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DIETARY IMPACT ON BILIARY LIPIDS AND GALLSTONES[1]

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CONTENTS

[1]Abbreviations: LI, lithogenic index; FC, free cholesterol; TGSR, triglyceride secretion rate; CE, cholesteryl ester; PUFA, polyunsaturated fatty acid; BA, bile acid; EFAD, essential fatty acid deficient; TG, triglyceride; LRP, LDL receptor-related protein; LDLr, LDL receptor; CSI, cholesterol saturation index; IgA, immunoglobin A.

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INTRODUCTION

This review summarizes current information concerning the dietary modulation of gallstone formation. The emphasis is on cholesterol gallstones associated with Western diets as opposed to less frequently encountered pigment gallstones. Although the genesis of cholesterol gallstones is multifactorial (19), basic physiological correlates associated with dietary modulation of biliary lipid metabolism (bile acids, phospholipids, cholesterol) are discussed in relation to gallstones. Several reviews on the subject of gallstones are available (24a, 27, 34, 58), and some include nutritional aspects of the problem (15, 36, 37, 85, 87, 122).

GENERAL PHYSIOLOGY OF ENTEROHEPATIC CIRCULATION

The liver and gut interact via the enterohepatic circulation to transport important lipid factors in a relatively closed circuit. Absorptive processes by the intestine and secretory processes by the liver are involved. Bile acids and lecithin secreted by the liver reach the intestine via the bile duct to solubilize fat and fat-soluble nutrients, thereby making their absorption possible. To that end, the liver must synthesize bile acids from free cholesterol as well as phospholipids (lecithin) from glycerol, choline, and fatty acids, including an important contribution from specific polyunsaturated fatty acids. In addition to these two lipids, bile contains a variable amount of free cholesterol. Lecithin is secreted in the form of lamellae (140) or together with free cholesterol as vesicles, via the smooth endoplasmic reticulum and Golgi apparatus of hepatocytes (29, 44, 94). Bile acids are secreted separately in much greater volume into the bile canaliculus, whereupon they disperse the lamellae and vesicles to form the micelles necessary for solubilizing and absorbing fat in the small intestine (59).

The ratio of bile salts to lecithin relative to the total lipid concentration is the overriding factor modulating the lithogenicity of bile (27). In other words, if cholesterol solubilization is inadequate, gallstones are apt to form. The relationship between the secretion of bile acids and either phospholipids or free cholesterol has been referred to in "linkage coefficients" representing important ratios of the secreted molecules that generally become distorted in lithogenic bile (59). The linkage coefficient between bile acid and free cholesterol secretion is highly species dependent (relative biliary cholesterol secretion is low in rats, high in humans) and, within certain species, readily affected by diet.

Bile Acids

Bile acids are formed exclusively in the liver via the rate-limiting initial hydroxylation of free cholesterol at the 7α position (161). The enzyme, cholesterol 7α-hydroxylase, forms the basic dihydroxy steroid molecule that gives rise to the primary bile acid known as (3,7-OH)chenodeoxycholic acid (cheno). A second hydroxylation at the twelfth position of the steroid nucleus (12C) leads to the other primary bile acid, cholic acid (3,7,12-OH). These bile acids rapidly conjugate with glycine or taurine by an amide linkage to form the respective conjugates of cholic or chenodeoxycholic acids, which are immediately secreted into bile. Taurine conjugation predominates if taurine is readily available from synthesis, but especially if the diet is rich in taurine (52). Since taurine is not available in plants, herbivorous animals (e.g. rabbits) tend to be glycine conjugators, whereas carnivores (e.g. cats) are mainly taurine conjugators. Species also vary substantially in their ability to synthesize taurine (54).

Once in the intestine, the distinct polarity of the two conjugates differentially impacts fat absorption. Thus, the taurine conjugates are more stable at low pH and less apt to be deconjugated during intestinal transit. They remain intraluminally and sustain fat absorption throughout the small intestine until they are actively absorbed in the terminal ileum. By contrast, glycine conjugates are more apt to be passively absorbed along the entire gut. Taurocholate is most hydrophilic and least able to solubilize cholesterol, whereas glycodeoxycholate and glycocheno (both dihydroxy bile acids) are least hydrophilic and solubilize fat and cholesterol more effectively (8).

The 3–5% of bile acids reaching the large bowel during each of the 5–7 daily circulations of the bile acid pool can be modified by bacterial flora that remove the 7-OH group, forming the secondary bile acids, deoxycholic (3,12-OH) and lithocholic acid (3-OH). About one third of these secondary bile acids are passively absorbed from the colon to be reincorporated into the bile acid pool. The amount depends, in part, on the mass of primary bile acids reaching the colon, colonic microflora activity, the concentration and type of dietary fiber, and fecal transit time. The bile acids in the gallbladder, gut, and liver constitute the bulk of the pool size, which is an important variable in lithogenesis, since gallstones often develop in individuals with a bile acid pool size insufficient to solubilize the biliary cholesterol present (19).

Bile acid synthesis and secretion follows a circadian rhythm that is closely associated with the feeding cycle and reflux of bile acids via the portal vein (113, 161). The nadir of 7α-hydroxylase activity occurs during fasting with peak activity postprandially. Although the ratio between primary bile acids may vary in normal individuals, an association between specific bile acids and cholesterol gallstones can be inferred from the literature. Healthy human bile

tends to have slightly more cholic acid than chenodeoxycholic acid, but this distribution is often reversed in patients with cholesterol gallstones (23, 59, 60). Hamsters (30) and prairie dogs (31) develop an exaggerated cheno profile as they develop cholesterol-induced gallstones, but cheno-supplemented hamsters fed a fat-free diet develop gallstones at an accelerated rate (37). On the other hand, cheno supplementation in humans desaturates bile and dissolves gallstones, in part, by decreasing hepatic cholesterol synthesis (2, 68, 124, 163).

The primary precursor pool for bile acid synthesis is thought to derive from lipoprotein cholesterol (51, 125, 127), but the exact mechanism of cholesterol delivery is unclear (Figures 1 and 2). Empirical evidence suggests that the two primary bile acids may receive substantial free cholesterol from different lipoproteins. Free cholesterol enters via the LDL receptor (LDLr), arriving with LDL under normal circumstances, and appears to be associated with cholate synthesis and glycine conjugation. By comparison, cheno synthesis appears to be enhanced by free cholesterol entering via HDL_2 or the apoE receptor (LDL receptor-related protein or LRP receptor), presumably during periods of lipoprotein cholesterol overload (HDL_2 postprandially) or from apoE-rich lipoproteins (HDL_2, IDL, or VLDL remnants) during dietary saturated fat feeding or cholesterol loading (9, 20, 48, 86, 88, 143). Cheno seems to prefer taurine conjugation. When taurine is available (high synthesis in rat or high diet source for carnivorous cat), it preferentially conjugates most bile acids, i.e. taurocholate predominates in normal cats and rats. In herbivorous animals such as rabbits, with a large cecum, minimal lipoprotein lipid pool and good LDLr activity, the main bile acid is glycodeoxycholate.

Humans and hamsters (the most widely studied model of cholelithiasis) have slightly more cholate than cheno, and glycine conjugation predominates under normolipemic conditions. A cheno profile typically develops as they become hyperlipemic from dietary excesses, in part because their relatively limited LDLr activity under normal circumstances is quickly down-regulated owing to their inability to compensate cholesterol balance by depressing an already marginal hepatic cholesterol synthesis (158). Furthermore, both hamsters and humans (women) have relatively generous HDL_2 profiles. The biological implications of the bile acid profile are unclear, but elucidation of the point may be revealing in terms of gallstone risk and comparative aspects of cholesterol flux across the liver (see below).

Lecithin

Lecithin (diacylphosphatidylcholine) is the predominant phospholipid in bile of humans and other animals, representing 15 to 25% of the total biliary lipids. In bile lecithin the sn-1 fatty acid tends to be the least hydrophobic, palmitic acid (16:0). The sn-2 position is unsaturated, generally with oleic

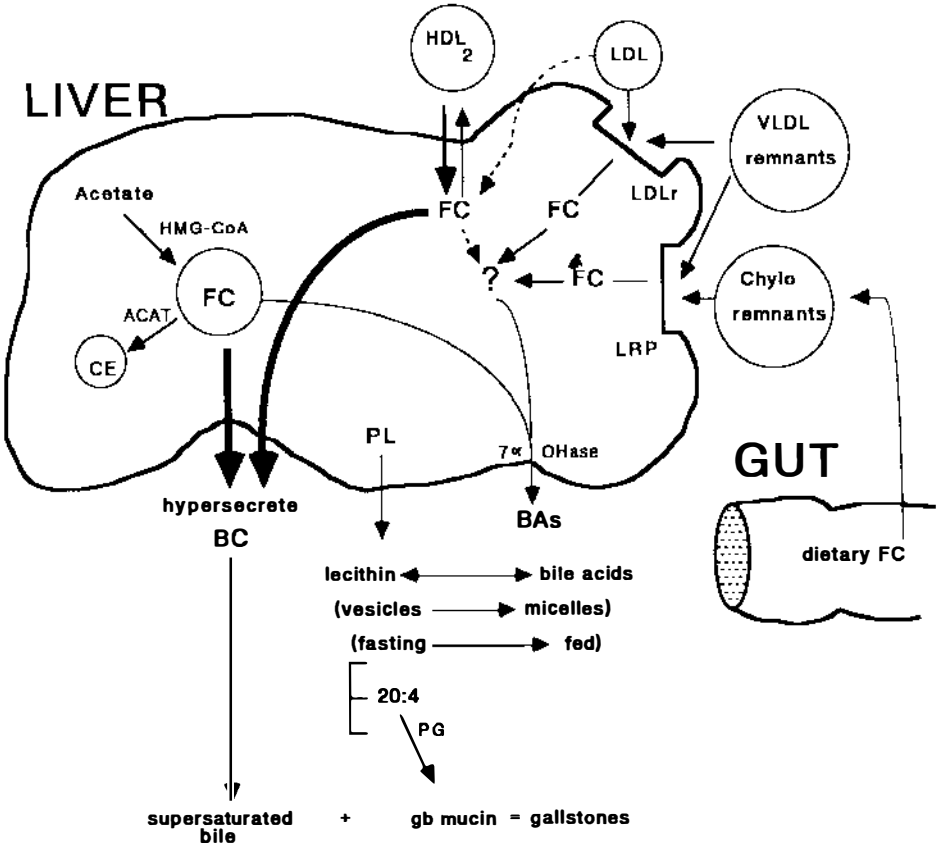

Figure 1 The basic metabolic determinants of hepatic biliary lipid secretion and cholesterol gallstone formation are depicted. The key variable is hypersecretion of biliary cholesterol (BC), which depends on the size and flux of the hepatic free cholesterol (FC) pool. The latter derives from free cholesterol returning to the liver via lipoproteins (LPs) plus newly synthesized cholesterol via HMG-CoA reductase activity. BC output can be modulated by diverting FC into cholesteryl esters (CE) via ACAT or into bile acids (BAs) via 7α-hydroxylase activity (7α-OHase). Lipoprotein-FC can enter the liver at least four ways; via the LDL receptor (LDLr), via apoE-rich LPs through the LDL receptor-like protein (LRP), from HDL_2-FC "exchange," or via nonreceptor-mediated LDL uptake *(dotted arrow)*. The exact distribution of these FC sources is uncertain, but HDL_2-FC is presumed to strongly influence BC secretion. Gallstones develop when BC is excreted disproportionally to phospholipids (PL) and bile acids (BAs), thus causing supersaturation of bile. Increasing arachidonate in biliary lecithin enhances gallbladder mucin production through increased prostaglandin (PG). Mucin acts as a nucleating matrix for gallstone formation. Diet manipulation impacts most of these variables (see text).

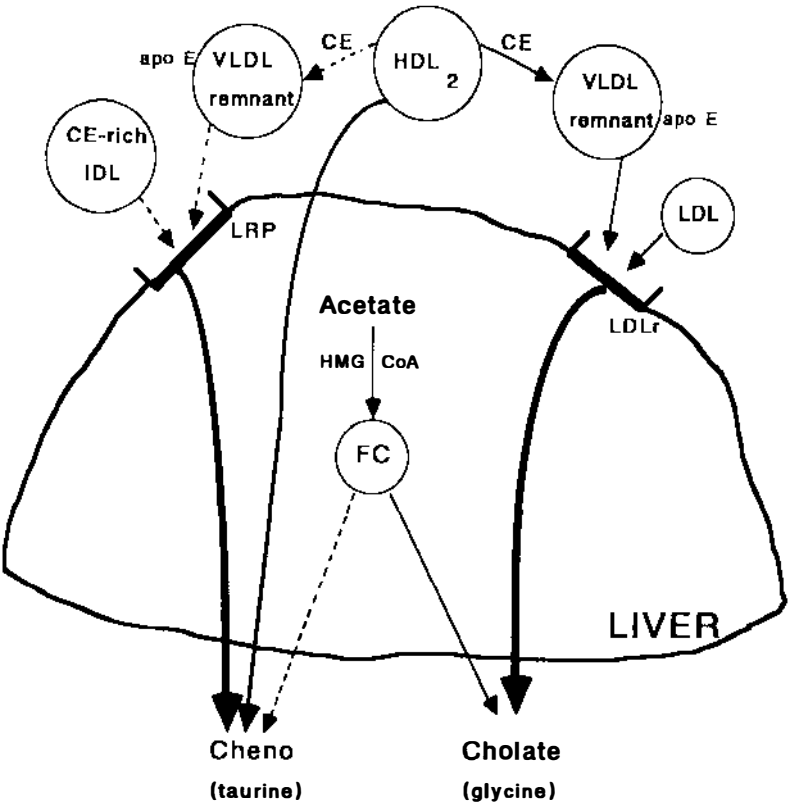

Figure 2 A scheme for the putative disposition of lipoprotein-free cholesterol (LP-FC) and newly synthesized cholesterol in the genesis of primary bile acids is depicted. The literature suggests that LP-FC, especially HDL_2-FC, represents the major precursor pool for bile acid synthesis. Under normolipemic conditions in most species, LDL receptors provide the route for LDL and VLDL remnant uptake that feeds preferentially into the cholate pool, which tends to be mostly glycine-conjugated. During enhanced (or increased) VLDL catabolism, HDL_2 increases and CE overflow into VLDL (IDL) occurs via transfer from HDL_2 *(broken arrow)*. Cholesterol uptake by the liver is increased by HDL_2 directly (process unclear) or indirectly via the apo E receptor (LRP). The LP-FC entering by this route putatively favors cheno synthesis, which tends to be taurine-conjugated. Gallstones develop in the second case, in part, because much of the HDL_2-FC appears to be secreted directly into bile (see Figure 1).

(18:1), linoleic (18:2), or arachidonic acid (20:4). The amount and exact fatty acid profile varies with species and diet. Lecithin composition is important because fatty acid saturation can greatly modify the micelle-vesicle equilibrium in bile to influence biliary cholesterol solubilization (33). In fact, nascent lecithin lamellae may be an important cholesterol carrier in bile (140). In addition, elevated arachidonic acid content can stimulate mucin production by the gallbladder mucosa, which enhances cholesterol crystal formation (28).

Biliary Cholesterol

Free cholesterol represents the least concentrated lipid in bile (1–5 mol% in healthy human bile, but generally < 1.0 mol% in most mammalian biles). Like bile acids, the principle source of biliary cholesterol appears to be lipoprotein free cholesterol coupled with a modest contribution from the newly synthesized pool of cholesterol. A growing body of evidence suggests that in humans (125, 126), rats (20, 116), and monkeys (131, 143), HDL_2-FC is the primary donor to the biliary free cholesterol pool. This contribution may be rather direct, bypassing general mixing with the hepatic intracellular pool of free cholesterol by lateral transfer through the cell membrane for direct secretion into the bile canaliculus (116, 131). Plasma cholesteryl esters, on the other hand, appear to contribute minimally to biliary sterols (17).

Biliary Proteins

In addition to lipids, a number of proteins, the character and importance of which are increasingly appreciated (24, 28, 50, 114, 139), are secreted in bile. Albumin is the most prevalent, constituting over 70% of bile protein. IgA, secretory protein, and apolipoproteins AI, AII, CIII, and E are also present, but their concentration is low and their role unknown (75, 136). Apo E and apo AI are the most prevalent apolipoproteins in nascent bile of hamsters (145). Although apolipoproteins are potential antinucleating proteins in bile, their functional role in vivo as a factor in the solubilization of biliary cholesterol is relatively unexplored (95). In vitro apo AI in low concentrations can delay the shift from micelles to vesicles, thereby enhancing the cholesterol-solubilizing capacity of bile acids (24a, 43). Immunoglobins (IgM, IgA) in bile can serve as pronucleating agents (53). Similarly, mucins and other glycoproteins in gallbladder bile are thought to provide a nucleating matrix for cholesterol crystal formation (50, 139), whereas other proteins inhibit crystalization (24). No evidence has been presented to indicate that the concentration of biliary proteins is affected by diet except indirectly by the fact that dihydroxy bile acids stimulate mucin secretion (105).

Bile Secretion

Bile secretion is stimulated by food intake. The presence of food in the duodenum (especially fat) induces secretion of cholecystokinin (CCK) by mucosal endocrine cells which, in turn, causes contraction and release of bile by the gallbladder (117). The failure of gallbladder contraction can result in stasis of gallbladder bile and can enhance the formation of biliary "sludge," characterized by the accumulation of a lipid-mucin-proteinaceous debris with calcium bilirubinate salts that generally precedes gallstone formation (82). Physiological events associated with digestion and absorption stimulate he-

patic bile synthesis and secretion to effectively increase the concentration of biliary micelles, i.e. phospholipid, bile acid, and free cholesterol, and decrease the relative biliary cholesterol concentration. As the postabsorptive condition develops, micelle formation gives way to biliary vesicles, which are phospholipid and cholesterol-rich but bile acid-poor (106).

Because vesicles do not solubilize cholesterol as effectively as micelles, they increase the chance of cholesterol crystalization during the fasting phase of the diurnal feeding cycle (44, 62). This is especially true in cholesterol gallstone disease, as bile acid and lecithin secretion rates tend to be depressed and cholesterol secretion rates elevated (29).

Gallstones

The pathogenesis of cholesterol gallstones involves a complex set of variables affecting the relative concentration of bile acid, phospholipid, free cholesterol, and protein during their collective flux through the biliary system. In essence, the supersaturation of bile with cholesterol results from increased biliary cholesterol secretion (28) and/or decreased secretion of bile acids and, to a lesser extent, lecithin. In addition to lipid changes, nucleating and/or antinucleating factors, coupled with stasis of gallbladder bile, are important considerations, since supersaturated bile does not always form crystals or gallstones (62).

BILIARY LIPID HOMEOSTASIS AND DIET

Since the above discourse indicates that the relative flux of cholesterol through biliary lipids is the critical issue in cholesterol gallstone disease, it is relevant to review nutritional factors that impact this relationship. In fact, diet affects most of the secreted components in bile.

Caloric Intake and Lipoprotein Profile

The most critical variable in lithogenic bile formation is the relative mass of free cholesterol fluxing through bile. In humans this correlates with the absolute load of calories consumed (89, 121, 122) and the resulting lipoprotein cholesterol that must eventually return to the liver and pass into bile as bile acids or, failing that, as free cholesterol itself (63). Not surprisingly, obesity represents the greatest risk factor for gallstones. Females whose body mass index (BMI) exceeded 32 have 6-times the risk of those with a BMI <22. Equally remarkable was the increased risk of gallstones associated with increasing caloric consumption in nonobese women in this cohort (89). However, short-term overconsumption by clinically normal, nonobese persons does not seem to be associated with increased cholesterol secretion (15),

in contrast to the situation for persons afflicted with gallstones and fed a high level of calories and protein (121).

The association of gallstones with obesity (and caloric overconsumption) presumably reflects cholesterol production, or flux, which is substantially elevated in the obese and gallstone patients (15, 119). This is further complicated by high insulin levels in obesity (63). Hyperinsulinemia itself is a risk factor for gallstones (133), possibly because insulin is known to stimulate hepatic cholesterol synthesis (99). Increased hepatic cholesterol synthesis and food intake have been linked to biliary cholesterol secretion in humans in certain circumstances but not in others (19, 112, 119). On the other hand, inhibition of cholesterol synthesis by lovastatin in humans depressed bile acid synthesis and secretion, but not biliary cholesterol secretion (98). However, in cholesterol-fed prairie dogs lovastatin actually prevented gallstone formation (123).

In essence, the liver of obese persons must cope with an increased flux of cholesterol derived both from newly synthesized cholesterol associated with elevated hepatic lipoprotein secretion (VLDL) as well as from an increment of exogenous cholesterol entering the lipoprotein cholesterol pool from the diet. Unlike rats, the ability of humans to dispose of this expanded cholesterol pool is limited by our ability to synthesize bile acids. As a consequence, free biliary cholesterol rises to dangerous concentrations, i.e. the cholesterol solubility index (CSI) or lithogenic index (LI) of gallbladder bile becomes supersaturated (>1.0) and readily exceeds the solubilization threshold, setting the stage for cholesterol nucleation and stone formation. By contrast, the rat (and most other species) readily convert any excess cholesterol to bile acids so that the biliary cholesterol concentration seldom approaches the saturation point.

Since the absolute mass of cholesterol fluxing through the liver into bile acids or biliary cholesterol depends, in part, upon energy intake and the lipoprotein cholesterol returning to the liver, dietary modification of the lipoproteins, both in terms of their relative distribution (VLDL, IDL, LDL, HDL_2, HDL_3) as well as their absolute mass, would appear to have an important bearing on lithogenesis. Unfortunately, minimal definitive information of a clinical nature is available on this subject (143).

The lipoprotein profile in persons with cholesterol gallstones is variable and probably complicated by gender differences. Correlations between gallstones and both depressed HDL (high VLDL) (41, 107, 134, 148a, 150, 151) and slightly elevated HDL (93) profiles have been reported in women and men, respectively. Although the LDL-C concentration appears to be related to the biliary cholesterol concentration in normal people (151), no correlation seems to exist between high LDL-C (type IIa) and gallstones. On the other hand, type IV individuals with elevated VLDL-TG and LDL-C have a high in-

cidence of gallstones (41, 46, 63). This concurs with human data correlating elevated VLDL-C with the CSI of bile (4, 107, 134) as well as with recent findings in cholesterol-fed hamsters that a VLDL-C/HDL-C ratio greater than 1.0 was highly associated with cholesterol gallstone formation (56). In both humans and hamsters under these conditions a cheno profile predominates, suggesting enhanced flow of lipoprotein cholesterol into the liver via non LDLr pathways (see Figures 1 and 2).

The association between gallstones and excessive caloric intake may also reflect increased cholesterol delivery to bile via VLDL remnants and HDL_2 because remnants and the HDL_2/HDL_3 ratio increase postprandially (105a, 143), which could represent a significant portion of the day in individuals with excessive energy consumption.

Rapid weight loss in the obese presents a high risk for lithogenic bile formation, but the lipoprotein dynamics of this situation have not been adequately characterized. It may seem ironic that severe caloric restriction and precipitous weight loss (the metabolic antithesis of obesity) should also induce a high degree of bile supersaturation and rapid gallstone induction (21, 84, 135). Presumably reverse transport of cholesterol as HDL_2 from the shrinking adipose pool (49) is coupled with enhanced biliary vesicle formation; and reduced micelle formation associated with decreased bile acid secretion is known to occur during prolonged periods of fasting, frequent dieting (137), or extended parenteral nutrition (96, 106). In addition, a reduced rate of enterohepatic circulation is associated with decreased gallbladder contraction and increased bile stasis (15, 84, 135), which are thought to increase cholesterol precipitation and gallstone nucleation.

The relevance of the lipoprotein profile to lithogenic bile is also supported by experiments with animals having HDL_2 as a prominent lipoprotein. The Brazilian squirrel monkey, but not the Bolivian strain, is prone to cholesterol gallstones when fed cholesterol. The susceptible Brazilian strain exhibits an HDL_2 profile (like women) while the resistant strain presents an HDL_3 profile (like men) (109, 110). In African green monkeys fed cholesterol-enriched diets, the total HDL-C also correlated well with bile lithogencity (132). Furthermore, a PUFA- and cholesterol-rich diet in these monkeys yielded a higher lithogenic index than the comparable saturated fat diet, a response similar to that of the gallstone-susceptible squirrel monkey but unlike the response of the resistant cebus monkey (9). In African green monkeys, PUFA plus cholesterol reduced the total HDL-C but increased the relative percentage of the HDL_{2a} subfraction compared to expansion of the HDL_{2b} subfraction by saturated fat and cholesterol (11). The hepatic CE pool in African green monkeys also increased with the PUFA-cholesterol diet (70), and in cebus monkeys the circulating cholesterol redistributed from the plasma (coconut oil-fed) into the enterohepatic circulation (corn oil-fed) (143) because polyun-

saturated fatty acids up-regulate the hepatic LDL receptors, thereby shrinking the plasma LDL cholesterol pool (103).

The relationship between HDL_2 and gallstones is further supported by studies in the Syrian hamster, which develops cholesterol gallstones under two experimental conditions, i.e. during cholesterol supplementation of a low-fat diet or, less predictably, when all fat and cholesterol are removed from the diet to induce essential fatty acid (EFA) deficiency (6, 30, 37, 55, 72). Although these two nutritional paradigms would seem diametrically opposed, they accomplish the same end by increasing the net flux of cholesterol across the liver and the relative concentration (mol%) of biliary cholesterol. In the EFAD hamster model this is the consequence of exaggerated fatty acid synthesis and a 2–3 fold increase in the hepatic trigylceride secretion rate (55), with the attendant increases in cholesterol synthesis needed for VLDL production (66) and HDL_2 reflux to the liver. In the cholesterol-supplemented model the dietary overload reaches the lipoprotein pool, dramatically expanding HDL_2 initially, followed by expansion of the VLDL cholesterol pool. Eventually, the hepatic FC and CE pools are increased and lithogenic bile and gallstones ensue. A minimum cholesterol concentration of 0.85 mg/kcal of diet appears to be the threshold at which biliary cholesterol saturation (CSI $>$ 1.0) is evidenced in most hamsters (56).

EFAD, obesity, and abrupt weight loss have a common metabolic theme in that all three include extreme reflux of free fatty acids through the liver accompanied by elevated trigylceride and VLDL output. VLDL secretion requires free cholesterol synthesis, which is often associated with bile supersaturation. At least two scenarios for lithogenic bile formation could result from such metabolism. First, as a consequence of increased cholesterol synthesis expanding hepatocyte pools, more HDL-FC may be diverted into bile. A second possibility might be increased secretion of the newly synthesized pool directly into bile. The first possibility seems more likely because HDL_2-FC is considered the preferred substrate for biliary cholesterol, and newly synthesized cholesterol favors bile acid synthesis, not the biliary cholesterol pool.

Whereas expanded pools of CE and FC in the liver are generally associated with gallstone development in most animal models (6), this is not particularly true for humans, and a high concentration of hepatic cholesterol can be maintained without gallstone formation in animals (155) if substantial sequestration and removal of bile acids and neutral sterols is achieved by dietary components (e.g. soluble fiber or sequestering agents). On the other hand, the hepatic cholesterol concentration in EFAD hamsters remains low even though hepatic synthesis of cholesterol is greatly elevated. Gallstones often form because biliary cholesterol saturation increases as phospholipid output declines and nucleating factors in the gallbladder are optimized (80,

115). Depressed lecithin output presumably reflects the lack of available polyunsaturated fatty acids needed for lecithin synthesis, since a minimal amount of polyunsaturated fat (2% w/w) added to the hamster diet greatly reduces the incidence of stones (37) and normalizes hepatic triglyceride secretion (55) along with cholesterol synthesis.

The relative depletion of essential fatty acids (18:2) may pertain to certain cases of human gallstone disease as well, since the unfavorable biliary lipid balance that ensues when cholesterol increases and phospholipids decrease in bile has been noted in humans (100). Collectively, the data suggest that a substantial increase in cholesterol transport to the liver (as apoE rich remnant particles and HDL_2, see Figure 1) may accentuate the flux of free cholesterol into bile. This possibility can be inferred from experiments in humans as well as in animals (116, 125, 126, 141, 143).

Dietary Fat

The overall impact of dietary fat on bile lipids in humans is unclear, possibly because gender and the inherent lipid metabolism, e.g., the lipoprotein profile, of the individual (or population) at the time of dietary challenge with polyenes or saturates may affect the outcome. In addition to the substantial energy load contributed by fat to caloric flux through the liver, the degree of fat saturation itself has been examined for its influence on lithogenesis with equivocal results. In one study, autopsied male patients who consumed a high PUFA diet to reduce their high risk for coronary heart disease (and who were also more obese!) had more gallstones than the leaner control population consuming the usual house diet containing more saturated fat (146). The tendency for PUFA to increase risk in men, but decrease risk in women, was noted by others (42). However, large-scale intervention or epidemiological studies either found no effect of dietary fat in men or women (97, 137) or a protective effect among women (89,000 nurses after 4 years) that was associated with vegetable oil consumption and could not be distinguished from the associated intake of vegetable protein (90). Also, female vegetarians were found to have one-half the incidence of gallstones of nonvegetarian controls (108). Thus, a gender difference may exist in the response to PUFA consumption, with a possible increased risk for men and decreased risk for women.

Metabolic studies of the impact of dietary fat on bile lipids are also equivocal, possibly because of inherent differences in lipid metabolism of the host at the time of dietary intervention. Both normal and hyperlipidemic subjects with and without gallstones have been studied. No consistent pattern has evolved (38, 77, 78), but in some cases (mostly men) PUFA has been found to increase the excretion of biliary cholesterol (15) while decreasing the moles percent of bile acids. A more consistent finding is the PUFA-induced increase in the moles percent of cholate (increased LDLr activity) and de-

crease in the moles percent of cheno associated with enhanced glycine conjugation (83, 86). A complicating possibility with most of these studies is that dietary PUFA may transiently increase the flux of biliary cholesterol or bile acids in the acute phase until the body has adjusted to a new steady-state of sterol distribution when the difference may no longer exist for one or both biliary sterols.

The protective role of adding PUFA to the EFAD-hamster gallstone model has been noted for its contribution to biliary phospholipid synthesis and secretion (79, 80, 115). Feeding humans diacyl linolyl lecithin enriched bile lecithin with 18:2 (120). Ironically, high PUFA intake reduces the conversion of linoleic acid to arachidonic acid as well as eicosanoid metabolism (44a), in part by limiting δ-6 desaturase activity. In fact, the substitution of butter for 18:2-rich margarine was found to increase the 20:4 content of bile lecithin in humans (38). Since the availability of arachidonate (20:4) for prostaglandin production and mucin secretion by gallbladder mucosa has been proferred as a risk factor for gallstones (28), the butter results would argue against the hypothesis that dietary PUFA (lower 20:4 content of bile lecithin) enhances lithogenesis, at least from the point of view of gallbladder mucin production. A comprehensive experiment examining a wide range of PUFA effects on gallbladder metabolism (prostaglandins) is not yet available.

On the other hand, fish oil, which interferes with normal prostaglandin metabolism, exerts a protective effect against gallstones in hamsters (36, 37) as well as in cholesterol-fed prairie dogs (18) and African green monkeys (129). This protective action was associated with increased 20:5 (n3) in biliary lecithin, which reduced prostaglandin-initiated mucin production by the gallbladder mucosa. Increased phospholipid and reduced biliary cholesterol saturation were noted as well. Fish oil also greatly reduces hepatic and intestinal triglyceride and cholesterol secretion in man and animals (101), which would ultimately reduce the return of lipoprotein cholesterol for excretion into bile. The n-6 polyenes may also reduce triglyceride synthesis and VLDL output relative to saturated fatty acids (70), and thereby act in a fashion similar to n-3-rich oils. In this sense, in the absence of an extreme dietary cholesterol load, polyenes might reduce lithogenesis both by decreasing lipoprotein cholesterol flux into bile and by favoring synthesis of biliary lecithin low in 20:4. On the negative side, fish oil has been reported to accelerate cholesterol nucleation in bile from healthy subjects to a rate comparable to that in gallstone patients, even though the saturation index decreased slightly (67). In rats fish oil increased biliary cholesterol concentration (12), whereas it prevented gallstones in the mouse model (13).

Whether or not fat saturation exerts an influence on bile acid synthesis and pool size has been examined in monkeys (9, 25, 111, 130). Although fat saturation (aside from n-3 fatty acids) does not seem to alter the lithogenic

index appreciably, polyunsaturates without added cholesterol stimulated bile acid synthesis and secretion in rhesus monkeys (25, 111). Adding cholesterol to squirrel monkey diets depressed the relative distribution of taurocheno by at least 30% relative to taurocholate (9), producing a shift in primary bile acid composition opposite to that found in hamsters (30), prairie dogs (31), and humans with gallstones (23, 59, 60). Polyenes plus cholesterol fed to African green monkeys increased the lithogenic index and gallstones relative to saturated fat plus cholesterol in association with a marked decrease in bile acid secretion (130). The most consistent production of gallstones in monkeys has been in Brazilian squirrel monkeys fed butter and cholesterol (0.9 mg/kcal), although the type of fat did not appear to have an appreciable effect when sufficient cholesterol was present (109).

Dietary Cholesterol

In epidemiological studies, high intake of cholesterol ironically appears to have a protective effect against gallstones (42, 108, 134). In contrast, the experimental consumption of cholesterol by men and women tends to increase the relative concentration of biliary cholesterol and decrease the moles percent of bile acids in both healthy persons (40, 81) and subjects with gallstones (81). Women have not responded to a cholesterol challenge in other studies (5, 37).

Feeding excessive cholesterol (> 0.85 mg/kcal or 2 g/day human equivalent) is essentially the only way to regularly induce gallstones in most animal models (see section on models below). Under these conditions bile is typically enriched with cholesterol and reduced in bile acids (e.g. see 56). A possible mechanism would be that inhibition of hepatic cholesterol synthesis impairs bile acid production in a manner similar to the coordinated inhibition of both cholesterol and bile acid synthesis by lovastatin in humans (98), while apoE-rich lipoproteins deliver the excess absorbed cholesterol directly to bile (Figure 1).

Ironically, adding dietary cholesterol to the EFAD hamster model decreases the incidence of cholesterol gallstones while enhancing pigment stone formation (37). This presumably reflects absorbed cholesterol causing feedback inhibition of hepatic cholesterol synthesis.

Dietary cholesterol also affects the amount and composition of lecithin in bile (38, 115). When prairie dogs are fed cholesterol, arachidonic acid in bile lecithin increases while linoleic acid decreases. In hamsters and patients with untreated gallstones, palmitic and arachidonic acids increase while linoleic acid decreases (1, 26, 27, 159, 160). The more hydrophobic bile acids (e.g. deoxycholate, cheno) are associated with biliary excretion of 20:4- and 18:0-rich lecithins, and arachidonic acid in bile might be expected to induce the production of prostaglandins and mucin (105, 159).

Dietary Protein

In humans no direct evidence indicates that the amount or type of protein consumed affects cholesterol gallstone incidence; however, epidemiological studies reveal a lower incidence of gallstones in vegetarians than in omnivores (108), and vegetable protein consumption may have a protective effect against gallstone occurrence in women (90). Distinguishing vegetable protein from the possible contribution by vegetable oil or fiber is difficult in such cases.

In hamster studies when dietary casein was replaced by soy protein or other vegetable proteins a "decreased incidence of gallstones" resulted (147), but the character and diversity of stones (cholesterol or pigment) has not been sufficiently documented to determine the protein impact on pure cholesterol lithogenesis.

Theoretically, it is possible that dietary sulfur amino acids, including taurine, could influence bile acid conjugation and secretion and/or lipoprotein clearance rates (144), but a relationship between taurine and cholesterol stones in humans is not evident from the literature. Although feeding taurine tends to increase the taurine conjugation of bile acids (14, 52, 76, 138, 141, 148), neither the biliary lipid profile nor bile lithogenicity appears to be affected in taurine-supplemented humans (52, 54, 156). A possible exception is the ameliorating effect on gallstones of a high taurine intake (5%) in mice consuming a lithogenic diet (162).

In infant monkeys the depletion of available taurine exerted a substantial influence on the bile acid profile without affecting the lithogenic index in either cebus or cynomolgus monkeys (141). Specifically, taurine-depleted cynomolgus reduced taurine conjugation of bile salts while increasing glycine conjugates, but the loss of taurine (gain in glycine) was almost exclusively observed in the cholate, and not cheno pool; the latter seemed to be preferentially conserved as taurocheno. Taurine depletion was linked to a relative increase in biliary phospholipid and a 4-fold increase in the taurocheno/taurocholate ratio. These data suggest that distortions in the bile acid profile (including conjugation pattern) of gallstone-bearing individuals may not be causative but rather may be indicative of abnormal hepatic sterol metabolism.

Why taurine appears closely linked to cheno metabolism and glycine to cholate, and whether these associations have any relevance to human gallstones, is unclear. Nevertheless, these relationships have been observed during manipulation of human (86) and monkey (9) bile lipids by dietary fat and of hamster bile lipids by dietary fiber (157). Specifically, humans and Brazilian squirrel monkeys fed corn oil lowered their plasma cholesterol, presumably increasing LDL receptor activity (103), which led to a rise in glycocholate. Coconut oil, which increases LDL, VLDL, and HDL (by down-regulating the LDL receptors), resulted in an expanded taurocheno pool. Thus, a VLDL/HDL_2-cheno-taurine precursor-product linkage appears

to be in contrast to an LDL-cholate-glycine connection. These observations reaffirm the association between gallstones, an HDL_2 profile (women), and an expanded cheno pool, whereas a low HDL_2 profile (males) relies on LDL-C delivery to yield more glycocholate and less gallstones, but more atherosclerosis, from the expanded plasma LDL pool.

Taurine depletion might decrease LDLr activity, divert lipoprotein clearance to HDL_2 for return to the liver, and favor the taurocheno profile observed in monkeys (141). In addition, taurine or cysteine supplementation of HEP-G2 cells increased LDLr activity and bile acid secretion independent of any effect of taurine conjugation, since HEP-G2 cells secrete unconjugated bile acids (144). Stimulation of bile acid synthesis by sulfur amino acids apparently is related to enhanced availability of a sulfhydryl protein (39), which may pertain to the whole animal as well, since hamsters fed a taurine supplement reportedly increased their bile acid output and reduced the biliary moles percent of cholesterol concentration (14). One potential advantage of taurine is that increased conversion of cholesterol to bile acids by taurine (especially taurine conjugates) would increase the opportunity for bile acid removal by naturally occurring dietary fibers, thus facilitating the steady removal of bile acids and diverting hepatic cholesterol from direct secretion into bile (see section on fiber below).

Dietary Carbohydrate

Certain epidemiological evidence suggests that persons consuming highly refined carbohydrates are more apt to have cholesterol gallstones than populations eating more complex forms of carbohydrate (42, 134, 149). However, when confounding factors were controlled in a large epidemiologic study, a carbohydrate effect was not apparent (90) but a protective influence of vegetable protein was noted that could not be separated from vegetable oil.

In contrast to the limited data on humans, the literature describing the impact of carbohydrate on gallstones in hamsters is extensive. Dam (36, 37) was the first to report that the development of cholesterol stones in Syrian hamsters fed the EFAD diet depended on all the carbohydrate being fed as glucose or sucrose, as opposed to other more complex sugars and starches. Lactose was also protective whereas a galactose and glucose mix was not, indicating that the protection afforded by lactose reflected its ability to partially bypass digestion and absorption in the small intestine, to reach the large bowel, and to stimulate bacterial flora activity. Lactose feeding results in lower plasma lipids, especially triglycerides, and increased bile acid turnover and secretion with a favorable impact on the lithogenic index, i.e. decreasing bile cholesterol supersaturation and preventing cholesterol gallstones in the EFAD model or pigment stones in hamsters fed a purified diet with fat added (55, 57). Cholesterol supplementation of the latter diet can eventually exceed the protection against cholesterol stones afforded by lac-

tose, even though lactose may increase large bowel size 3–4-fold (55). To a certain extent various complex carbohydrates (rice flour, cornstarch) exert a similar protective effect depending on the amount of digesta that reaches the cecum.

In the absence of sufficient nutrients reaching the large bowel, cecal flora activity wanes and the cecum atrophies, eventually resulting in diarrhea and death. This "wet tail" syndrome appears in a variety of forms, but is widely recognized as a deterrent to sustained nutritional studies in hamsters (57).

In fact, the cecum is larger than the liver in the suckling hamster at the time of weaning, presumably due to the lactose in suckled milk (K. C. Hayes, unpublished data). Thus lactose may assure the early, rapid development of normal large bowel flora to act as a counterbalance [via bile acid catabolism and generation of short-chain fatty acids to modulate hepatic lipid metabolism (153)] to an expanding lipoprotein cholesterol pool in the newborn. If lactose is continued as the major source of carbohydrate in the post-weaning diet, the cecum continues to outweigh the liver, plasma triglycerides and cholesterol concentrations are reduced, and cholesterol gallstones are prevented or reduced in number (37, 57). Ordinarily, a small amount of complex carbohydrate and an increment of dietary fiber would reach the cecum in mature hamsters to sustain the cecum. Note that once the cecum "reaches maturity" it becomes extremely difficult to induce cholesterol gallstones in the EFAD hamster model (37). Based on relative cecal weight, the "stressed" cecal flora would appear to fare better with dietary PUFA, possibly delivered by the fiber component of the diet (57). Previous reports also demonstrate the differential effect of polyenes and saturates on cultures of large bowel bacteria (104). The protective role of age on gallstone susceptibility in EFAD hamsters may reflect the accumulation of PUFAs in adipose reserves that protect against the metabolic consequences of EFAD. These PUFA stores also may impact the metabolism of the large bowel flora.

Dietary Fiber

From the above discussion on carbohydrates it should be apparent that dietary fiber might influence gallstone incidence via its impact on large bowel metabolism. This hypothesis was first expounded by Burkitt & Trowell (22) for humans when they noted the relative absence of chronic diseases (including diabetes, gallstones, coronary heart disease, appendicitis, diverticulitis) in Third World populations and attributed this to the consumption of complex carbohydrates and fiber. The fiber hypothesis is supported by some epidemiologic data (134), but not by others (100). Dietary fiber includes a wide array of complex substances commonly divided into soluble and insoluble components.

A number of metabolic studies have attempted to establish the fiber-gallstone link in humans (71, 73). Insoluble and soluble fibers exert different

effects. It is known that slow fecal transit time (constipation) is associated with elevated deoxycholate (a secondary bile acid) and gallstones (91). In general, insoluble fibers are good bulking agents and increase fecal transit rate and bile acid removal. Simply doubling the dietary mixed-fiber load (12 to 25 g/per day) increased fecal bile acid output 13% (74). Several investigators have examined the effects of wheat bran with varied results. Humans with gallstones or highly lithogenic bile tend to improve when fed wheat bran, associated with a decrease in deoxycholate, whereas normal persons are not affected (71, 73). However, normal individuals fed high mixed-fiber diets or pectin tend to increase deoxycholate while decreasing cheno (71, 73). Experiments designed to vary bowel transit time (92) indicate that rapid transit decreases the opportunity for colonic flora to generate secondary bile acids. Deoxycholate can be considered lithogenic, in part, because it is associated with increased arachidonic acid in biliary lecithin and because it can transport the most cholesterol into bile (8, 105, 159).

Western diets high in sucrose and low in fiber may also increase gallstones because such a diet is highly glycemic and insulinemic, the latter stimulating cholesterol synthesis (99) in conjunction with elevated triglyceride (VLDL) secretion by the liver. Fiber, especially soluble fibers like pectin and psyllium, lowers the glycemic index and reduce insulin secretion (69), which decreases an important risk factor for gallstones (133).

Soluble fibers (64, 155, 157) also exert an interesting selective removal of taurine-conjugated bile acids by virtue of their gelling action. Because taurine conjugates are not passively absorbed by the small intestine and thus remain longer intraluminally than glycine conjugates, taurine-conjugated bile acids (especially cheno) become entrapped and bulk-removed by fiber gels. In hamsters the net effect was to reduce lithogenic bile and gallstone formation (155). Whether this is applicable to humans is unexplored, although psyllium fed to hypercholesteremic humans does increase LDL receptor activity and decreases plasma LDL without increasing cholesterol synthesis or absorption (7, 47).

Colonic fermentation of nonstarch polysaccharides (including fibers) is a rapidly evolving area sure to expand our knowledge of bile lipid metabolism and lithogenesis. Currently it is unclear how volatile short chain fatty acids, released during bacterial colonic fermentation, impact cholesterol metabolism, but research is actively exploring this aspect of lipid metabolism (153).

Experiments in hamsters have demonstrated the protective role of both soluble (psyllium) and insoluble (lignin, "lactulose") fibers as well as artificial resins (cholestyramine) against cholesterol gallstone formation (16, 118, 155). The mechanism of this protection is not fully appreciated, but fibers fed to hamsters have revealed a variety of effects on the bile acid profile, and cholestyramine is known for its efficient binding of bile acids in the gut (45). Hamsters fed 1% cholestyramine revealed dramatic lowering of chenode-

oxycholate, which appeared to interfere with cholesterol absorption. The result was lower plasma cholesterol, prevention of hepatic cholesterol accumulation, an increase in the cholate bile acid profile, and lower LI while preventing gallstones. By comparison, cholesterol-fed (1.12 mg/kcal) control hamsters experienced elevated lipids, hepatic cholesterol deposition, a high cheno profile, bile supersaturated with cholesterol, and a high incidence of cholesterol stones (155). Psyllium, on the other hand, prevented cholesterol gallstones by the less efficient, but selective removal of taurine-conjugated bile acids. Psyllium was associated with moderately lower blood lipids without prevention of hepatic cholesterol accumulation, but the LI was reduced to the point of minimal supersaturation.

In rats citrus pectin mediated an increase in bile acid secretion that depleted the hepatic taurine pool available for bile acid conjugation and increased glycine conjugation of bile acids (64). Rats fed methionine and taurine in addition to pectin experienced a striking increase in hepatic taurine concentration, and glycine conjugation of bile acids was almost totally replaced by taurine conjugates (65).

Thus, it appears that the fiber effect is multifaceted and potentially extremely complex in its influence on lipoproteins, bile lipids, and intestinal sterols, including metabolism by the large bowel flora.

Alcohol

Although a protective effect of alcohol consumption has been noted in an epidemiologic study (134) as well as experimentally in cholesterol-fed prairie dogs (128) and in humans in terms of the bile LI (152), other experimental results are inconsistent as to possible mechanisms. An acute increase in bile acid synthesis (10) and output with a minimal change in total sterol excretion has been noted in some hyperlipidemic patients (102) and pigs (154), but not in other individuals (35). Again, the metabolic status of the host may be an important variable.

ANIMAL MODELS

Selection of an animal model for gallstone study is limited by the fact that relatively few species readily develop cholesterol gallstones under any circumstance, and none, other than humans, are thought to spontaneously develop supersaturated gallbladder bile (58). The reason for this has not been fully delineated, but a key limitation is the failure of most species to secrete supersaturated bile, i.e. conversion of hepatic free cholesterol to cholesteryl esters and bile acids is sufficient to preclude excess free cholesterol from entering bile (3).

The fact that none of the models (with the exception of the EFAD hamster) develop gallstones without extraordinary cholesterol loading is probably a

serious limitation of their relevance to the actual human experience unless careful monitoring of the prevailing lipoprotein dynamics elicited by the total dietary regimen is included for comparison. As indicated earlier, the influence of PUFA on bile acid secretion may be totally reversed by the presence of excess dietary cholesterol. Since humans are not "cholesterol loaded" in the way animals models tend to be, results from animal studies must be interpreted with care.

Among species whose bile becomes supersaturated with cholesterol feeding (hamster, prairie dog, ground squirrel, and certain monkeys), not all are equally endowed with the "other" prerequisites of sufficient gallbladder mucin production such as sludge formation, bile stasis, nucleating factors, etc, to allow cholesterol nucleation and stone formation once cholesterol supersaturation is achieved (61).

Consequently, only two species are commonly used for gallstone modeling based on their size, availability, and relative cost effectiveness. These are the Syrian hamster (30, 37, 56, 85) and the prairie dog (31), both of which develop lithogenic bile and stones in 3 to 8 weeks depending primarily on the amount of cholesterol fed (0.3–2.0%). Although of limited availability, the cholesterol-fed ground squirrel responds to cholesterol feeding as well.

The Brazilian squirrel monkey fed cholesterol (0.3% or more) is also an excellent model (109, 110), but these monkeys are no longer available for general study. Other monkeys such as the African green monkey (129, 130) and baboon develop stones albeit at a reduced incidence, whereas others such as rhesus, cynomolgus, Bolivian squirrel monkey, and cebus monkeys, often used in cardiovascular research, do not readily form cholesterol stones even when fed cholesterol. The reason for this comparative resistance or susceptibility has not been studied in detail, but the cholesterol-fed cebus (HDL_3 predominates) has a relatively greater bile acid pool size than the Brazilian squirrel monkey (HDL_2 profile), which increases the moles percent of biliary cholesterol under comparable conditions of cholesterol feeding (9). The fasting cebus also has three times the bile acid secretion rate of the cynomolgus, another resistant species (142). On the other hand, Brazilian squirrel monkeys, but not the Bolivian strain, reportedly expand their HDL and VLDL cholesterol pools like the cholesterol-fed hamster (109, 110).

Syrian hamsters have the decided advantage of availability, cost effectiveness, and relative uniformity, but their size (adult 125–160 gm) and inconsistent induction of gallstones can limit their general applicability in certain experiments. The complicating factor of the high incidence of pigment gallstones in hamsters has been noted (37) and described in detail (32, 57). The pathogenesis of pigment stones is relatively unexplored, but the high incidence of these stones serves to underscore the facility with which hamsters develop them. Feeding hamsters purified diets, per se, without added cholesterol, seems to enhance pigment stone formation (32, 55, 57). Many

reports in hamsters are sufficiently vague about the composition of the induced stones to render their results of questionable value in a discussion focusing specifically on cholesterol gallstones.

Until recently (57) the inability to prevent the lethal enteritis "wet tail" in hamsters fed purified diets has limited long-term (several weeks) study of nutritional factors, which necessitates the use of purified diets. Prairie dogs are larger (1–2 kg), but their nutritional requirements and the application of purified diets in this model are poorly defined. Mice hold great promise for study of the genetic variables involved in lithogenesis but they only develop cholesterol stones when fed high (0.4–2.0%) levels of dietary cholesterol, usually with cholic acid added to the diet to enhance cholesterol absorption and depress bile acid synthesis (3).

SUMMARY

Although dietary factors influence bile lithogenicity and gallstone formation, the main dietary effect appears to be indirect, depending on an interaction between caloric consumption and gender-specific aspects of lipoprotein metabolism. Excessive energy intake elicits its deterimental effect by altering lipoprotein and hepatic cholesterol metabolism in association with hyperinsulinemia.

Factors, dietary and genetic, that favor elevated hepatic cholesterol synthesis and production of a bile acid profile in which chenodeoxycholic acid predominates appear to be associated with lithogenic bile. An inconsistent effect of dietary fat saturation on gallstones is that polyunsaturates possibly increase risk in men and decrease risk in women. Vegetable protein may reduce the risk of choletithiasis. Whereas both the amount and type of dietary fiber influence cholesterol and bile lipid metabolism, specific associations between fiber and gallstones in humans remain elusive.

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