

Review

Diet as a Risk Factor for Cholesterol Gallstone Disease

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Cholesterol gallstone disease is a common condition in western populations. The etiology is multifactorial with interaction of genetic and environmental factors. Obesity, aging, estrogen treatment, pregnancy and diabetes are consistently associated to a higher risk. A number of dietary factors have been involved in the pathogenesis of cholelithiasis. In this article we summarize several studies that have evaluated the role of diet as a potential risk factor for gallstone formation, including energy intake, cholesterol, fatty acids, fiber, carbohydrates, vitamins and minerals, and alcohol intake. Consumption of simple sugars and saturated fat has been mostly associated to a higher risk, while fiber intake and moderate consumption of alcohol, consistently reduce the risk. The association between cholesterol intake and gallstone disease has been variable in different studies. The effects of other dietary factors are less conclusive; additional studies are therefore necessary to clarify their relevance in the pathogenesis of gallstone disease. Recent discoveries of the role of orphan nuclear receptors in the regulation of fatty acid and hepatic cholesterol metabolism and excretion open new perspectives for a better understanding of the role of dietary constituents on cholesterol gallstone formation.

Key teaching points:

- The etiology of cholesterol gallstone disease is multifactorial with interaction between genome and environment.
- It has been postulated that dietary constituents are important determinants for the formation of lithogenic bile.
- Intake of high energy, simple sugar and saturated fat favors gallstone formation. Fiber and moderate consumption of alcohol reduce the risk.
- The role of orphan nuclear receptors in the regulation of hepatic cholesterol metabolism and excretion open new leads for understanding the role of dietary constituents on cholesterol gallstone formation.

Introduction

The etiology of cholesterol cholelithiasis is considered to be a multifactorial, with interaction of genetic and environmental factors [1]. Most exogenous factors are a consequence of westernization of modern societies, including a high intake of refined carbohydrates and a high prevalence of obesity, non-insulin dependent diabetes, atherosclerosis and sedentary life-style [2]. A gallstone is a solid mass that forms in the gallbladder from cholesterol, bilirubin and calcium salts precipitated from the bile. The large majority of gallstones found in Western countries have cholesterol as their primary component, whereas a much smaller number are composed primarily of calcium salts, of bilirubin and

phosphate [1]. Interestingly, both metabolic abnormalities of cholesterol metabolism in humans, atherosclerosis and cholesterol cholelithiasis, share similar metabolic risk factors including age, obesity, non-insulin dependent diabetes, hyperlipidemia of the type high serum triglyceride and low HDL serum cholesterol, hyperinsulinism and sedentary life-style [1–3].

The pathophysiological conditions that predispose to cholesterol cholelithiasis are the formation of a lithogenic bile, short cholesterol crystallization time and gallbladder stasis. Lithogenicity of the bile is mainly determined by the concentrations of their principal three lipid components: cholesterol, bile acids and phospholipids. Relative increase concentrations of cholesterol in bile, or supersaturation, are a *sine qua non*

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condition for gallstones formation. It is apparent that the confluence of all lithogenic conditions in a relatively short period of time is necessary for gallstone formation. Cholesterol crystallization is accelerated by gallbladder mucins. Gallbladder stasis is associated to still poorly understood gallbladder motility abnormalities, leading to incomplete emptying of the bile and therefore facilitating the appearance of cholesterol crystals within the gallbladder, crystal agglomeration and stone growth as a function of time. For example, a sluggish gallbladder is commonly found during pregnancy, fasting and during caloric restriction, all conditions associated to gallstone formation [1–3]. Interestingly, deterioration of gallbladder contractions by alterations in the cholecystokinin (CCK)-AR gene knockout mice that lack the CCK-AR receptor, facilitated the induction of cholesterol gallstone formation [4].

Cholesterol cholelithiasis is particularly prevalent in some specific populations including Mapuche and North American Indians, Chilean and Mexican Hispanics. The disease reaches epidemic characteristics among Native Americans [5,6]. Therefore, identification of risk factors that predispose to gallstone formation in order to develop preventive strategies is crucial in the prevention of this disease. Amerindian traits, independently of obesity, aging and diabetes, are consistently associated with a higher risk of cholesterol gallstone formation by increasing cholesterol saturation in gallbladder bile [5–8].

A number of epidemiologic studies have linked obesity, diabetes type 2 and hyperlipidemia (of the type high serum triglyceride and low HDL serum cholesterol) to cholesterol gallstone formation [1–3]. These conditions are commonly included under the heading of the metabolic syndrome or Syndrome X [9–11]. The basic pathophysiological abnormality underlying the metabolic syndrome is insulin resistance, which represents a generalized derangement in metabolic processes linked to nutrition and energy storage [9–11]. The major clinical consequences of the metabolic syndrome are coronary heart disease and stroke, type 2 diabetes and its complications [9–11], fatty liver [12,13] and cholesterol gallstones [14,15]. It is still unknown what the pathophysiological links are between hyperinsulinemia, diet, metabolic syndrome and cholesterol gallstone formation.

The association of diet and cholesterol cholelithiasis has been investigated in prospective, cross sectional and case-control studies and in experimental animal models. Numerous studies have examined the role of specific dietary constituents as a potential risk factor for gallstone formation in humans. Some of these studies have included the intake of types of fatty acids, cholesterol, fiber, carbohydrates, alcohol and some vitamins, and minerals. A major limitation of studies on diet and gallstone disease in humans involves two inherent difficulties related to gallstone pathogenesis. First, gallstones are usually asymptomatic and are formed through many years. The estimated growth rate of gallstones was found to be approximately 2 mm per year [3]. This fundamental characteristic of cholelithiasis makes impossible to know the time of initiation of the

disease, therefore the environmental factors including diet that might have been interacting in the process of favoring biliary cholesterol supersaturation. Secondly, measurement of energy intake and estimates of dietary constituents effectively ingested by individuals is very indirect and subject to uncontrolled error given the difficulties involved in accurately recalling past diet intake.

The purpose of this article is first to review the available epidemiological and experimental evidence pointing at specific dietary constituents that might contribute to cholesterol gallstone formation. Secondly, to see in perspective the potential new pathogenic links between dietary constituents and regulation of hepatic cholesterol metabolism and secretion in light of the new discoveries of the role of orphan nuclear receptors factors on the regulatory mechanisms of cholesterol and fatty acid metabolism.

The Role of Specific Dietary Constituents

The principal dietary hypotheses found in the literature are summarized in Table 1. Epidemiological studies have been performed in ultrasonographic population-based surveys and in prospective, cross sectional or case-control studies in which the case groups are constituted by subjects harboring symptomatic gallbladder disease. The following dietary factors have been correlated to gallstone disease: energy intake, fatty acids, cholesterol, highly refined carbohydrates, alcohol, micronutrients and dietary fiber.

Energy Intake. Energy intake has been directly associated to cholelithiasis risk, mainly by contributing to development of obesity. Obesity is a well known risk factor of gallstones primarily acting by increasing cholesterol synthesis, biliary cholesterol secretion and cholesterol supersaturation [15]. A large number of epidemiological studies have examined the association between energy intake and cholesterol cholelithiasis, but their findings are controversial (Table 1). A French study of 76 subjects with cholelithiasis detected by ultrasound, compared with paired control subjects, showed a higher risk associated to a high calorie diet (>2500 kcal/day), but only significant for men [16]. Similarly, a Spanish study showed a higher consumption of calories in patients with cholelithiasis compared to control subjects [17]. Studies in North American Indians and Caucasians also showed that a hypercaloric diet correlated with a higher prevalence of gallstones. However, other studies did not found a relationship between caloric intake and cholelithiasis. In contrast, an Italian large cross-sectional study detected a significant negative association between energy intake and risk of cholelithiasis in men [18]. These discrepancies can be attributed to differences in study designs, energy intake assessment, and methods used to diagnose gallstone disease. However, other possible factors could be considered; for example, obese people who are prone to

Table 1. Epidemiological Studies of Association between Dietary Factors and Gallbladder Disease

Author Ref Year	Study Design	Population	Dietary Assessment	Energy	Total Fat	Protein	Refined Sugar	Cholesterol	Fiber	Alcohol
A. Population-Based Ultrasonographic Studies										
Jorgensen [22] 1989	Cross Sectional	3,608 control 47 cases	Diet history	NA	(+)	NA	(+)	NA	(-)	(-)
Attili [18] 1998	Prospective	29, 584 controls 1,801 cases	FFQ	(-)&	(-)	(+)&	NE	NE	(-)#	(-)&
Ortega [17] 1998	Case-control	46 controls 54 cases	24 hr recall	(+)	(+)	NA	NE	(+)	(-)	NA
Misciagna [24] 1999	Case-control	290 controls 100 cases	FFQ	(-)	(+) ^{SAT} (-) ^{MUFA}	NE	(+)	(-)	(-)	(-)
B. Studies on Symptomatic Gallstone Patients.										
Scrabb [56] 1984	Case-control	359 controls 241 cases	FFQ	(+)*	(+)*	NA	(+)	(+) [#]	(-)	(-)
Diehl [47] 1989	Cross Sectional	2,064 controls 189 cases	24 hrs recall	NE	(+)& (-) [#]	NA	(+)	(-)	(+)	(-)& NA [#]
Maclure [19] 1990	Prospective	88,837 controls 612 cases	FFQ	(+)	NA	NA	NA	NA	(-)	(-) [#]
Sichiere [20] 1991	Prospective	4,730 controls 216 cases	24 hr recall	(-) ^{#*}	NA	NE	NE	NA	(-)	NE
Moerman [21] 1994	Prospective	860 controls 54 cases	Diet history	NA	NA	NA	(+)	(-)	(-)	(-)
Tandon [46] 1996	Case-control	98 controls 200 cases	Diet history	(+)	(+)	NA	NE	(+)&	NA	NE
Caroli [16] 1998	Case-control	78 controls 76 cases	FFQ	(+)&	(+)	NE	NE	NE	NE	(-)

FFQ = Food frequency questionnaire.

w = Women.

NA = No association.

* = Under 50 years old.

= Only for women.

& = Only for men.

Sat = Saturated fat.

MUFA = Monounsaturated fat.

NE = Not evaluated.

gallstones tend to minimize their caloric intake, with a subsequent underestimation of the true calorie intake in those subjects.

Fatty Acids. The studies of the role of total fat intake on cholesterol gallstones have also shown controversial results (Table 1). Several epidemiological studies have not detected a significant positive association between total fat intake and risk of cholelithiasis [19–21]. However, other studies reported that subjects with cholelithiasis exhibit a higher consumption of total lipids, mainly saturated fatty acids. A large cross-sectional study among Danish, found a positive, but non-significant association between total fat intake (mostly saturated fats) and gallstones [22]. Similarly a French study showed a positive association between gallstone disease and total and saturated fat intake [16]. Results regarding other fatty acids different from saturated fats are less conclusive. Animal studies have shown that feeding monounsaturated fatty acids (MUFA), compared to saturated fats, may decrease the risk of gallstone formation [23]. Moreover, epidemiological studies suggest that

MUFA may have a protective role on gallbladder disease [24], while others have shown an opposite effect [16,17].

Studies of polyunsaturated fatty acids n-6 (PUFA n-6) consumption on gallbladder disease are fewer and inconclusive. One study in animals reported that PUFA n-6 compared to saturated fat induced changes in biliary lipid secretion that could reduce gallstone formation [25]. However, another study in animals receiving a lithogenic diet did not detect modifications in biliary lipid composition and cholesterol gallstone formation when PUFA n-6, MUFA or saturated fat were fed as the main fat component. Instead, when PUFA n-3 was used, an increase in biliary phospholipid concentration was observed [26].

Experimental studies have brought attention to fish oil as a protective factor. It has been reported that populations consuming a diet rich in fish oil and n-3 fatty acids exhibit a low incidence of cholesterol cholelithiasis [27]. In animal studies, feeding a lithogenic diet with fish oil supplementation induced a significant reduction of both cholesterol monohydrate crystal

nucleation and gallstone formation [28,29]. Similarly, Booker *et al.*, noted a significant decrease in the incidence of biliary cholesterol crystals in prairie dogs fed a lithogenic diet supplemented with fish oil [30]. Human studies have shown controversial results. Berr *et al.* [31] detected that n-3 PUFA decreased biliary cholesterol saturation in the bile of patients with gallstones; however, no differences in the incidence of cholesterol monohydrate crystal or in cholesterol nucleation time were observed. Another study showed that nucleation time was significantly prolonged in patients with gallstones treated with fish oil compared to untreated patients [32]. A more recent study suggested that n-3 PUFA supplementation of obese women on weight reduction treatment could prevent the decrease in nucleation time and the increase of cholesterol saturation index (observed during rapid loose of weight), resulting in the prevention of cholesterol gallstones formation [33]. Different mechanisms may explain the inhibitory effect of fish oil on gallstones formation, such as changes in cholesterol metabolism that could reduce biliary cholesterol saturation [30,31], reduction in cholesterol precipitation by changes in the composition of biliary phospholipids [28,30,31] and reduction in biliary protein concentration [31]. Additionally, a decrease in biliary calcium levels that might also reduce cholesterol precipitation has been reported [34].

Cholesterol and Plant Sterols. It has been hypothesized that a high cholesterol intake may predispose to gallstones disease. However, the results of different studies are contradictory. Diets supplemented with cholesterol have been shown to produce lithogenic bile and gallstones in experimental animals including prairie dogs, squirrel monkeys and hamsters [35–37]. Cholesterol gallstones have also been induced in mice [38–39] by administration of cholic acid plus cholesterol. In contrast, Ho *et al.* found no significant increase in biliary cholesterol saturation after one month of cholesterol feeding to chickens, rabbits and rats [40]. Interpretation of results obtained with cholesterol-fed animal models of cholelithiasis must be cautious, since the amounts of cholesterol fed to the experimental animals are several orders of magnitude over the amount present in the normal human diet.

Studies in human subjects on the effect of dietary cholesterol on biliary lipids have also yielded conflicting results. Den Besten fed 10 healthy normolipemic men with a eucaloric cholesterol-free liquid formula for 3 weeks. Cholesterol (750 mg/day) in the form of egg yolk was added at expense of protein and fat for another 3 weeks. On the high-cholesterol diet, the mean biliary cholesterol saturation increased, four subjects developed lithogenic bile and three developed cholesterol crystals [41]. Grundy and co-workers [42] studied two obese men on diets which were cholesterol-free or contained 3 g of cholesterol daily; they found increased secretion of biliary cholesterol with the high cholesterol diet. Lee *et al.* found an increase in biliary cholesterol saturation with modest increments in dietary cholesterol for short periods in patients with and without gallstones [43]. Dam *et al.* studied nine

healthy female college students before and 3 to 6 weeks after addition of egg yolk (1 or 2 g cholesterol daily) to solid diets, while keeping total amounts of fat, protein and calories content. Unexpectedly, they found no increase in biliary cholesterol saturation with the high cholesterol diet; in fact some of the individuals actually decreased biliary cholesterol saturation [44]. Similarly, Andersen and Helstrom found no change in biliary cholesterol saturation in six normolipidemic women and six hyperlipidemic patients without gallstones, when dietary cholesterol was increased from 300 mg daily to 1.5 g daily [45]. The reason for the discrepant observations may be due partly to interindividual differences in the capacity of intestinal cholesterol absorption in different populations and, or differences among the specific dietary constituents present in the cholesterol-rich diets used in the experiments. DenBesten and colleagues [41] investigated healthy men, using fiber-free liquid formula diets, while Dam and coworkers [44] studied female college students ingesting solid diets. The subjects of Andersen and Helstrom [45] were healthy, middle-aged women and patients with hyperlipidemia.

Similarly, epidemiological studies detected positive [17,46], negative [12,47] or no association [19,20,22] between cholesterol consumption and gallstone disease (Table 1). Unpublished results from an ongoing population-based survey have shown that Chileans unaware of harboring gallstones in their gallbladders consumed a diet with significantly lower amounts of cholesterol than a control group of individuals, as shown in Table 2. This unexpected observation is intriguing because it is not consistent with the majority of the experimental studies in humans [43,46–48] and experimental animals [29–32]. One explanation is that some populations, particularly those of Amerindian origin like Chileans, when exposed to low dietary cholesterol intake, will respond by increasing cholesterol synthesis and the flux of cholesterol into the bile, favoring the formation of supersaturated bile and increasing thereby the probability of forming cholesterol gallstones.

Regarding to the effect of plant sterols on gallstone formation, Goswami *et al.* [48] showed that β -sitosterol prevented gallstone formation in mice fed with a lithogenic diet, presumably by decreasing intestinal cholesterol absorption. Similarly, in apolipoprotein E*3 Leiden transgenic mice, plant stanol ester feeding dose-dependently lowered biliary cholesterol output, indicative of a reduced lithogenicity of bile [49]. Plant sterols, like diosgenin and other sapogenins, induce massive secretion of cholesterol into rat bile [50]. Legumes that contain significant amounts of sapogenins [51] increase biliary cholesterol saturation and secretion in men [52,53] and simultaneously, decreases serum LDL cholesterol concentration. A similar effect is found in bean-fed rats [54], an effect dependent of the sapogenin content of beans [55]. Interestingly, Pima Indians, who have one of the highest prevalence of cholesterol gallstones in the world, consume large amounts of beans [52], a condition shared by Chilean Mapuche Indians (unpublished observations from this Department).

Table 2. Energy Intake and Dietary Variables in Men and Women according to Gallbladder Status in La Florida, Santiago, Chile

	Men			Women		
	Without GD (n = 299)	With GD (n = 62)	<i>P</i>	Without GD (n = 343)	With GD (n = 232)	<i>P</i>
Energy intake (kcal/day)	2901 ± 1069	3108 ± 1363	0.19	2345 ± 943	2274 ± 1037	0.39
Dietary protein (%) ^{&}	11.2 ± 2.8	10.1 ± 2.5*	0.006	11.3 ± 3.2	11.6 ± 3.4	0.41
Dietary carbohydrate (%) ^{&}	65.1 ± 9.8	67.4 ± 9.5	0.09	66.1 ± 9.3	65.7 ± 11.1	0.59
Dietary fat (%) ^{&}	22.1 ± 7.5	20.5 ± 7.9	0.12	23.4 ± 7.8	23.5 ± 9.5	0.9
P/S [†]	1.14 ± 0.64	1.12 ± 0.83	0.83	1.27 ± 0.76	1.32 ± 0.78	0.44
Dietary alcohol (%) ^{&}	2.44 ± 4.8	1.33 ± 2.02	0.08	0.16 ± 0.55	0.19 ± 0.56	0.52
Dietary Cholesterol (mg/day)	277.6 ± 214.3	218.6 ± 148.6*	0.04	204.4 ± 181.1	175.0 ± 125.4*	0.03
Fiber (g/day)	5.5 ± 1.9	5.3 ± 2.7	0.58	5.4 ± 2.3	5.0 ± 2.5	0.08
Legume (g/day)	23.0 ± 22.1	27.3 ± 21.3	0.16	18.5 ± 18.6	16.7 ± 17.4	0.27

The target population of the dietary survey was a group of 2,558 lower middle socioeconomic group of Chilean Hispanics, selected from an urban area of south Santiago (La Florida) [109]. Of these, 1,678 (66%) agreed to participate in the study. A randomly selected sample of subjects unaware of harbouring gallstones detected by ultrasonography and a group without gallstones was included in a dietary survey. Nutritional data were obtained by means of semi-quantitative food-frequency questionnaire with a 24-hour dietary recall. All variables are age adjusted.

[&] = Percent of total energy intake.

* *p* < 0.01.

[†] P/S = polyunsaturated/saturated fatty acid ratio of diet.

Carbohydrates. The effect of carbohydrates on gallstone disease has been evaluated in several studies. In general terms, most of the studies revealed that consumption of refined sugars is directly associated with gallbladder disease (Table 1). Misciagna *et al.* [24] detected that a high intake of refined sugars may increase the risk of gallstone development. This finding was attributed to a higher synthesis of cholesterol in the liver secondary to an increase in insulin secretion. Another large epidemiological study showed a positive association between risk of gallstone disease and carbohydrates consumption analyzed by a food-frequency questionnaire [56]. Similarly, Morman *et al.* [21] in a long term follow-up study of middle aged men detected that consumption of monosaccharides and disaccharides was positively associated to gallstones incidence. They hypothesized that carbohydrates may induce changes in lipoprotein metabolism that induce modifications in the bile composition. However, another clinical study in patients with gallstones did not detect changes in bile composition when a diet rich in refined sugars was administered [57]. In conclusion, most authors agree that a diet rich in refined sugar and beverages containing saccharose could represent a risk factor for gallstones in both sexes. It has been estimated that the equivalent of 40 grams of sugar per day doubles the risk of symptomatic gallstones [56].

Fiber. A large number of epidemiological studies have shown that insoluble fiber intake is inversely associated to gallbladder disease (Table 1). In cross-sectional study in Italy, a significant negative association between fiber intake and gallstones risk was detected [18]. Contrarywise, an increased risk of gallstones for low fiber intake was seen in a prospective cohort study conducted in the Netherlands [21]. Moreover, a clinical interventional study showed that fiber supplementation of obese patients who are losing weight, could prevent gallstones development [58].

Fiber may protect against gallstone formation by speeding intestinal transit and reducing the generation of secondary bile acids such as deoxycholate [59,60], which has been associated with increased cholesterol saturation of the bile [15,61]. It has been reported that fiber supplementation to prairie dogs placed on a lithogenic diet, inhibited cholesterol stone formation by reducing biliary cholesterol saturation [62]. However, it is important to keep in mind that a diet poor in fiber is usually associated with a high intake of refined sugars and/or fat; therefore, an independent effect of fiber on gallbladder disease needs to be carefully analyzed.

Vitamins and Minerals. Vitamin C deficiency in guinea pigs reduces cholesterol 7 α -hydroxylase activity, leading to cholesterol supersaturation of bile and formation of cholesterol gallstones [63]. Consistently, an experimental study in patients with gallstones and supplemented with vitamin C (2 g per day during two weeks) induced changes in bile composition and prolongation of nucleation time, suggesting that vitamin C supplementation may also influence the conditions for cholesterol crystal formation in humans [64]. Data from more than 13,000 American adults showed that serum ascorbic acid levels was inversely related to prevalence of clinical and asymptomatic gallbladder disease among women, but not among men. They also observed that ascorbic acid supplementation among women was associated with a lower prevalence of clinical gallbladder disease [65]. Other epidemiological studies showed similar results [17,66,67]. A small case-control study found an association between lower dietary intake of ascorbic acid and gallbladder disease in women, but not men [17]. Another study, Simon *et al.* [66], examined ascorbic acid supplement use as a correlate of clinical gallbladder disease among postmenopausal women with coronary heart disease. They detected that among women that consumed alcohol, ascorbic acid supplement was independently associated with a 50% reduction in prevalence of

self-reported gallstones and a 62% reduction in cholecystectomy. Thus, the association between ascorbic acid status and cholelithiasis has been reported only in women, and may be the result of a biological interaction between ascorbic acid status and sex hormones [17,66,67]. However, these findings may also reflect the lower prevalence of gallbladder disease among men.

There are few studies on other vitamins and minerals. Calcium has been hypothesized to protect against gallstones by binding secondary bile acids including deoxycholate in the small intestinal lumen, thus reducing the deoxycholate and cholesterol content of the bile. Some studies found an inverse association between dietary calcium and gallbladder disease [17,21,68], but others found no association [20,69–71]. An inverse association has also been observed with dairy products in some studies [21,72,73]. Other authors have reported associations with folate, vitamin E or magnesium deficiency, but these are small and therefore inconclusive studies [17,70].

Alcohol. Several epidemiological studies have indicated a reduced risk for gallstones in subjects with moderate alcohol consumption [19,22,56,74–76]. Alcohol lowers bile cholesterol saturation with the result of a reduction in cholesterol gallstone formation. A protective effect of alcohol against gallstones has also been explained by an increased conversion of cholesterol to bile acids and by alterations in the type of bile acids undergoing enterohepatic circulation [76–78]. In addition, it has been shown that low plasma high density lipoprotein (HDL) cholesterol concentrations increase the risk for the formation of cholesterol stones [79]. Therefore, increase of HDL cholesterol plasma levels, induced by moderate alcohol consumption [80,81], may reduce the risk of gallbladder disease. In contrast, alcoholism is a major risk factor for the development of liver cirrhosis, which on its own is associated with pigmentary gallstones [56].

Coffee. Coffee affects several hepatobiliary processes that are involved in cholesterol gallstone formation. Coffee components stimulate cholecystokinin release [82], enhance gallbladder motility [82,83], inhibit gallbladder fluid absorption [84], decrease cholesterol crystallization in bile [85], and perhaps increase intestinal motility [86]. Moreover, coffees diterpenes may down-regulate the hepatic low density lipoprotein receptor [87] and decrease 3-hydroxyl-3-methylglutaryl Co A reductase activity [88]. Thus, metabolic studies suggest that coffee consumption may influence gallstone formation [82–88]. Epidemiological studies are not conclusive; some studies have shown a positive association between coffee intake and risk of gallbladder disease [89–95], one study in both genders reported an odds ratio of 0.62 for any *versus* no coffee drinking [89]. One study in men observed a relative risk of 0.67 comparing ≥ 4 cups of coffee per day with abstention from coffee [96], and another study in women found a trend to decrease prevalent gallbladder disease with increasing coffee consumption [91]. A recent survey, in a cohort of almost 81,000 women followed during 20 years, detected a lower risk of cholecystectomy in women that consume caffeinated coffee; a consistent intake

of ≥ 4 cups of coffee per day was associated with a 25% overall risk reduction [96]. However, results of other studies did not support a protective role of coffee consumption on clinical gallbladder disease [72, 97,98]. In addition, it is important to consider the possibility that the observed relationship between coffee intake and gallstones may be attributed to a coffee avoidance between individuals with symptomatic disease or due to the existence of upper gastrointestinal symptoms related to both coffee use and gallstones.

New Leads from Nutrigenomics

In the last few years the identification of a growing list of regulatory genes involved in lipid metabolism has opened new horizons in the investigation of the relationship between diet, lipid homeostasis and disease. Theoretically these regulatory genes related to the regulation of lipid metabolism could also be involved in biliary lipid secretion; they could, therefore, play a role in cholesterol gallstone formation. For this reason, it is possible to hypothesize that diet modulation of these regulatory genes leading to an increase in biliary cholesterol secretion and/or a decrease in the ratio bile salt/cholesterol in bile could favor cholesterol gallstone formation. Evidence supporting this hypothesis is still lacking, but some interesting advances have been reported in this field.

One of the most important groups of regulatory genes involved in lipid metabolism corresponds to sterol regulatory element binding proteins (SREBPs) [99]. This group is formed by three different SREBPs isoforms (SREBP1a, SREBP1c and SREBP2) that directly activate the expression of more than 30 genes controlling production and transport of cholesterol and fatty acids [99]. Interestingly, transcriptional regulation of SREBP-1c, the lipolytic member of the SREBP group, by glucagon and insulin has been reported [100, 101]. Multiple lines of evidence suggest that insulin's hepatic stimulatory effect on fatty acid synthesis is mediated by an increase in SREBP-1c [99–101]. SREBP-1c expression is also modulated by polyunsaturated fatty acids since rodents fed diets enriched in this type of lipids showed reduced SREBP1-c mRNA levels and low rates of lipogenesis [102]. Although various transgenic mice that overexpress the three different SREBPs isoforms have been generated [99] and although their role in lipid metabolism was well-evaluated, biliary cholesterol secretion and gallstone formation has not been investigated yet.

The adopted orphan nuclear receptor group of regulatory factors that functions as heterodimers with the retinoid X receptor (RXR) also has an important role in lipid metabolism and potentially in the regulation of biliary lipid secretion. Members of this group include receptors for fatty acids (PPARs), oxysterols (LXR), bile acids (FXR) and xenobiotics such as the steroid xenobiotic receptor/pregnane X receptor (SXR/PXR) [103]. The receptors of this group are activated by lipid ligands; theoretically, therefore, they could be modulated by dietary intake. These receptors appear to function as lipid

sensors that activate metabolic pathways intended to maintain homeostatic balance by controlling the expression of key genes involved in lipid production, transport and elimination [103,104].

LXR could have a potential role in cholesterol gallstone formation since this transcription factor regulates *cyp7A1*, the limiting enzyme in bile salt synthesis, in response to cholesterol feeding in mice [103,104]. However, in the LXR α $-/-$ mice biliary cholesterol or gallstone formation has not been evaluated yet. Interestingly, LXR also activates SREBP-1c and lipogenesis [99,103,104], and some evidence suggests that this activation can be antagonized by certain unsaturated fatty acids [105]. FXR is an essential regulator that maintains bile acid homeostasis [103,104,106]. This factor regulates key genes involved in bile acid biosynthesis and transport and thus could potentially have a role in cholesterol gallstone formation.

PPAR α is another key lipid sensor since this factor regulates the expression of several genes related to fatty acid oxidation and peroxisome proliferation. Recently, it has been demonstrated that PPAR α also modulates positively the expression of several genes related to biliary lipid secretion in association with fasting, including the hepatic canalicular phospholipid and cholesterol transporters ABCB4 and ABCG5/G8, respectively, FXR and LXR [107]. PPAR α regulation of these genes was associated with increased amounts of phospholipid and bile salts in bile. Since hypolipidemic compounds, like fibrates, activate PPAR α , these could potentially modulate biliary lipid secretion and gallstone formation. Interestingly, in humans administration of fibrates reduces *cyp7a1* expression and increases the risk for gallstone formation [108]. PPAR γ gene is also a potential candidate involved in gallstone pathogenesis since this nuclear transcription factor regulates the expression of multiple genes involved in lipid metabolism, and it is associated with hyperlipidemia, obesity, insulin resistance and type 2 diabetes [7,8], all factors that are related to cholesterol gallstone disease in humans.

Conclusion and Perspectives

It is apparent that energy intake related to obesity and energy storage represents an important risk factor for the formation of gallstones, presumably through hyperinsulinism. Of the specific dietary constituents, consumption of simple sugars and saturated fat has been found consistently associated to a higher risk of gallstone. Interestingly, elements of a “healthy” diet also recommended for the prevention of atherosclerosis, including fiber and moderate consumption of alcohol, also appear to reduce the risk of cholesterol gallstone formation. We envision for the near future that new knowledge related to the molecular genetic regulatory mechanisms of hepatic cholesterol metabolism and secretion into bile and the modulatory interactions between dietary lipids and orphan nuclear receptors will open new leads to better understand the role of diet in cholesterol gallstone formation.

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