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The Syrian golden hamster strain LPN: a useful animal model for human cholelithiasis

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Abstract

The purpose of this study was to specify the main mechanisms at the origin of gallstone formation in very young (5-week old) or young adult (9-week old) LPN hamsters fed a sucrose-rich (normal lipid) lithogenic diet for one and four weeks, respectively. It was also to compare these mechanisms in the two strains of hamsters (LPN and Janvier) or when an anti-lithiasic diet was given by substituting 10% of the sucrose by β cyclodextrin. The LPN strain of hamsters showed a very high incidence of cholesterol gallstones (73%) after receiving the lithogenic diet. The gallstone formation is very rapid and occurs in less than one week in very young hamsters which show a high cholesterol synthesis rate in the liver. The cholesterol and phospholipid concentrations in the bile, cholesterol saturation index (CSI) and hydrophobic index (HI) increased significantly, concomitantly with a higher liver cholesterol synthesis in very young hamsters and with a lower bile acid synthesis (neutral pathway: cholesterol 7α-hydroxylase, CYP7A1 and acidic pathway: sterol 27 hydroxylase, CYP27A1) in young adult hamsters. No significant changes in the lipoprotein receptor expression (LDLr, SR-BI) were observed after feeding the lithogenic diet. Adding ten per cent β-cyclodextrin, a cyclic oligosaccharide that binds cholesterol and bile acids to the lithogenic diet at the expense of sucrose, induced a decrease in cholesterol bile secretion and in the CSI and HI and prevented cholesterol gallstone formation. Similarly, another strain of Syrian Golden hamsters (" Janvier ") which originally exhibited a smaller bile cholesterol concentration, lower liver cholesterol synthesis and higher CYP7A1/CYP27A1 activity ratio did not carry cholesterol gallstones when fed the lithogenic diet. The main parameters always found at the origin of cholelithiasis in the Hamster are discussed: a higher hepatic cholesterogenesis (HMGCoAR), a higher HMGCoAR/CYP7A1 activity ratio, a lower cholesterol ester storage capacity, a higher CYP27A1/CYP7A1 activity ratio correlated to a higher cholesterol secretion in the bile and higher CSI and HI. In LPN hamsters, the incidence of cholesterol gallstones is nil when CSI + HI < 0.8 and positive for CSI + HI > 0.9. An overall comparison of the data obtained in LPN Hamsters and in Man suggests that this hamster strain appears to be an interesting model for human cholelithiasis.

Keywords: LDL receptor; SR-BI; HMGCoAR; CYP7A1; CYP27A1; Activity and mRNA; Beta-cyclodextrin (BCD); Sucrose; Starch

1. Introduction

Cholesterol cholelithiasis constitutes a major health problem in developed countries. It constitutes the third most common cause of hospitalization. A slowly evolving disease, the latency between gallstone formation is 8 to 10 years [1]. The prevalence of this pathology is three times higher in women than in men and increases regularly with age [2]. Currently, there is no animal model which can spontaneously develop cholesterol gallstones. Several animal models for human cholelithiasis have been proposed: the prairie dog [3,4], ground squirrel [5], mouse [6], hamster [7–11] and primates [12]. The choice of hamsters as a model for studying human cholelithiasis was made because numerous similar physiological and metabolic processes have been found between these two species. Gilloteaux et al. have also proposed the hamster as a good cholelithiasis model and have used sexual hormones as cholelithiasis inducers [13,14].

The Golden Syrian Hamster (*Mesocricetus auratus*) is a small, easily handled animal. As is the case in man, it has a gallbladder. It is noteworthy that the cholesterol metabolism

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Abbreviations: 3-hydroxyl-3-methylglutaryl-CoA reductase: HMG-CoAR; cholesterol 7α-hydroxylase: CYP7A1; sterol 27 hydroxylase: CYP27A1; Acyl-coenzymeA-cholesterol acyltransferase: ACAT; sterol carrier protein 2: SCP2; LDL receptor: LDLr; scavenger receptor, class B type I : SR-BI; cholesterol saturation index: CSI; hydrophobic index: HI.

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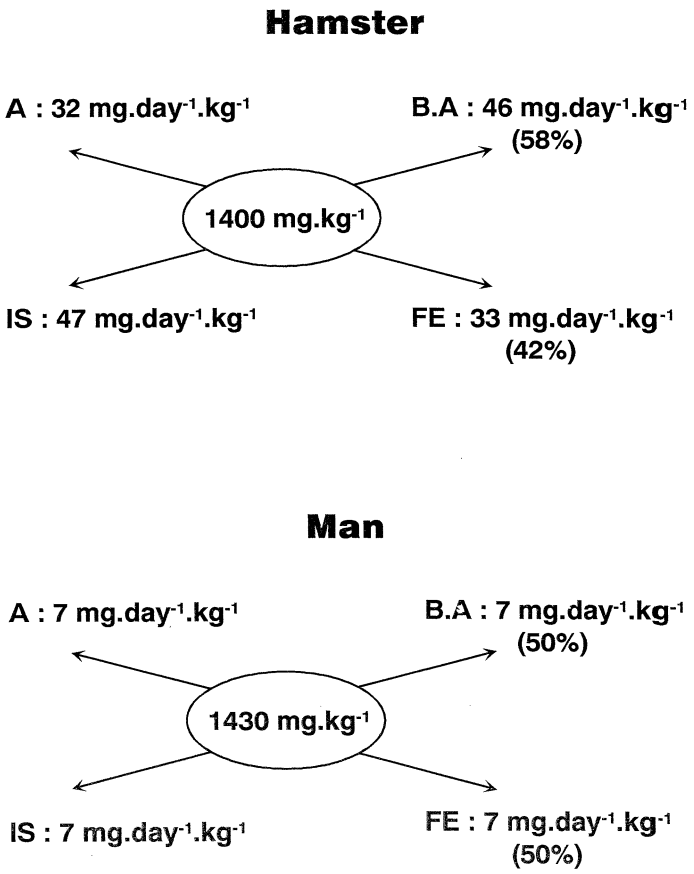

Fig. 1. Input and output rates ($mg.day^{-1}.kg^{-1}$) of cholesterol metabolism in Hamsters and Man (data derived from refs. 15, 17, 44, 53). A = Absorption; BA Bile acid synthesis; IS: internal synthesis: FE: faecal cholesterol excretion.

of the hamster has been shown to be similar to that of humans [15]. This is particularly true for the two cholesterol outputs (faecal excretion, FE, and faecal bile acid excretion, BA) (Fig. 1). Conversely the faecal bile acid excretion rate observed in the rat corresponds to 80% of the total cholesterol output [16]. Furthermore, although the total lipid concentration appears to be generally lower in normal hamsters (2–5 g/dl) than in Man (9 ± 5 g/dl) [17–25], the nature and composition of very hydrophobic bile acids and the ratio of tauro and glycoconjugated bile acids are similar in both species (Fig. 2).

The first lithogenic diet, described by Dam [7] was characterized by a large proportion of sucrose (72.5%) and a total absence of lipids (casein 20%, mineral mix 5%, vitamin mix 2.5%). We first used it in 4-month old adult hamsters [15] and showed that faecal cholesterol excretion as well as the cholesterol biosynthesis rate more than doubled with this diet, while the percentage of cholesterol output as bile acids decreased considerably (35% instead of 52%). A major drawback of this diet was the total absence of essential fatty acids. Thus, we looked for a more "physiological" lithogenic diet, still rich in sucrose but containing 5 (L) or 10% lipids (LI) at the expense of sucrose and inducing a mean cholelithiasis of 73 and 50%, respectively, in the young adult hamster. Using the lithogenic diet (LI) containing 10% lipids, we developed a new method for visualizing cholesterol gallstones by magnetic resonance imaging in the anesthetized animal [25]. However, the prevalence of cholelithiasis with this diet appeared to decrease with age [24–28] and biliary cholesterol secretion as well as the CSI was less "overstimulated" than with the Dam diet. With the lithogenic diet (L) containing only 5% lard, the prevalence of cholesterol gallstones was very high (73%) and appeared more stable with age.

The purpose of the present study was to specify the main mechanisms at the origin of gallstone formation in very young (5-week old) and young adult (9-week old) LPN hamsters receiving this sucrose-rich lithogenic diet for one and four weeks, respectively. It was also to compare these mechanisms when an anti-lithiasic diet was given by substituting 10% of the sucrose by β cyclodextrin. Gallstone formation was also compared in two strains of hamsters since the ability to induce gallstones can be highly dependent on the strain [10,11]. An overall comparison of the data obtained in LPN Hamsters and in Man suggests that this hamster strain appears to be an interesting model for human cholelithiasis.

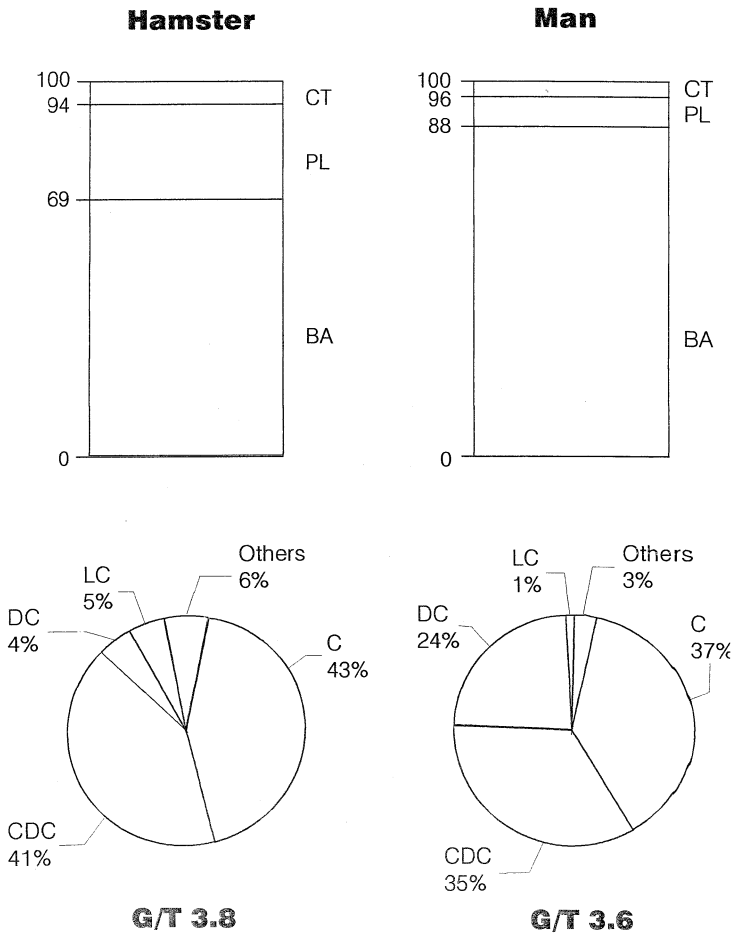

Fig. 2. Percentage distribution of lipids and bile acids in the vesicular bile of normal Hamsters and Man (data derived from refs. 17–25). a) Percentage distribution of lipids (CT: cholesterol; PL: phospholipids; BA: bile acids) in the vesicular bile of normal Hamsters and Man. b) Percentage distribution of bile acids (C; cholic; CDC: chenodeoxycholic; DC: deoxycholic; LC: lithocholic acid) in vesicular bile of hamsters and Man. G/T = glyco/tauro conjugated ratio.

2. Materials and methods

2.1. Chemicals and isotopes

4 ^{14}C Cholesterol was purchased from DuPont-NEN Products (Les Ulis, France). β-Cyclodextrin (BCD) (Kleptose^{R,} water content 12%, purity 99%) was kindly provided by Société Roquette Frères, 62136 Lestrem, France

2.2. Diets

The percentage composition of the lithogenic diet (**L**) was: sucrose 67.5, casein 20, lard 5, mineral mix 5 [29], vitamin- cellulose mix 2.5 [29]. That of the semi-purified diet (SP) was sucrose 53, casein 23, lard 9.2, mineral mix 5, defatted milk 4, yeast 2.3, vitamin-cellulose mix 2.5, walnut oil 0.8 and cystin 0.2. The commercial (C) hamster diet, purchased from UAR, Epinay/Orge, France, contained 12% moisture, 53% carbohydrate, 20% proteins, 5% lipids, 4%cellulose, and 6% mixture of mineral salts and vitamins. The L BCD10 diet contained 10% β-Cyclodextrin as a substitute for sucrose in the L diet.

2.3. Animals

The male LPN hamsters were bred in our own breeding unit. The constant temperature of the air-conditionned nursery was 23 $\pm$ 1°C. The animals were exposed to a lighting cycle (lights on from 6 am to 8 pm). The hamsters were individually caged from weaning (when approximately 3 weeks old) and had access to water and commercial food for one week and then the lithogenic L (or experimental) diet for 1 or 4 weeks.

The male Janvier hamsters were purchased from a commercial breeding unit (Center d'Elevage Janvier, Le Genest-St Isle, France). They were individually caged from weaning (when approximately 3 weeks old) and had access to water and commercial food for one week and then the lithogenic L (or experimental) diet for 4 weeks. During the experimental period, Janvier hamsters had a higher body

growth (1.33 ± 0.07 g/day) than LPN hamsters (0.82 ± 0.05 g/day). The care and use of the animals for experimental purposes were carried out according to the ethical standards set by the French decree 87–849 (October 19, 1989).

2.4. Plasma, bile and organ sampling

At the end of the experimental period, the non-fasting animals were anesthetized at 9 am with an intramuscular injection of a solution containing Tiletamine and Zolazepam (Zoletil 50, Virbac, 06116 Carros, France) at a dose of 100 mg/kg body weight. The abdomen was opened by a midline incision, the bile was directly aspirated from the gallbladder, diluted (1:20) with a 0.9% saline solution and stored at −20°C for further analysis. The liver was carefully excised and weighed and aliquots were taken for lipid measurement, immunodetection of lipoprotein receptors and enzymatic assays.

2.5. Chemical and biochemical assays

2.5.1. Lipid analysis

Phospholipid, cholesterol and bile acid concentrations in the diluted bile samples were measured using commercial kits. The cholesterol saturation index (CSI) was calculated according to the Carey method [30]. Bile acid composition was determined by gas-liquid chromatography (GLC) after deconjugation by choloylglycine hydrolase EC 3.5.1.24 (Sigma Chemical, St. Louis, MO), extraction by diethylether, methylation with diazomethane and sylilation with deriva-sil and BSTFA [24]. The hydrophobicity index of the bile acid pool was calculated as described [31].

Liver samples (0.5 g) were homogenized in 5 ml isopropanol, using an Ultra-Turrax apparatus. After incubation at 60°C for 1h and centrifugation for 5 min at 3000 g, the supernatant was collected and the pellet was re-extracted with 5 ml isopropanol**.** Radioactive tracers have shown that extraction of lipids reached 100%. Triglycerides, cholesterol and plasma lipids were measured as described previously [24].

2.5.2. Enzymes activities

Microsomes and mitochondria were prepared from fresh liver samples (1 g) by homogenization in 7 ml buffer (Tris/Hcl 50 mM, sucrose 300 mM, DTT 10 mM, NaCl 50 mM, pH 7.4) at 4°C, with a Teflon pestle. Microsomal and mitochondrial fractions were isolated according to the procedure described by Einarsson et al. [32] and Souidi et al. [28], respectively. HMG-CoA reductase activity was determined in the microsomes, in the presence of alkaline phosphatase using Philipp and Shapiro's radioisotopic technique [33]. Two optimal standard assays for ACAT activity were determined in microsomes [26], one for endogenous cholesterol and the other containing exogenous cholesterol. Cholesterol 7α-hydroxylase was assayed in the microsomal fractions by a radioisotopic method using [4-^{14}C] cholesterol, solubilised and carried by hydroxypropyl-β-cyclodextrin [27]. An NADPH regenerating system was used to inhibit the 3β-hydroxy-Δ5-C27-steroid oxydoreductase which is able to convert 7α-OH-cholesterol into 7α-OH-4-cholest-3-one, in the presence of NADP+. Sterol 27-hydroxylase was assayed in the mitochondrial fractions by a radioisotopic method using [4-^{14}C] cholesterol, solubilised in hydroxypropyl-β-cyclodextrin [28].

2.5.3. Immunoassays

Total membranes from fresh liver samples (1 g) were prepared according to Kovanen et al. [34]. This preparation has been described in detail previously [35]. Membrane proteins were solubilised in a buffer containing Triton-X100 2% [36] and assayed by the Lowry method using bovine serum albumin as a standard [37]. They were electrophoresed on polyacrylamide gels and electrotransferred onto nitrocellulose membranes.

The quantity of LDLr was determined by immunoassay using the antibody raised in rabbits against the LDLr purified from bovine adrenal cortex (gift from P. Roach, Adelaide, Australia). Nitrocellulose membranes were incubated overnight at 4°C in a quenching buffer (5% non-fat dry milk, TrisHCl 60 mM, NaCl 25 mM, $CaCl_2$ 2mM, pH8). They were then washed three times with TTBS (Tris-HCl 20 mM, pH 7.5; NaCl 500 mM; Tween 20 0.05%). The rabbit anti-LDLr immunoserum, diluted 1/5000 in buffer A (quenching buffer but with only 0.1% non-fat dry milk) was then added. After 1h30 incubation and 3 washes with TTBS, the membranes were incubated for 1h30 with the second antibody; goat anti-rabbit IgG conjugated with horseradish peroxidase and diluted 1/2000 in buffer A. After 3 washes with TTBS, detection was performed using the enhanced chemiluminescence method (ECL, Amersham). Films were scanned in a LKB 2222 ultroscan XL laser densitometer which was interfaced with a microcomputer. Peak areas were integrated and expressed in arbitrary units. The value 100 was attributed to control 9- week old hamsters. A "normalization" of the multiple films obtained throughout the different experiments was obtained by putting in each film a same control sample, systematically.

The quantity of SR-BI was measured by immunoblotting on liver membranes, using a rabbit polyclonal antibody raised against a peptide corresponding to the carboxyl terminus of murine SR-BI and kindly prepared by André Mazur (Laboratoire des Maladies métaboliques, INRA, Theix, France) [38]. The western blot showed a single protein band of apparent MW 82 KDa.

2.6. Liver mRNA levels of HMGCoAR, CYP7A1 and SR-BI

Total RNA was extracted from frozen (−80°C) liver samples (100 mg) using a commercial kit (Quickprep Total RNA Extraction, Pharmacia Biotech, USA) and stored at

Table 1
Lipid composition of the vesicular bile in very young (5-week old) or young adult (9-week old) LPN hamsters fed a control (C), semi-purified (SP) or lithogenic diet (L)

	5-week old		9-week old		
	C	L	C	SP	L
Total lipids (μmole/ml)	175 ± 10*	125 ± 22	126 ± 8	165 ± 5	169 ± 12
CT (mole %)	2.1 ± 0.3^{bc}	4.0 ± 0.5^{bc}	1.0 ± 0.1^{a}	1.8 ± 0.1^{a}	2.4 ± 0.2^{bc}
PL (mole %)	4.2 ± 0.5^{bc}	6.4 ± 1.0^{cd}	3.0 ± 0.4^{a}	6.9 ± 0.4^{d}	6.7 ± 0.6^{d}
BA (mole %)	93.5 ± 11.7	89.5 ± 19.6	95.9 ± 6.0	91.4 ± 3.0	90.9 ± 7.0
CSI**	0.39 ± 0.04^{b}	0.70 ± 0.14^{d}	0.36 ± 0.02^{a}	0.49 ± 0.02^{bcd}	0.66 ± 0.4^{cd}
HI***			0.15 ± 0.01	0.23 ± 0.01	0.37 ± 0.01
PL/CT ratio	1.9	1.6	2.9	3.9	2.8
BA/CT ratio	43	22	92	52	38
BA/CT + PL ratio	14.5	8.5	23.7	10.5	9.9

* Mean ± SEM $n = 5$ to 8 per group. Values without a common superscript in the same line are significantly different as determined by ANOVA and Student-Newman Keuls ($P < 0.05$). CT: cholesterol; PL: phospholipids; BA: bile acids.
** CSI (according to Carey Ref 30).
*** according to Heuman (Ref 31).

−80°C. The RNA concentration was determined by measuring the absorbance at 260 nm. All the samples had an A260/A280 ratio of about 1.8–2.0.

Quantification of HMGCoAR, CYP7A1 and SR-BI mRNA was performed by RT-PCR using a Retroscript TM-kit (Ambion, Clinisciences, France) and thermophilus aquaticus DNA polymerase (Qiagen, Courtaboeuf, France). For semi-quantitative purposes, G3PDH was co-amplified with either HMGCoAR, CYP7A1 or SR-BI. The choice of oligonucleotide sequences (Oligo-express, Paris, France) was guided by the hamster cDNA sequences of CYP7A1 [39], HMGCoAR [40], G3PDH [41] and SR-BI [42] as follows: HMGCoAR : 5′ AGG AAG AGG AAA GAC 3′ : 3′ CGG TAG GAG GTT GGT 5′. CYP7A1 : 5′ TTT GGA CAC AGA AGC ATT 3′ : 3′ GCC ATG TCA TCA AAG GTA 5′. SR-BI : 5′ TCG GTG TGG TTA TGA TC 3′ : 3′ CAT CAT CAG CTT CAG GC 5′. G3PDH : 5′ GGC TCT CTG CTC CTC 3′ : 3′ CAG CCC CAG CAT CAA 5′. After denaturation at 94°C for 5 min, the PCR procedure which has already been detailed [43] was used.

2.7. Statistical analysis

Results are given as mean values ± SEM. Statistical analyses were performed by Anova and Student Neuman Keuls test.

3. Results

The data of the present studies are shown in Tables 1–5.

3.1. Comparison of the lipid composition of vesicular bile in very young or young adult LPN hamsters fed a control, semi-purified or lithogenic diet (Table 1)

The incidence of cholesterol gallstones in very young or young adult LPN hamsters was high (50–80%) when they were fed the lithogenic diet and nil when fed the C or SP diet.

In 5 or 9-week old hamsters, the sucrose-rich lithogenic diet (L) induced an increase in the cholesterol (CT) and phospholipid (PL) proportions, leading to a decrease in the mean bile acid (BA)/CT and BA/PL+CT ratios. Similarly, the CSI or the HI went up from C to L.

In young adult 9-week old hamsters fed a semi-purified diet (SP) containing 53% sucrose, the values of lipid parameters in the bile as well as the CSI or HI were intermediate between those of the C or L hamsters.

3.2. Hepatic enzyme activities and mRNA levels in very young or young adult LPN hamsters fed a control or lithogenic diet (Table 2)

In 5 or 9-week old hamsters fed a control diet, age induced a decrease in ACAT activity (-39%), a large decrease in HMGCoAR activity (2.6 fold) and a slight increase in CYP7A1 activity (+29%). Consequently, we observed a decrease in the HMGCoAR/CYP7A1 or HMGCoAR/(CYP7A1+ACAT) activity ratios with age.

In very young (5-week old) hamsters, the lithogenic diet induced a 2–3 fold stimulation of the already high HMGCoAR activity in the liver. Conversely, it induced a decrease (-34 to 21%) in the CYP7A1 and ACAT activities. Therefore, the HMGCoAR/CYP7A1 activity ratio in the hamster liver increased from 6 to 24 and HMGCoAR/(CYP7A1+ACAT) from 0.85 to 2.9 in the C and L diets respectively.

In the young adult hamster however, liver HMGCoAR activity already low in the control, was significantly reduced by the L diet. The CYP7A1 as well as CYP27A1 activities decreased equally with the lithogenic diet in the 9-week old hamsters (and slightly for CYP7A1 in the 5-week old ham-

Table 2
Hepatic enzyme activities and mRNA (arbitrary units) levels in very young (5-week old) or young adult (9-week old) LPN hamsters fed a control (C) diet or lithogenic (L) diet

	5-week old		9-week old	
	C	L	C	L
ACAT + ch (%)	162 ± 8.8[b]	128 ± 6	100 ± 2[a]	n.d.
− ch	2.8 ± 0.5	5.3 ± 0.7	1.2 ± 0.1	n.d.
HMGCoAR (%)	264 ± 36[c]	690 ± 111[d]	100 ± 11[a]	18 ± 3[b]
*mRNA***	n.d.	n.d.	2.38 ± 0.57[a]	0.98 ± 0.27[b]
CYP7A1 (%)	77 ± 6[c]	51 ± 4[d]	100 ± 10[a]	21 ± 2[b]
*mRNA***	n.d.	n.d.	3.09 ± 0.31[b]	1.67 ± 0.21[b]
CYP27A1 (%)	n.d.	n.d.	100 ± 7	59 ± 12
HMGCoAR/CYP7A1 ratio	6.3	24.5	1.8	1
HMGCoAR/CYP7A1 + ACAT ratio	0.85	2.9	0.45	n.d.
CYP27A1/CYP7A2 ratio			1	2.8

* Expressed as percentage of the control 9-week old values (M ± SEM, $n = 6$ to 10). Two optimal assays were determined for ACAT activity, one with exogenous cholesterol (+ ch) one for endogenous cholesterol (− ch) [26].

Values without a common superscript in the same line are significantly different as determined by ANOVA and Student-Newman Keuls ($P < 0.05$).

n.d.: not determined.

** *mRNA*: arbitrary units.

In order to compare the four groups of animals, the enzyme activities were expressed as percentage of the control 9-week old values.

sters). The control and lithogenic diets modulated in parallel the HMGCoAR and CYP7A1 activities and mRNA in young adult hamsters.

3.3. Hepatic lipoprotein receptor proteins and mRNA levels in very young or young adult LPN hamsters fed a control or lithogenic diet (Table 3)

In hamsters fed a control or lithogenic diet, the amount of LDLr protein practically doubled between 5 and 9-week old hamsters.

The lithogenic diet induced a significant increase (+ 59%) in the LDLr protein content of the 5 week-old hamsters but not in that of the young adult hamsters. In the 9-week old adult hamsters, the lithogenic diet induced a slight decrease (-30 to 40%) in the amounts of SR-BI protein and mRNA compared to the control animals, while no significant changes were seen in the LDLr protein.

Table 3
Hepatic lipoprotein receptor proteins and mRNA levels in very young (5-week old) and young adult (9-week old) LPN hamsters fed a control (C) or lithogenic (L) diet

	5-week old		9-week old	
	C	L	C	L
LDLr	44 ± 6[a]	70 ± 9[b]	100 ± 12[c]	116 ± 2[c]
SR-B1	n.d.	n.d.	100 ± 3[b]	77 ± 11[a]
*mRNA**	n.d.	n.d.	5.18 ± 0.25[b]	3.20 ± 0.61[a]

(M ± SEM, $n = 9$ to 10 per group) expressed as percentage of C 9-week old values.

Values without a common superscript in the same line are significantly different as determined by ANOVA and Student-Newman Keuls ($P < 0.05$).

n.d.: not determined

* *mRNA*: arbitrary units.

In order to compare the four groups of animals, the lipoprotein receptors were expressed as percentage of the control 9-week old values.

3.4. Lipid composition of the vesicular bile in young adult LPN hamsters fed a lithogenic or lithogenic + 10% BCD diet (Table 4)

While the incidence of gallstones was high (more than 73%) with the L diet, it was nil when 10% β-cyclodextrin (instead of sucrose) was added to the L diet. β-cyclodextrin supplementation did not modify bile secretion (271 ± 20

Table 4
Lipid composition of the vesicular bile in young adult (9-week old) LPN hamsters, fed an L diet or lithogenic + 10% beta-cyclodextrin (L BCD10) diet, and in young adult Janvier hamsters fed an L diet

	LPN strain		JANVIER strain
	L	L, BCD	L
White ch gallstones*	18/25	0/29	0/24
Total lipids (μmole/ml)	167.1 ± 12.7[a]	144.2 ± 9.5[b]	100 ± 9.0[c]
CT (mole %)	2.5 ± 0.1[a]	1.8 ± 0.1[b]	1.4 ± 0.1
PL (mole %)	6.8 ± 0.5[a]	5.3 ± 0.3[b]	4.9 ± 0.5
BA (mole %)	90.7 ± 0.6[a]	92.8 ± 0.3[b]	93.7 ± 2.0
CSI**	0.66 ± 0.04[a]	0.51 ± 0.04[c]	0.46 ± 0.02[b]
HI***	0.37 ± 0.01[a]	0.19 ± 0.01[b]	0.29 ± 0.01[a]
PL/CT ratio	2.8	2.8	35
BA/CT	38	49	67
BA/CT + PL	10	13	15

Mean ± SEM ($n = 25$ to 29). Values without a common superscript in the same line are significantly different as determined by ANOVA and Student-Newman Keuls ($P < 0.05$).

CT: cholesterol; PL: phospholipids; BA: bile acids.

* These gallstones contain > 88% cholesterol, w/w.

** CSI (according to Carey Ref 30).

*** According to Heuman (Ref 31).

Table 5
Hepatic enzyme activities and mRNA (arbitrary units) levels in young adult hamsters (LPN and JANVIER strain) fed a lithogenic diet L

	LPN strain	JANVIER strain
HMGCoAR (pmole/min.mg)	168 ± 38[a]	55 ± 11[b]
*mRNA**	0.98 ± 0.27	0.83 ± 0.23
CYP7A1 (pmole/min.mg)	30 ± 3[a]	15 ± 1[b]
*mRNA**	1.67 ± 0.21[a]	0.77 ± 0.20[b]
CYP27A1 (pmole/min.mg)	47 ± 20[a]	10 ± 7[b]
Mean HMGCoAR/CYP7A1 ratio	5.6	3.6
HMGCoAR/CYP7A1 + CYP27A1	2.2	2.2
CYP27A1/CYP7A1	1.5	0.66

Mean ± SEM ($n = 6$).

Values without a common superscript in the same line are significantly different as determined by Students' t test ($P < 0.05$).

* *mRNA*: arbitrary units.

versus 298 ± 42 μl/h in L and LBCD10, respectively) but lowered the CT and PL concentrations and proportions in the bile (−28 and 32%, respectively). Consequently, the PL/CT ratio in the bile did not significantly change while the BA/CT and BA/CT+PL ratios significantly increased in the BCD10 group, leading to a lower CSI and a reduced HI due to more hydrophilic bile acids in the bile (not shown).

3.5. Comparisons between two strains of hamsters (LPN and Janvier) fed a lithogenic diet (Tables 4 and 5)

The sucrose-rich lithogenic diet (L) induced a high incidence of cholesterol gallstones in male Syrian hamsters from the LPN strain but not in hamsters from the commercial breeding unit Janvier (no white cholesterol gallstones and 14 black stones/24).

LPN hamsters, which have a lower plasma cholesterol level than Janvier hamsters (3.39 ± 0.12 mM versus 4.77 ± 0.12, not shown), showed higher lipid concentrations as well as higher cholesterol and phospholipid proportions and lower BA/CT and BA/CT+PL mean ratios in the gallbladder bile.

LPN hamsters displayed higher hepatic activities of HMGCoAR (but no significant increase in the mRNA levels of HMGCoAR was detected), a slightly higher CYP7A1 activity correlated with higher mRNA levels of CYP7A1, and a higher CYP27A1 activity and CYP27A1/CYP7A1 ratio than in Janvier hamsters.

4. Discussion

4.1. Different sensitivities towards gallstone induction between hamster strains (LPN/Janvier)

The results of this study first show that LPN hamsters are far more susceptible to cholelithiasis than Janvier hamsters. This is mainly due to the fact that LPN hamsters secrete more cholesterol and have lower molar BA/CT and BA/PL + CT ratios in their bile. They have also a higher rate of cholesterol synthesis in the liver, a higher HMGCoAR/CYP7A1 ratio, a lesser capacity to store ester cholesterol in the liver without significant modification of the bile acid spectrum in this case.

These main characteristics (with a higher HI) are also observed when LPN hamsters receive the lithogenic sucrose-rich diet instead of the commercial one. They are also often observed, all together or only a few, in lithiasic man compared to "normal" man [44]. Bile saturation with cholesterol is an essential prerequisite for gallstone development [45]. If the lipid composition of human and hamster vesicular bile is compared in lithiasic versus normal subjects, the total lipid and cholesterol concentrations and proportions in hamsters as well as humans tend to be higher in lithiasic subjects, with a significant increase in the molar per cent of cholesterol and a decrease in the molar per cent of PL and BA. Consequently, the BA/CT and BA/CT+PL ratios drop, the CSI rises, as does the mean hydrophobic index since CDC proportions in vesicular bile usually increase slightly or significantly as do the CYP27A1/CYP7A1 ratios in the lithiasic liver. If these hamster results are extrapolated to Man, then the human predisposition for gallstones could be found in subjects with basically higher hepatic cholesterol synthesis, lower ACAT activity and higher CYP27A1/CYPA1 activities.

The hypothesis that the rate of biliary cholesterol secretion can be controlled by the avaibility of a hepatic, metabolically active, free cholesterol pool whose size is determined by the rates of cholesterol synthesis and ACAT has already been tested [46]. The experiments using ACAT or HMGCoAR inhibitors suggest the existence of a metabolically active pool of free cholesterol that could be used as a precursor pool for biliary cholesterol secretion. In patients with cholesterol gallstones, ACAT activity decreased to one third of that in the controls [47]. In very young lithiasic hamsters, ACAT activity was also lower (−39%) than in the controls. Furthermore, the cholesterol concentration of the cytosolic fraction of the liver shows a marked increase compared to the controls (6.84 ± 0.31 versus 3.63 ± 0.14 nmole/mg protein). This also was observed by J. Smith et al. in gallstone versus control patients [47]. This cholesterol concentration in the cytosolic fraction could represent the cholesterol carried by cytosolic lipid carriers such as SCP2 which is involved in the rapid transport of newly synthesized cholesterol in the bile and in cytosolic transport to ACAT [48,49]. However, the present data show no direct relationship between the cholesterol concentration in the bile and hepatic HMGCoAR activity.

4.2. Mechanisms of cholelithiasis in hamsters and man

One of the key factors demonstrated by the present data is the following: in very young (5-week old) hamsters, the lithogenic sucrose-rich diet induces rapidly (less than one

week) a 2–3 fold stimulation of the already high HMG-CoAR activity and a decrease (−35 and −39%, respectively) in the CYP7A1 and ACAT activities in the liver. It induces a four-fold increase in the HMGCoAR/CYP7A1 activities ratio (from 6 (control) to 24 (lithiasic)) and a 3-fold increase in the HMGCoAR/(CYP7A1+ACAT) activities ratio (from 0.85 to 2.9). Thus, the hyper insulin secretion induced by the consumption of a sucrose-rich diet induces a stimulation of cholesterol biosynthesis in Hamsters as is the case in Man [15,50,51].

In adults (Hamster, Man . . .), the cholesterol biosynthesis cellular rate drops since the cholesterol need for cell division decreases. LDL receptor expression, and probably other lipoprotein receptors, also increase strongly in the late suckling stage [52] and assure a substantial delivery of lipoprotein cholesterol to the liver. The delicate cholesterol balance in the hepatic cell results from numerous mechanisms (synthesis, lipoprotein uptake, esterification, catabolism into bile acids, bile secretion) but cholesterol and bile acid secretion represent the major pathway for the net excretion of this steroid in the body.

The importance of lipoprotein cholesterol as a precursor to biliary cholesterol and bile acids has been demonstrated in animals and man [15,53,54] and recently confirmed in patients with complete biliary diversion [55]. Among the main lipoprotein receptors, the scavenger SR-BI receptor could play an important role in the secretion of cholesterol in the bile. Overexpression of this receptor in mice results in a two-fold increase in biliary free cholesterol [56,57]. Our present results in Hamsters however do not show any substantial modulation of SR-BI in L versus control adult animals, while the concentration of cholesterol in the bile is multiplied by 3. After using an ACAT inhibitor, we recently observed a 3-fold increase in the amount of hepatic SR-BI without changes in the biliary cholesterol concentration [58].

The bile acid pathway accounts for most of the cholesterol output in mammals and plays a crucial role in cholesterol homeostasis. Half of the total daily cholesterol output in Man are bile acids as is the case in Hamsters. It is also the pathway which responds the most rapidly to a change in cholesterol metabolism [59]. In the liver, two pathways (neutral or classical in which CYP7A1 is the rate-limiting enzyme and acidic or alternative in which CYP27A1 is the rate-limiting enzyme) are now considered as playing a role in the transformation of cholesterol into cholic and chenodeoxycholic acids in Hamsters and in Man [60]. The relative importance of these two pathways in each species and the regulatory role of CYP27A1 in bile acid metabolism has yet to be debated. The interesting works on CYP7A1 or CYP27A1 KO mice do not provide any estimation concerning the relative participation of these two pathways in Man because Mice and Rats have very hydrophilic bile acids, far too different from those found in Hamsters and Man [61, 62].

Although the changes in CYP27A1 activity generally seem to be less marked than for CYP7A1, several sterol inhibitors of CYP7A1 activity (7β-hydroxycholesterol, cholestanol) similarly inhibit CYP27A1 [60,28]. In this last study, we found that epicoprostanol specifically inhibits CYP27A1, suggesting that the two enzymes are separately regulated in Hamsters. This hypothesis has been confirmed by the fact that the circadian rhythm of both enzymes is totally different in Hamsters, and that the lowest value for CYP7A1 (20h) corresponds to the peak for CYP27A1 (unpublished results).

With our more sensitive assays for measuring CYP7A1 and CYP27A1 activities [27,28], we have shown that the lithogenic sucrose-rich diet significantly lowers both enzyme activities (more efficiently for CYP7A1 than for CYP27A1), increasing the CSI and HI and favoring a higher chenodeoxycholic acid concentration in the bile. Several epidemiological studies have shown a statistical association between consumption of rapidly digestible sugars and a high gallstone incidence while resistant carbohydrates were associated with a lower frequency of cholelithiasis [63–69]. The present study and previous ones [24] have shown that BCD lowers CSI and HI, increases the CYP7A1 and CYP27A1 activities and inhibits cholesterol gallstone incidence in LPN hamsters as do resistant carbohydrates [67].

It is noteworthy that the relative increase in CYP27A1/CYP7A1 in females compared to males [28], with a sucrose-rich diet versus the control (Table 2), or in sensitive versus less sensitive strains (Tables 4 and 5) is consistent with the well known higher incidence of gallstones in developed compared to less developed countries or to women compared to men. A declining ovarian function (decreased production of estrogen) in baboons has been positively correlated with a lower level of 27-hydroxycholesterol and diminished hepatic CYP27A1 activity [70], demonstrating the positive effect of female hormones on the acidic bile acid pathway. In cultured rat hepatocytes, Twisk at al. demonstrated that physiological concentrations of insulin suppress bile acid synthesis by downregulating CYP7A1 and CYP27A1 gene transcription [71]. Both enzymes are inhibited in hamsters as well as in rats, but CYP7A1 is inhibited to a greater extent than CYP27A1. Although CYP7A1 appears to be highly regulated by changes in mRNA levels ([71] and our results), the different modulation of HMGCoAR activity between the two strains of hamsters is not accompanied by parallel changes in mRNA levels suggesting that HMGCoAR regulation can be largely posttranscriptional in hamsters as well as in rats, as discussed by Spady et al [72].

5. Conclusion

The results of this study show that the main mechanisms at the origin of a sucrose-rich induced-cholelithiasis (compared to a commercial or semi-purified or antilithiasic diet)

is a higher hepatic cholesterogenesis (in very young hamsters) or a higher HMGCoAR/CYP7A1 activity ratio (very young and young adult hamsters), a lower cholesterol ester storage capacity, a higher CYP27A1/CYP7A1 activity ratio, correlated to higher cholesterol secretion in the bile and a higher CSI. A comparison between two strains of hamsters also shows that the more sensitive to induced gallstones has the highest bile HI, confirming the importance of both CSI and HI. In LPN hamters, the incidence of cholesterol gallstones is nil when CSI (according to Carey) + HI < 0.8 and positive for CSI + HI > 0.9. Therefore the LPN hamster appears to be a particularly interesting animal model for inducing and studying the numerous mechanisms at the origin of gallstones in vesicular bile. It shows the same pattern of biliary bile acids as that of man, similar conjugation, similar CSI and HI. Dietary or hormonal factors involved in inducing human cholelithiasis are also very efficient in increasing gallstone formation in this strain.

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References

[1] N.Y.I. Mok, E.R.M. Druffel, W.M. Pampone, Chronology of cholelithiasis. Dating gallstones from atmospheric radiocarbon produced by nuclear bomb explosions, N. Engl. J. Med. 314 (1986) 1075–1077.
[2] J.P. Capron, Qui forme des calculs de cholestérol?, Gastroenterol. Clin. Biol. 18 (1994) 988–995.
[3] D.E. Brenneman, W.E. Connor, E.L. Forker, L. DenBensten, The formation of abnormal bile and cholesterol gallstones from dietary cholesterol in the prairie dog, J. Clin. Invest. 51 (1972) 1495–1503.
[4] R.T. Holzbach, C. Corbusier, M. Marsh, H.K. Naito, The process of cholesterol cholelithiasis induced by diet in the prairie dog: a physicochemical characterization, J. Lab. Clin. Med. 87 (1976) 987–998.
[5] B.R. McPherson, R.S. Pemsingh, Ground squirrel model for cholelithiasis role of epithelial glycoproteins, Microsc. Res. Technique 39 (1997) 39–55.
[6] J. Tepperman, F. Caldwell, H.M. Tepperman, Induction of gallstones in mice by feeding a cholesterol-cholic acid containing diet, Am. J. Physiol. 206 (1964) 628–634.
[7] H. Dam, F. Christensen, Alimentary production of gallstones in hamsters, Acta Pathol. Microbiol. Scand. 30 (1952) 236–242.
[8] A.K. Singhal, B.I. Cohen, J. Finver-Sadowsky, C.K. McShery, E.H. Mosbach, Role of hydrophilic bile acids and of sterols on cholelithiasis in the hamster, J. Lipid Res. 25 (1984) 564–570.
[9] B.J. Pearlman, G.G. Bonorris, M.J. Phillips, A. Chung, S. Vimadalal, J.W. Marks, L.J. Schoenfield, Cholesterol gallstone formation and prevention by chenodeoxycholic and ursodeoxycholic acids. A new hamster model, Gastroenterology 77 (1979) 634–641.
[10] B.I. Cohen, N. Matoba, E.H. Mosbach, C.K. MacSherry, Dietary induction of cholesterol gallstones in hamsters from three different sources, Lipids 24 (1989) 151–156.
[11] E.A. Trautwein, J. Liang, K.C. Hayes, Cholesterol gallstone induction in hamsters reflects strain differences in plasma lipoproteins and bile acid profiles, Lipids 28 (1993) 305–312.
[12] R.N. Redinger, D.M. Grace, Cholesterol gallstones and biliary lipid metabolism in the primate, Gastroenterology 74 (1978) 201–204.
[13] J. Gilloteaux, E. Kosek, T.R. Kelly, Sex steroid induction of gallstones in the male Syrian hamster, J. Submicrosc. Cytol. Pathol. 25 (1993) 157–172.
[14] S. Karkare, J. Gilloteaux, Gallstones induced by sex steroids in the female Syrian hamster: duration effects, J. Submicrosc. Cytol. Pathol. 27 (1995) 53–74.
[15] J. Khallou, M. Riottot, M. Parquet, C. Verneau, C. Lutton, Biodynamics of cholesterol and bile acids in the lithiasic hamster, Brit. J. Nutrition 66 (1991) 479–492.
[16] F. Chevallier, C. Lutton, Vitesses des processus de renouvellement du cholestérol contenu dans son espace de transfert, chez le rat. I Méthodes et résultats obtenus dans le cas d'un régime semi-synthétique témoin, Bull. Soc. Chim. Biol. 48 (1966) 507–524.
[17] W.C. Duane, Effects of legume consumption on serum cholesterol, biliary lipids and sterol metabolism in humans, J. Lipid Res. 38 (1997) 1120–1128.
[18] Y.C.A. Keulemans, K.S. Mok, L.T. de Wit, D.J. Gouma, A.K. Groen, Hepatic bile versus gallbladder bile: a comparison of protein and lipid concentration and composition in cholesterol gallstone patients, Hepatology 28 (1998) 11–16.
[19] E.R.M. Eckhardt, B.J.M. van de Heijning, K.J. van Erpecum, W. Renooij, G.P. VanBerge-Henegouwen, Quantitation of cholesterol-carrying particles in human gallbladder bile, J. Lipid Res. 39 (1998) 594–603.
[20] D.Q.H. Wang, M.C. Carey, Characterization of crystallization pathways during cholesterol precipitation from human gallbladder biles: identical pathways to corresponding model biles with three predominating sequences, J. Lipid Res. 37 (1996) 2539–2549.
[21] P.R.C. Harvey, G. Somjen, T. Gilat, S. Gallinger, S.M. Strasberg, Vesicular cholesterol in bile. Relationship to protein concentration and nucleation time, Biochim. Biophys. Acta, 958 (1988) 10–18.
[22] S. Gallinger, P.R.C. Harvey, C.N. Petrunka, R.G. Ilson, S.M. Strasberg, Biliary proteins and the nucleation defect in cholesterol cholelithiasis, Gastroenterology 92 (1987) 867–875.
[23] E.A. Trautwein, A. Kunathran, J. Dietrich, S. Drusch, H.F. Erbersdobler, Effects of dietary fats rich in lauric, myristic, palmitic, oleic or linoleic acid on plasma, hepatic and biliary lipids in cholesterol-fed hamsters, Br. J. Nutr. 77 (1997) 605–620.
[24] N. Boehler, M. Riottot, J. Férézou, M. Souidi, F. Milliat, C. Sérougne, J.L. Smith, C. Lutton, Antilithiasic effect of β-cyclodextrin in LPN hamster: comparison with cholestyramine, J. Lipid Res. 40 (1999) 726–734.
[25] C. Cohen-Solal, M. Parquet, B. Tiffon, A. Volk, M. Laurent, C. Lutton, Magnetic resonance imaging for the visualization of cholesterol gallstones in hamster, J. Hepatology 22 (1995) 486–494.
[26] J. Smith, C. Lutton, Determination of hepatic acyl-coenzymeA:cholesterol acyltransferase activity in LPN hamsters: a model for cholesterol gallstone formation, J. Gastroenterol. Hepatol. 12 (1997) 877–886.
[27] M. Souidi, M. Parquet, C. Lutton, Improved assay of hepatic microsomal cholesterol 7α hydroxylase activity by use of hydroxypropyl-β−cyclodextrin and an NADPH regenerating system, Clin. Chim. Acta 269 (1997) 201–217.
[28] M. Souidi, M. Parquet, J. Férézou, C. Lutton, Modulation of cholesterol 7α hydroxylase and sterol 27-hydroxylase activities by steroids and physiological conditions in the hamster liver, Life Sci. 64 (1999) 1585–1593.
[29] J. Khallou, M. Riottor, M. Parquet, C. Verneau, C. Lutton, Antilithiasic and hypocholesterolemic effects of diets containing autoclaved amylomaize starch in hamster, Digestive Diseases and Sciences 40 (1995) 2540–2548.

[30] M.C. Carey, Critical tables for calculating the cholesterol saturation of native bile, J. Lipid Res. 19 (1978) 945–955.
[31] D.M. Heuman, Quantitative estimation of the hydrophilic balance of mixed bile salt solutions, J. Lipid Res. 30 (1989) 719–730.
[32] K. Einarsson, B Angelin, S. Ewerth, K. Nilsell, I. Björkhem, Bile acid synthesis in man: assay of hepatic microsomal cholesterol 7α-hydroxylase activity by isotope dilution-mass spectrometry, J. Lipid Res. 27 (1986) 82–88.
[33] B.W. Philipp, D.J. Shapiro, Improved method for the assay and activation of 3-hydroxy-3-methylglutaryl CoA reductase, J. Lipid Res. 20 (1979) 588–593.
[34] P.T. Kovanen, M.J. Brown, J.L. Goldstein, Increased binding of low density lipoprotein to liver membranes from rats treated with 17α-ethinyl estradiol, J. Biol. Chem. 254 (1979) 11367–11373.
[35] C. Felgines, C. Sérougne, A. Mazur, J. Férézou, C. Lutton, Y. Raysiguier, Hepatic apolipoprotein and LDL receptor gene expression in the genetically hypercholesterolemic (Rico) rat, Atherosclerosis 117 (1995) 15–24.
[36] W.J. Schneider, U. Beisiegel, J.L. Goldstein, M.S. Brown, Purification of the low density lipoprotein receptor, an acidic glycoprotein of 164,000 molecular weight, J. Biol. Chem. 257 (1982) 2664–2673.
[37] O.H. Lowry, N.J. Rosebrough, A.L. Farr, R.J. Randall, Protein measurement with the Folin phenol reagent, J. Biol. Chem. 192 (1951) 265–275.
[38] S. Acton, A. Rigotti, K.T. Landschulz, S. Xu, H.H. Hobbs, M. Krieger, Identification of scavenger receptor SRB1 as a high density lipoprotein receptor, Science 271 (1996) 518–520.
[39] M. Crestani, G. Galli, J.Y.L. Chiang, Genomic cloning, sequencing and analysis of the hamster 7α-hydroxylase gene (CYP7), Arch. Biochem. Biophys. 306 (1993) 451–460.
[40] D. Skalnik, R.D. Simoni, The nucleotide sequence of syrian hamster HMGCoAR cDNA, DNA 4 (1985) 439–444.
[41] S. Vincent, P. Fort, Nucleotide sequence of hamster G3PDH mRNA, Nucl. Acids Res. 18 (1990) 3054–3060.
[42] S. Acton, P.E. Sherer, R.H. Lodish, M. Krieger, Expression cloning of SRB1, as CD36-related class B scavenger receptor, J. Biol. Chem. 33 (1994) 21003–21009.
[43] J. Ferezou, M. Combettes-Souverain, M. Souidi, J.L. Smith, N. Boehler, F. Milliat, E. Eckardt, G. Blanchard, M. Riottot, C. Sérougne, C. Lutton, Cholesterol, bile acid, and lipoprotein metabolism in two strains of hamster, one resistant, the other sensitive (LPN) to sucrose-induced cholelithiasis, J. Lipid Res. 41 (2000) 2042–2054.
[44] A.F. Hofman, Bile acid secretion, bile flow and biliary lipid secretion in humans, Hepatology 12 (1990) S17–S25.
[45] N. Mendez-Sanchez, R. Cardenas-Vazquez, G. Ponciano-Rodriguez, M. Uribe, Pathophysiology of cholesterol gallstone disease, Arch. Med. Res. 27 (1996) 443–441.
[46] B.G. Stone, S.K. Eriksson, W.Y. Craig, A.D. Cooper, Regulation of biliary cholesterol secretion by agents that alter intrahepatic cholesterol metabolism, J. Clin. Invest. 76 (1985) 1773–1781.
[47] J.L. Smith, I.R. Hardie, S.P. Pillay, J. de Jersay, Hepatic acyl-coenzyme A:cholesterol acyltransferase activity is decreased in patients with cholesterol gallstones, J. Lipid Res. 31 (1990) 1993–2000.
[48] L. Puglielli, A. Rigotti, L. Amigo, L. Nunez, A.V. Greco, M.J. Santos, F. Nervi, Modulation of intrahepatic cholesterol trafficking: evidence by in vivo antisense treatment for the involvement of SCP2 in newly synthetized cholesterol transport into rat bile, Biochem. J. 317 (1996) 681–687.
[49] E.J. Murphy, F. Schroeder, SCP2 mediated cholesterol esterification in transfected L-cell fibroblasts, Biochim. Biophys. Acta 1345 (1997) 283–292.
[50] M. Griffin, A. Frazer, A. Jonhson, P. Collins, D. Owens, G.H. Tomkin, Cellular cholesterol synthesis. The relationship to postprandial glucose and insulin following weight loss, Atherosclerosis 138 (1998) 313–318.
[51] A.F. De Santis Aattili, S.G. Corradini, E. Scafato, A. Cantagalli, C. De Luca, G. Pinto, D. Lisi, L. Capocaccia, Gallstones and diabetes: a case-control study in a free-living population sample, Hepatology 25 (1997) 787–790.
[52] J.L. Smith, S.R. Lear, S.K. Erikson, Developmental expression of elements of hepatic cholesterol metabolism in the rat, J. Lipid Res. 36 (1995) 641–652.
[53] C. Lutton, Dynamique du cholesterol et des acides biliaires. Aspects comparatifs, Reprod. Nutr. Dev. 30 (1990) 145–160.
[54] C.C. Schwartz, Z.R. Vlacehvic, L.G. Halloran, L. Swell, An in vivo evaluation in man of the transfer of esterified cholesterol between lipoproteins and into the liver and bile, Biochim. Biophys. Acta 663 (1981) 143–162.
[55] C.G. Hillebrant, B. Nyberg, K. Einarsson, N. Einarsson, The effect of plasma low density lipoprotein apheresis on the hepatic secretion of biliary lipids in humans, Gut 41 (1997) 700–709.
[56] K.F. Kozarski, M.H. Donahee, A. Rigotti, S.N. Iqbal, E.R. Edelman, M. Krieger, Overexpression of the HDL receptor SRB1 alters plasma HDL and bile cholesterol levels, Nature 387 (1997) 414–417.
[57] E. Sehayek, J.G. Ono, S. Shefer, L.B. Nguyen, N. Wang, A.K. Batta, G. Salen, J.D. Smith, A.R. Tall, J.L. Breslow, Biliary cholesterol excretion: a novel mechanism that regulates dietary cholesterol absorption, Proc. Natl. Acad. Sci. USA 95 (1998) 10194–10199.
[58] F. Milliat, J. Férézou, A. Delhon, C. Lutton, Overexpression of SR-BI in hamsters treated with a novel ACAT inhibitor (F12511), C. R. Acad. Sci. Paris, in press.
[59] G.C. Ness, L.C. Pendleton, Y.C. Li, J.Y.L. Chiang, Effect of thyroid hormone on hepatic cholesterol 7α-hydroxylase, LDLr, HMGCoAR, farnesyl pyrophosphate synthetase and apo A1 mRNA levels in hypophysectomized rats, Biochim. Biochem. Res. Commun. 172 (1990) 1150–1156.
[60] H.M.G. Princen, S.M. Post, J. Twisk, Regulation of bile acid biosynthesis, Curr. Pharmaceutic Design 3 (1997) 59–84.
[61] M. Schwarz, E.G. Lund, K.D.R. Setchell, H.J. Kayden, J.E. Zerwek, I. Björkhem, J. Herz, D.W. Russell, Disruption of cholesterol 7a-Hydroxylase gene in mice. II Bile acid deficiency is overcome by induction of oxysterol 7a-hydroxylase, J. Biol. Chem. 30 (1996) 18024–18031.
[62] H. Rosen, A. Reshef, N. Maeda, A. Lippold, S. Shpizen, L. Triger, G. Eggertsen, I. Bjôrkhem, E. Leitersdorf, Markedly reduced bile acid synthesis but maintained levels of cholesterol and vitamin D metabolites in mice with disrupted sterol 27-hydroxylase gene, J. Biol. Chem. 24 (1998) 14805–14812.
[63] H. Sarles, J. Hauton, N.E. Planche, H. Lafont, A. Gerolami, Diet, gallstones, and composition of bile, Am. J. Dig. Dis. 15 (1970) 251–260.
[64] K. Heaton, The role of diet in the ætiology of cholelithiasis, Nutr. Abstr. Rev. 54 (1984) 549–560.
[65] A.K. Diehl, S.M. Haffner, J.A. Knapp, H.P. Hazuda, M.P. Stern, Dietary intake and the prevalence of gallbladder disease in Mexican Americans, Gastroenterology 97 (1989) 1527–1533.
[66] R.K.R. Scragg, A.J. McMichael, P.A. Baghurst, Diet, alcohol, and relative weight in gallstone disease: case control study, Br. J. Med. 288 (1984) 1113–1119.
[67] J.R. Thornton, P.M. Emmett, K.W. Heaton, Diet and gallstones: effects of refined and unrefined carbohydrate diets on bile cholesterol saturation and bile acid metabolism, Gut 34 (1983) 2–6.
[68] L. Barbara, S. Sama, A.M. Morselli-Labate, F. Taroni, A.G. Rusticalli, D. Festi, C. Sapio, E. Roda, A population study on the prevallence of gallstone disease: the sirmione study, Hepatology 7 (1987) 913–917.
[69] R.E. Sampliner, P.H. Bennet, L.J. Comess, F.A. Rose, T.A. Burch, Gallbladder disease in Pima indians, N. Engl. J. Med. 283 (1970) 1358–1376.
[70] L.D. Chen, R.S. Kushawaha, H.C. Mcgill, K.S. Rice, K.D. Carey, Effect of naturally reduced ovarian function on plasma lipoprotein and S27-hydroxycholesterol levels in baboons (Papio sp.), Atherosclerosis 136 (1998) 89–98.

[71] J. Twisk, M. Hoekman, E. Lehmann, P. Meijer, W. Mager, H. Princen, Insulin suppresses bile acid synthesis in cultured rat hepatocytes by down-regulation of cholesterol 7α hydroxylase and sterol 27-hydroxylase gene transcription, Hepatology 21 (1995) 501–510.

[72] D.K. Spady, J.A. Cuthbert, Regulation of hepatic sterol metabolism in the rat. Parallel regulation of activity and mRNA for 7α-hydroxylase but not 3-hydroxy-3-methylglutarylCoA reductase or LDLr, J. Biol. Chem. 267 (1992) 5584–5591.