincasa P, Wang DQ. New insights into the molecular mechanisms underlying effects of estrogen on cholesterol gallstone formation. *Biochim Biophys Acta* 2009; 1791: 1037-47.
11. Angelin B, Olivecrona H, Reihner E, Rudling M, Stahlberg D, Eriksson M, Ewerth S, et al. Hepatic cholesterol metabolism in estrogen-treated men. *Gastroenterology* 1992; 103: 1657-63.
12. Novacek G. Gender and gallstone disease. *Wien Med Wochenschr* 2006; 156: 527-33.
13. Diehl AK. Epidemiology and natural history of gallstone disease. *Gastroenterol Clin North Am* 1991; 20: 1-19.
14. Barbara L, Sama C, Morselli Labate AM, Taroni F, Rusticali AG, Festi D, Sapio C, et al. A population study on the prevalence of gallstone disease: the Sirmione Study. *Hepatology* 1987; 7: 913-7.
15. Layde PM, Vessey MP, Yeates D. Risk factors for gallbladder disease: a cohort study of young women attending family planning clinics. *J Epidemiol Community Health* 1982; 36: 274-8.
16. Friedman GD, Kannel WB, Dawber TR. The epidemiology of gallbladder disease: observations in the Framingham Study. *J Chronic Dis* 1966; 19: 273-92.
17. Scragg RK, McMichael AJ, Seamark RF. Oral contraceptives, pregnancy, and endogenous oestrogen in gall stone disease—a case-control study. *Br Med J* 1984; 288: 1795-9.
18. Dhiman RK, Chawla YK. Is there a link between oestrogen therapy and gallbladder disease? *Expert Opin Drug Saf* 2006; 5: 117-29.
19. Kern F Jr., Everson GT, DeMark B, McKinley C, Showalter R, Braverman DZ, Szczepanik-Van Leeuwen P, et al. Biliary lipids, bile acids, and gallbladder function in the human female: effects of contraceptive steroids. *J Lab Clin Med* 1982; 99: 798-805.
20. Maringhini A, Ciambra M, Baccelliere P, Raimondo M, Orlando A, Tine F, Grasso R, et al. Biliary sludge and gallstones in pregnancy: incidence, risk factors, and natural history. *Ann Intern Med* 1993; 119: 116-20.
21. Bolukbas FF, Bolukbas C, Horoz M, Ince AT, Uzunkoy A, Ozturk A, Aka N, et al. Risk factors associated with gallstone and biliary sludge formation during pregnancy. *J Gastroenterol Hepatol* 2006; 21: 1150-3.
22. Maringhini A, Marceno MP, Lanzarone F, Caltagirone M, Fusco G, Di Cuonzo G, Cittadini E, et al. Sludge and stones in gallbladder after pregnancy. Prevalence and risk factors. *J Hepatol* 1987; 5: 218-23.
23. Valdivieso V, Covarrubias C, Siegel F, Cruz F. Pregnancy and cholelithiasis: pathogenesis and natural course of gallstones diagnosed in early puerperium. *Hepatology* 1993; 17: 1-4.
24. Basso L, McCollum PT, Darling MR, Tocchi A, Tanner WA. A study of cholelithiasis during pregnancy and its relationship with age, parity, menarche, breast-feeding, dysmenorrhea, oral contraception and a maternal history of cholelithiasis. *Surg Gynecol Obstet* 1992; 175: 41-6.
25. Stauffer RA, Adams A, Wygal J, Lavery JP. Gallbladder disease in pregnancy. *Am J Obstet Gynecol* 1982; 144: 661-4.
26. Ros E, Navarro S, Bru C, Garcia-Puges A, Valderrama R. Occult microlithiasis in 'idiopathic' acute pancreatitis: prevention of relapses by cholecystectomy or ursodeoxycholic acid therapy. *Gastroenterology* 1991; 101: 1701-9.
27. Ko CW, Beresford SA, Schulte SJ, Matsumoto AM, Lee SP. Incidence, natural history, and risk factors for biliary sludge and stones during pregnancy. *Hepatology* 2005; 41: 359-65.
28. Glasinovic JC, Mege R, Ferreiro O, Rodríguez N, Marinovic I, Villarroel L, Vela P. Cholelithiasis in Chilean female population. Prevalence and associated risk factors. *Gastroenterology* 1986; 96: A601.
29. Van Bodegraven AA, Bohmer CJ, Manoliu RA, Paalman E, Van der Klis AH, Roex AJ, Kruishoop AM, et al. Gallbladder contents and fasting gallbladder volumes during and after pregnancy. *Scand J Gastroenterol* 1998; 33: 993-7.
30. Everhart JE, Khare M, Hill M, Maurer KR. Prevalence and ethnic differences in gallbladder disease in the United States. *Gastroenterology* 1999; 117: 632-9.
31. Thijs C, Knipschild P, Leffers P. Pregnancy and gallstone disease: an empiric demonstration of the importance of specification of risk periods. *Am J Epidemiol* 1991; 134: 186-95.
32. The Rome Group for Epidemiology and Prevention of Cholelithiasis (GREPCO).The epidemiology of gallstone disease in Rome, Italy. Part II. Factors associated with the disease. *Hepatology* 1988; 8: 907-13.
33. Stampfer MJ, Maclure KM, Colditz GA, Manson JE, Willett WC. Risk of symptomatic gallstones in women with severe obesity. *Am J Clin Nutr* 1992; 55: 652-8.

34. Lindseth G, Bird-Baker MY. Risk factors for cholelithiasis in pregnancy. *Res Nurs Health* 2004; 27: 382-91.
35. Ko CW. Risk factors for gallstone-related hospitalization during pregnancy and the postpartum. *Am J Gastroenterol* 2006; 101: 2263-8.
36. Lydon-Rochelle M, Holt VL, Martin DP, Easterling TR. Association between method of delivery and maternal rehospitalization. *JAMA* 2000; 283: 2411-6.
37. Printen KJ, Ott RA. Cholecystectomy during pregnancy. *Am Surg* 1978; 44: 432-4.
38. Carpenter MW. Gestational diabetes, pregnancy hypertension, and late vascular disease. *Diabetes Care* 2007; 30 (Suppl. 2): S246-S250.
39. Valdiviezo C, Garovic VD, Ouyang P. Preeclampsia and hypertensive disease in pregnancy: their contributions to cardiovascular risk. *Clin Cardiol* 2012; 35: 160-5.
40. Bennion LJ, Ginsberg RL, Gernick MB, Bennett PH. Effects of oral contraceptives on the gallbladder bile of normal women. *N Engl J Med* 1976; 294: 189-92.
41. Lynn J, Williams L, O'Brien J, Wittenberg J, Egdahl RH. Effects of estrogen upon bile: implications with respect to gallstone formation. *Ann Surg* 1973; 178: 514-24.
42. Pertsemlidis D, Panveliwalla D, Ahrens EH, Jr. Effects of clofibrate and of an estrogen-progestin combination on fasting biliary lipids and cholic acid kinetics in man. *Gastroenterology* 1974; 66: 565-73.
43. Kern F Jr., Everson GT. Contraceptive steroids increase cholesterol in bile: mechanisms of action. *J Lipid Res* 1987; 28: 828-39.
44. Bennion LJ, Mott DM, Howard BV. Oral contraceptives raise the cholesterol saturation of bile by increasing biliary cholesterol secretion. *Metabolism* 1980; 29: 18-22.
45. van der Werf SD, van Berge Henegouwen GP, Ruben AT, Palsma DM. Biliary lipids, bile acid metabolism, gallbladder motor function and small intestinal transit during ingestion of a sub-fifty oral contraceptive. *J Hepatol* 1987; 4: 318-26.
46. Heuman R, Larsson-Cohn U, Hammar M, Tiselius HG. Effects of postmenopausal ethinylestradiol treatment on gallbladder bile. *Maturitas* 1980; 2: 69-72.
47. Anderson A, James OF, MacDonald HS, Snowball S, Taylor W. The effect of ethynyl oestradiol on biliary lipid composition in young men. *Eur J Clin Invest* 1980; 10: 77-80.
48. Vazquez MC, Rigotti A, Zanlungo S. Molecular mechanisms underlying the link between nuclear receptor function and cholesterol gallstone formation. *J Lipids* 2012; 2012: 547643.
49. Portincasa P, Di Ciaula A, Wang HH, Palasciano G, van Erpecum KJ, Moschetta A, Wang DQ. Coordinate regulation of gallbladder motor function in the gut-liver axis. *Hepatology* 2008; 47: 2112-26.
50. Everson GT. Pregnancy and gallstones. *Hepatology* 1993; 17: 159-61.
51. Everson GT, McKinley C, Lawson M, Johnson M, Kern F, Jr. Gallbladder function in the human female: effect of the ovulatory cycle, pregnancy, and contraceptive steroids. *Gastroenterology* 1982; 82: 711-9.
52. Braverman DZ, Johnson ML, Kern F, Jr. Effects of pregnancy and contraceptive steroids on gallbladder function. *N Engl J Med* 1980; 302: 362-4.
53. Wang HH, Afdhal NH, Wang DQ. Estrogen receptor alpha, but not beta, plays a major role in 17beta-estradiol-induced murine cholesterol gallstones. *Gastroenterology* 2004; 127: 239-49.
54. Wang HH, Portincasa P, Wang DQ. Molecular pathophysiology and physical chemistry of cholesterol gallstones. *Front Biosci* 2008; 13: 401-23.
55. Silverthorn DU. Human Physiology: An Integrated Approach. 6th ed. Glenview, IL: Pearson Education, Inc.; 2013.
56. Speroff L GR, Kase NG. Clinical gynecologic endocrinology and infertility. 6th Ed. Baltimore; 1999.
57. Ferin M JR, Warren M. The menstrual cycle. In Physiology, Reproductive Disorders, and Infertility. New York; 1993.
58. Senie RT, Tenser SM. The timing of breast cancer surgery during the menstrual cycle. *Oncology* 1997; 11: 1509-17.
59. Pike MC, Spicer DV, Dahmoush L, Press MF. Estrogens, progestogens, normal breast cell proliferation, and breast cancer risk. *Epidemiol Rev* 1993; 15: 17-35.
60. Chikazawa K, Araki S, Tamada T. Morphological and endocrinological studies on follicular development during the human menstrual cycle. *J Clin Endocrinol Metab* 1986; 62: 305-13.
61. Tsimoyiannis EC, Antoniou NC, Tsaboulas C, Papanikolaou N. Cholelithiasis during pregnancy and lactation. Prospective study. *Eur J Surg* 1994; 160: 627-31.
62. Hay JE. Liver disease in pregnancy. *Hepatology* 2008; 47: 1067-76.
63. Mendez-Sanchez N, Chavez-Tapia NC, Uribe M. Pregnancy and gallbladder disease. *Ann Hepatol* 2006; 5: 227-30.
64. Scott LD. Gallstone disease and pancreatitis in pregnancy. *Gastroenterol Clin North Am* 1992; 21: 803-15.
65. Trotman BW, Soloway RD. Pigment vs cholesterol cholelithiasis: clinical and epidemiological aspects. *Am J Dig Dis* 1975; 20: 735-40.
66. Neil Bajwa RB, Ambrish Ghumman, Agrawal R. M. . The Gallstone Story: Pathogenesis and Epidemiology. *Practical gastroenterology* 2010; XXXIV.
67. The epidemiology of gallstone disease in Rome, Italy. Part I. Prevalence data in men. The Rome Group for Epidemiology and Prevention of Cholelithiasis (GREPCO). *Hepatology* 1988; 8: 904-6.
68. Rome Group for the Epidemiology and Prevention of Cholelithiasis (GREPCO). Prevalence of gallstone disease in an Italian adult female population. *Am J Epidemiol* 1984; 119: 796-805.
69. Attili AF, Capocaccia R, Carulli N, Festi D, Roda E, Barbara L, Capocaccia L, et al. Factors associated with gallstone disease in the MICOL experience. Multicenter Italian Study on Epidemiology of Cholelithiasis. *Hepatology* 1997; 26: 809-18.
70. Pazzi P, Gamberini S, Buldrini P, Gullini S. Biliary sludge: the sluggish gallbladder. *Dig Liver Dis* 2003; 35(Suppl. 3): S39-S45.
71. Davis M, Ryan JP. Influence of progesterone on guinea pig gallbladder motility in vitro. *Dig Dis Sci* 1986; 31: 513-8.
72. Gilat T, Konikoff F. Pregnancy and the biliary tract. *Can J Gastroenterol* 2000; 14(Suppl. D): 55D-59D.
73. Acalovschi M. Cholesterol gallstones: from epidemiology to prevention. *Postgrad Med J* 2001; 77: 221-9.
74. Cohen S. The sluggish gallbladder of pregnancy. *N Engl J Med* 1980; 302: 397-9.
75. Ko CW, Sekijima JH, Lee SP. Biliary sludge. *Ann Intern Med* 1999; 130: 301-11.
76. Sali A, Oats JN, Acton CM, Elzarka A, Vitetta L. Effect on pregnancy on gallstone formation. *Aust N Z J Obstet Gynaecol* 1989; 29: 386-9.
77. Kern F, Jr., Everson GT, DeMark B, McKinley C, Showalter R, Erfling W, Braverman DZ, et al. Biliary lipids, bile acids, and gallbladder function in the human female. Effects of pregnancy and the ovulatory cycle. *J Clin Invest* 1981; 68: 1229-42.
78. Kuiper GG, Carlsson B, Grandien K, Enmark E, Haggblad J, Nilsson S, Gustafsson JA. Comparison of the ligand binding specificity and transcript tissue distribution of

estrogen receptors alpha and beta. *Endocrinology* 1997; 138: 863-70.
79. Iavarone M, Lampertico P, Seletti C, Francesca Donato M, Ronchi G, del Ninno E, Colombo M. The clinical and pathogenetic significance of estrogen receptor-beta expression in chronic liver diseases and liver carcinoma. *Cancer* 2003; 98: 529-34.
80. Gruber CJ, Tschugguel W, Schneeberger C, Huber JC. Production and actions of estrogens. *N Engl J Med* 2002; 346: 340-52.
81. Coyne MJ, Bonorris GG, Chung A, Winchester R, Schoenfield LJ. Estrogen enhances dietary cholesterol induction of saturated bile in the hamster. *Gastroenterology* 1978; 75: 76-9.
82. Maclure KM, Hayes KC, Colditz GA, Stampfer MJ, Speizer FE, Willett WC. Weight, diet, and the risk of symptomatic gallstones in middle-aged women. *N Engl J Med* 1989; 321: 563-9.
83. Wang HH, Afdhal NH, Wang DQ. Overexpression of estrogen receptor alpha increases hepatic cholesterogenesis, leading to biliary hypersecretion in mice. *J Lipid Res* 2006; 47: 778-86.
84. Duan LP, Wang HH, Ohashi A, Wang DQ. Role of intestinal sterol transporters Abcg5, Abcg8, and Npc1l1 in cholesterol absorption in mice: gender and age effects. *Am J Physiol* 2006; 290: G269-G276.
85. Nervi FO, Del Pozo R, Covarrubias CF, Ronco BO. The effect of progesterone on the regulatory mechanisms of biliary cholesterol secretion in the rat. *Hepatology* 1983; 3: 360-7.
86. Down RH, Whiting MJ, Watts JM, Jones W. Effect of synthetic oestrogens and progestagens in oral contraceptives on bile lipid composition. *Gut* 1983; 24: 253-9.
87. Heuman DM, Hernandez CR, Hylemon PB, Kubaska WM, Hartman C, Vlahcevic ZR. Regulation of bile acid synthesis. I. Effects of conjugated ursodeoxycholate and cholate on bile acid synthesis in chronic bile fistula rat. *Hepatology* 1988; 8: 358-365.
88. Heuman DM, Vlahcevic ZR, Bailey ML, Hylemon PB. Regulation of bile acid synthesis. II. Effect of bile acid feeding on enzymes regulating hepatic cholesterol and bile acid synthesis in the rat. *Hepatology* 1988; 8: 892-7.
89. Shefer S, Nguyen L, Salen G, Batta AK, Brooker D, Zaki FG, Rani I, et al. Feedback regulation of bile-acid synthesis in the rat. Differing effects of taurocholate and tauroursocholate. *J Clin Invest* 1990; 85: 1191-8.
90. Stange EF, Scheibner J, Ditschuneit H. Role of primary and secondary bile acids as feedback inhibitors of bile acid synthesis in the rat in vivo. *J Clin Invest* 1989; 84: 173-80.
91. Wang DQ, Afdhal NH. Genetic analysis of cholesterol gallstone formation: searching for Lith (gallstone) genes. *Curr Gastroenterol Rep* 2004; 6: 140-50.
92. Portincasa P, Moschetta A, Palasciano G. Cholesterol gallstone disease. *Lancet* 2006; 368: 230-9.
93. Carey MC, Cahalane MJ. Whither biliary sludge? *Gastroenterology* 1988; 95: 508-23.
94. Ryan JP. Effect of pregnancy on gallbladder contractility in the guinea pig. *Gastroenterology* 1984; 87: 674-8.
95. Ryan JP. Calcium and gallbladder smooth muscle contraction in the guinea pig: effect of pregnancy. *Gastroenterology* 1985; 89: 1279-85.
96. Ryan JP, Pellecchia D. Effect of progesterone pretreatment on guinea pig gallbladder motility in vitro. *Gastroenterology* 1982; 83: 81-3.
97. Chen Q, Chitinavis V, Xiao Z, Yu P, Oh S, Biancani P, Behar J. Impaired G protein function in gallbladder muscle from progesterone-treated guinea pigs. *Am J Physiol* 1998; 274: G283-G289.
98. Newbern D, Freemark M. Placental hormones and the control of maternal metabolism and fetal growth. *Curr Opin Endocrinol Diabetes Obes* 2011; 18: 409-16.
99. Murphy VE, Smith R, Giles WB, Clifton VL. Endocrine regulation of human fetal growth: the role of the mother, placenta, and fetus. *Endocr Rev* 2006; 27: 141-69.
100. Barbour LA, McCurdy CE, Hernandez TL, Kirwan JP, Catalano PM, Friedman JE. Cellular mechanisms for insulin resistance in normal pregnancy and gestational diabetes. *Diabetes Care* 2007; 30(Suppl. 2): S112-S119.
101. Lain KY, Catalano PM. Metabolic changes in pregnancy. *Clin Obstet Gynecol* 2007; 50: 938-48.
102. Zavalza-Gomez AB, Anaya-Prado R, Rincon-Sanchez AR, Mora-Martinez JM. Adipokines and insulin resistance during pregnancy. *Diabetes Res Clin Pract* 2008; 80: 8-15.
103. Mor G, Cardenas I. The immune system in pregnancy: a unique complexity. *Am J Reprod Immunol* 2010; 63: 425-33.
104. Jamieson DJ, Theiler RN, Rasmussen SA. Emerging infections and pregnancy. *Emerg Infect Dis* 2006; 12: 1638-43.
105. Koren O, Goodrich JK, Cullender TC, Spor A, Laitinen K, Backhed HK, Gonzalez A, et al. Host remodeling of the gut microbiome and metabolic changes during pregnancy. *Cell* 2012; 150: 470-80.
106. Ko CW, Beresford SA, Schulte SJ, Lee SP. Insulin resistance and incident gallbladder disease in pregnancy. *Clin Gastroenterol Hepatol* 2008; 6: 76-81.
107. Collado MC, Isolauri E, Laitinen K, Salminen S. Distinct composition of gut microbiota during pregnancy in overweight and normal-weight women. *Am J Clin Nutr* 2008; 88: 894-9.
108. Di Cianni G, Miccoli R, Volpe L, Lencioni C, Del Prato S. Intermediate metabolism in normal pregnancy and in gestational diabetes. *Diabetes Metab Res Rev* 2003; 19: 259-70.
109. Nelson SM, Matthews P, Poston L. Maternal metabolism and obesity: modifiable determinants of pregnancy outcome. *Hum Reprod Update* 2010; 16: 255-75.
110. Lawlor DA, Fraser A, Lindsay RS, Ness A, Dabelea D, Catalano P, Davey Smith G, et al. Association of existing diabetes, gestational diabetes and glycosuria in pregnancy with macrosomia and offspring body mass index, waist and fat mass in later childhood: findings from a prospective pregnancy cohort. *Diabetologia* 2010; 53: 89-97.
111. Menzies FM, Shepherd MC, Nibbs RJ, Nelson SM. The role of mast cells and their mediators in reproduction, pregnancy and labour. *Hum Reprod Update* 2011; 17: 383-96.
112. Catalano PM, Kirwan JP. Clinical utility and approaches for estimating insulin sensitivity in pregnancy. *Semin Perinatol* 2002; 26: 181-9.
113. Kirwan JP, Hauguel-De Mouzon S, Lepercq J, Challier JC, Huston-Presley L, Friedman JE, Kalhan SC, et al. TNF-alpha is a predictor of insulin resistance in human pregnancy. *Diabetes* 2002; 51: 2207-13.
114. Jumpertz R, Le DS, Turnbaugh PJ, Trinidad C, Bogardus C, Gordon JI, Krakoff J. Energy-balance studies reveal associations between gut microbes, caloric load, and nutrient absorption in humans. *Am J Clin Nutr* 2011; 94: 58-65.
115. Ley RE, Turnbaugh PJ, Klein S, Gordon JI. Microbial ecology: human gut microbes associated with obesity. *Nature* 2006; 444: 1022-3.

116. Turnbaugh PJ, Ley RE, Mahowald MA, Magrini V, Mardis ER, Gordon JI. An obesity-associated gut microbiome with increased capacity for energy harvest. *Nature* 2006; 444: 1027-31.
117. Turnbaugh PJ, Gordon JI. The core gut microbiome, energy balance and obesity. *J Physiol* 2009; 587: 4153-8.
118. Backhed F, Ding H, Wang T, Hooper LV, Koh GY, Nagy A, Semenkovich CF, et al. The gut microbiota as an environmental factor that regulates fat storage. *Proc Natl Acad Sci USA* 2004; 101: 15718-23.
119. Gielkens HA, Lam WF, Coenraad M, Frolich M, van Oostayen JA, Lamers CB, Masclee AA. Effect of insulin on basal and cholecystokinin-stimulated gallbladder motility in humans. *J Hepatol* 1998; 28: 595-602.
120. Haffner SM, Diehl AK, Mitchell BD, Stern MP, Hazuda HP. Increased prevalence of clinical gallbladder disease in subjects with non-insulin-dependent diabetes mellitus. *Am J Epidemiol* 1990; 132: 327-35.
121. Ruhl CE, Everhart JE. Association of diabetes, serum insulin, and C-peptide with gallbladder disease. *Hepatology* 2000; 31: 299-303.
122. Wu T, Zhang Z, Liu B, Hou D, Liang Y, Zhang J, Shi P. Gut microbiota dysbiosis and bacterial community assembly associated with cholesterol gallstones in large-scale study. *BMC Genomics* 2013; 14: 669.
123. Sayin SI, Wahlstrom A, Felin J, Jantti S, Marschall HU, Bamberg K, Angelin B, et al. Gut microbiota regulates bile acid metabolism by reducing the levels of tauro-beta-muricholic acid, a naturally occurring FXR antagonist. *Cell Metab* 2013; 17: 225-35.
124. Sekirov I, Russell SL, Antunes LC, Finlay BB. Gut microbiota in health and disease. *Physiol Rev* 2010; 90: 859-904.
125. Diamant M, Blaak EE, de Vos WM. Do nutrient-gut-microbiota interactions play a role in human obesity, insulin resistance and type 2 diabetes? *Obes Rev* 2011; 12: 272-81.
126. Van Erpecum KJ, Van Berge-Henegouwen GP. Gallstones: an intestinal disease? *Gut* 1999; 44: 435-38.
127. Wang DQ, Cohen DE, Carey MC. Biliary lipids and cholesterol gallstone disease. *J Lipid Res* 2009; 50 (Suppl.): S406-S411.
128. Portincasa P, Ciaula AD, Bonfrate L, Wang DQ. Therapy of gallstone disease: What it was, what it is, what it will be. *World J Gastrointest Pharmacol Ther* 2012; 3: 7-20.
129. Maurer KJ, Ihrig MM, Rogers AB, Ng V, Bouchard G, Leonard MR, Carey MC, et al. Identification of cholelithogenic enterohepatic helicobacter species and their role in murine cholesterol gallstone formation. *Gastroenterology* 2005; 128: 1023-33.
130. Shaffer EA. Gallbladder sludge: what is its clinical significance? *Curr Gastroenterol Rep* 2001; 3: 166-73.
131. Filly RA, Allen B, Minton MJ, Bernhoft R, Way LW. In vitro investigation of the origin of echoes with biliary sludge. *J Clin Ultrasound* 1980; 8: 193-200.
132. Lee SP, Nicholls JF. Nature and composition of biliary sludge. *Gastroenterology* 1986; 90: 677-86.
133. Ko CW, Schulte SJ, Lee SP. Biliary sludge is formed by modification of hepatic bile by the gallbladder mucosa. *Clin Gastroenterol Hepatol* 2005; 3: 672-8.
134. Lee SP, Maher K, Nicholls JF. Origin and fate of biliary sludge. *Gastroenterology* 1988; 94: 170-6.
135. Strasberg SM. Cholelithiasis and acute cholecystitis. *Baillieres Clin Gastroenterol* 1997; 11: 643-61.
136. Jamidar PA, Beck GJ, Hoffman BJ, Lehman GA, Hawes RH, Agrawal RM, Ashok PS, et al. Endoscopic retrograde cholangiopancreatography in pregnancy. *Am J Gastroenterol* 1995; 90: 1263-7.
137. Tham TC, Vandervoort J, Wong RC, Montes H, Roston AD, Slivka A, Ferrari AP, et al. Safety of ERCP during pregnancy. *Am J Gastroenterol* 2003;98:308-11.
138. ACOG Committee on Obstetric Practice. ACOG Committee Opinion. Number 299, September 2004 (replaces No. 158, September 1995). Guidelines for diagnostic imaging during pregnancy. *Obstet Gynecol* 2004; 104: 647-51.
139. Barthel JS, Chowdhury T, Miedema BW. Endoscopic sphincterotomy for the treatment of gallstone pancreatitis during pregnancy. *Surg Endosc* 1998; 12: 394-9.
140. Sungler P, Heinerman PM, Steiner H, Waclawiczek HW, Holzinger J, Mayer F, Heuberger A, et al. Laparoscopic cholecystectomy and interventional endoscopy for gallstone complications during pregnancy. *Surg Endosc* 2000; 14: 267-71.
141. Kahaleh M, Hartwell GD, Arseneau KO, Pajewski TN, Mullick T, Isin G, Agarwal S, et al. Safety and efficacy of ERCP in pregnancy. *Gastrointest Endosc* 2004; 60: 287-92.
142. Ko C. Biliary sludge and acute pancreatitis during pregnancy. *Nat Clin Pract Gastroenterol Hepatol* 2006; 3: 53-7.
143. Sharp HT. Gastrointestinal surgical conditions during pregnancy. *Clin Obstet Gynecol* 1994; 37: 306-15.
144. Angelico M, De Santis A, Capocaccia L. Biliary sludge: a critical update. *J Clin Gastroenterol* 1990; 12: 656-62.
145. Sitzmann JV, Pitt HA, Steinborn PA, Pasha ZR, Sanders RC. Cholecystokinin prevents parenteral nutrition induced biliary sludge in humans. *Surg Gynecol Obstet* 1990; 170: 25-31.
146. Friedman JM, Little BB, Brent RL, Cordero JF, Hanson JW, Shepard TH. Potential human teratogenicity of frequently prescribed drugs. *Obstet Gynecol* 1990; 75: 594-9.
147. Glasgow RE, Visser BC, Harris HW, Patti MG, Kilpatrick SJ, Mulvihill SJ. Changing management of gallstone disease during pregnancy. *Surg Endosc* 1998; 12: 241-6.
148. Smoleniec JS, James DK. Gastro-intestinal crises during pregnancy. *Dig Dis* 1993; 11: 313-24.
149. Lu EJ, Curet MJ, El-Sayed YY, Kirkwood KS. Medical versus surgical management of biliary tract disease in pregnancy. *Am J Surg* 2004; 188: 755-9.
150. Dixon NP, Faddis DM, Silberman H. Aggressive management of cholecystitis during pregnancy. *Am J Surg* 1987; 154: 292-4.
151. Bellows CF, Berger DH, Crass RA. Management of gallstones. *Am Fam Physician* 2005; 72: 637-42.
152. Swisher SG, Schmit PJ, Hunt KK, Hiyama DT, Bennion RS, Swisher EM, Thompson JE. Biliary disease during pregnancy. *Am J Surg* 1994; 168: 576-9.
153. Cosenza CA, Saffari B, Jabbour N, Stain SC, Garry D, Parekh D, Selby RR. Surgical management of biliary gallstone disease during pregnancy. *Am J Surg* 1999; 178: 545-8.
154. Rollins MD, Chan KJ, Price RR. Laparoscopy for appendicitis and cholelithiasis during pregnancy: a new standard of care. *Surg Endosc* 2004; 18: 237-41.
155. McKellar DP, Anderson CT, Boynton CJ, Peoples JB. Cholecystectomy during pregnancy without fetal loss. *Surg Gynecol Obstet* 1992; 174: 465-8.
156. Lee SP, Nicholls JF, Park HZ. Biliary sludge as a cause of acute pancreatitis. *N Engl J Med* 1992; 326: 589-93.
157. Gouldman JW, Sticca RP, Rippon MB, McAlhany JC, Jr. Laparoscopic cholecystectomy in pregnancy. *Am Surg* 1998; 64: 93-7.
158. Lanzafame RJ. Laparoscopic cholecystectomy during pregnancy. *Surgery* 1995; 118: 627-31.

159. Robertson KW, Stewart IS, Imrie CW. Severe acute pancreatitis and pregnancy. *Pancreatology* 2006; 6: 309-15.
160. Affleck DG, Handrahan DL, Egger MJ, Price RR. The laparoscopic management of appendicitis and cholelithiasis during pregnancy. *Am J Surg* 1999; 178: 523-29.
161. Geisler JP, Rose SL, Mernitz CS, Warner JL, Hiett AK. Non-gynecologic laparoscopy in second and third trimester pregnancy: obstetric implications. *JSLS* 1998; 2: 235-8.
162. Stepp K, Falcone T. Laparoscopy in the second trimester of pregnancy. *Obstet Gynecol Clin North Am* 2004; 31: 485-96, vii.
163. Gurusamy KS, Koti R, Fusai G, Davidson BR. Early versus delayed laparoscopic cholecystectomy for uncomplicated biliary colic. *Cochrane Database Syst Rev* 2013; 6: CD007196.
164. Allmendinger N, Hallisey MJ, Ohki SK, Straub JJ. Percutaneous cholecystostomy treatment of acute cholecystitis in pregnancy. *Obstet Gynecol* 1995; 86: 653-4.
165. Mazzella G, Rizzo N, Azzaroli F, Simoni P, Bovicelli L, Miracolo A, Simonazzi G, et al. Ursodeoxycholic acid administration in patients with cholestasis of pregnancy: effects on primary bile acids in babies and mothers. *Hepatology* 2001; 33: 504-8.
166. Allen B, Bernhoft R, Blanckaert N, Svanvik J, Filly R, Gooding G, Way L. Sludge is calcium bilirubinate associated with bile stasis. *Am J Surg* 1981; 141: 51-6.
167. Glantz A, Reilly SJ, Benthin L, Lammert F, Mattsson LA, Marschall HU. Intrahepatic cholestasis of pregnancy: Amelioration of pruritus by UDCA is associated with decreased progesterone disulphates in urine. *Hepatology* 2008; 47: 544-51.
168. Imam MH, Gossard AA, Sinakos E, Lindor KD. Pathogenesis and management of pruritus in cholestatic liver disease. *J Gastroenterol Hepatol* 2012; 27: 1150-8.
169. de Bari O, Neuschwander-Tetri BA, Liu M, Portincasa P, Wang DQ. Ezetimibe: its novel effects on the prevention and the treatment of cholesterol gallstones and nonalcoholic fatty liver disease. *J Lipids* 2012; 2012: 302847.
170. Ballantyne CM. Role of selective cholesterol absorption inhibition in the management of dyslipidemia. *Curr Atheroscler Rep* 2004; 6: 52-9.
171. Di Ciaula A, Wang DQ, Wang HH, Bonfrate L, Portincasa P. Targets for current pharmacologic therapy in cholesterol gallstone disease. *Gastroenterol Clin North Am* 2010; 39: 245-264, viii-ix.
172. Wang HH, Portincasa P, de Bari O, Liu KJ, Garruti G, Neuschwander-Tetri BA, Wang DQ. Prevention of cholesterol gallstones by inhibiting hepatic biosynthesis and intestinal absorption of cholesterol. *Eur J Clin Invest* 2013; 43: 413-26.
173. Wang DQ, Tazuma S. Effect of beta-muricholic acid on the prevention and dissolution of cholesterol gallstones in C57L/J mice. *J Lipid Res* 2002; 43: 1960-8.
174. Broomfield PH, Chopra R, Sheinbaum RC, Bonorris GG, Silverman A, Schoenfield LJ, Marks JW. Effects of ursodeoxycholic acid and aspirin on the formation of lithogenic bile and gallstones during loss of weight. *N Engl J Med* 1988; 319: 1567-72.
175. Shiffman ML, Kaplan GD, Brinkman-Kaplan V, Vickers FF. Prophylaxis against gallstone formation with ursodeoxycholic acid in patients participating in a very-low-calorie diet program. *Ann Intern Med* 1995; 122: 899-905.
176. Worobetz LJ, Inglis FG, Shaffer EA. The effect of ursodeoxycholic acid therapy on gallstone formation in the morbidly obese during rapid weight loss. *Am J Gastroenterol* 1993; 88: 1705-10.
177. Wang HH, Portincasa P, Mendez-Sanchez N, Uribe M, Wang DQ. Effect of ezetimibe on the prevention and dissolution of cholesterol gallstones. *Gastroenterology* 2008; 134: 2101-10.
178. Lanzini A, Jazrawi RP, Kupfer RM, Maudgal DP, Joseph AE, Northfield TC. Gallstone recurrence after medical dissolution. An overestimated threat? *J Hepatol* 1986; 3: 241-6.
179. Rabenstein T, Radespiel-Troger M, Hopfner L, Benninger J, Farnbacher M, Greess H, Lenz M, et al. Ten years experience with piezoelectric extracorporeal shockwave lithotripsy of gallbladder stones. *Eur J Gastroenterol Hepatol* 2005; 17: 629-39.