Cholesterol, bile acid, and lipoprotein metabolism in two strains of hamster, one resistant, the other sensitive (LPN) to sucrose-induced cholelithiasis[1]

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Abstract A comprehensive study of cholesterol, bile acid, and lipoprotein metabolism was undertaken in two strains of hamster that differed markedly in their response to a sucrose-rich/low fat diet. Under basal conditions, hamsters from the LPN strain differed from Janvier hamsters by a lower cholesterolemia, a higher postprandial insulinemia, a more active cholesterogenesis in both liver [3- to 4-fold higher 3-hydroxy 3-methylglutaryl coenzyme A reductase (HMG-CoAR) activity and mRNA] and small intestine, and a lower hepatic acyl-coenzyme A:cholesterol acyltransferase activity. Cholesterol saturation indices in the gallbladder bile were similar for both strains, but the lipid concentration was 2-fold higher in LPN than in Janvier hamsters. LPN hamsters had a lower capacity to transform cholesterol into bile acids, shown by the smaller fraction of endogenous cholesterol converted into bile acids prior to fecal excretion (0.34 vs. 0.77). In LPN hamsters, the activities of cholesterol 7α-hydroxylase (C7OHase) and sterol 27-hydroxylase (S27OHase), the two rate-limiting enzymes of bile acid synthesis, were disproportionably lower (by 2-fold) to that of HMG-CoAR. When fed a sucrose-rich diet, plasma lipids increased, dietary cholesterol absorption improved, hepatic activities of HMG-CoA reductase, C7Ohase, and S27OHase were reduced, and intestinal S27OHase was inhibited in both strains. Despite a similar increase in the biliary hydrophobicity index due to the bile acid enrichment in chenodeoxycholic acid and derivatives, only LPN hamsters had an increased lithogenic index and developed cholesterol gallstones (75% incidence), whereas Janvier hamsters formed pigment gallstones (79% incidence). These studies indicate that LPN hamsters have a genetic predisposition to sucrose-induced cholesterol gallstone formation related to differences in cholesterol and bile acid metabolism. — Férézou, J., M. Combettes-Souverain, M. Souidi, J. L. Smith, N. Boehler, F. Milliat, E. Eckhardt, G. Blanchard, M. Riottot, C. Sérougne, and 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.* 2000. **41: 2042–2054.**

Supplementary key words bile • SR-BI • sterol 27-hydroxylase • cholesterol 7α-hydroxylase

The Syrian golden hamster (*Mesocricetus auratus*) is now the most widely used animal model for studying cholesterol gallstone development because it has important aspects of cholesterol and bile acid metabolism similar to that of humans (1, 2). Following the pioneering studies of Dam and Christensen (3), who induced cholesterol gallstones with a lipid-free sucrose-based diet, other diets for the induction of cholesterol gallstones have emerged (4, 5) and many contain unrealistic levels of cholesterol (6–8). These studies on the whole have demonstrated that the ability to induce gallstones is variable and dependent on the sex and strain of the hamster (9, 10). Studies from our laboratory have demonstrated that male hamsters raised in our breeding unit [Laboratoire de Physiologie de la Nutrition (LPN) strain] have a high susceptibility to develop cholesterol gallstones when fed various sucrose-rich diets. While gallstones were found in 100% of adult LPN animals fed the lipid-free Dam diet over several weeks (11), a 50% gallstone incidence was observed if immature hamsters were fed a sucrose-based diet containing 10% lipids for 1 week, an incidence that consistently declined if animals were fed beyond 1 week (12). Further studies showed that long-lasting lithiasis could be achieved by using a sucrose-based diet containing 5% lard instead of the more usual 10%, resulting in about 65% of adult animals developing
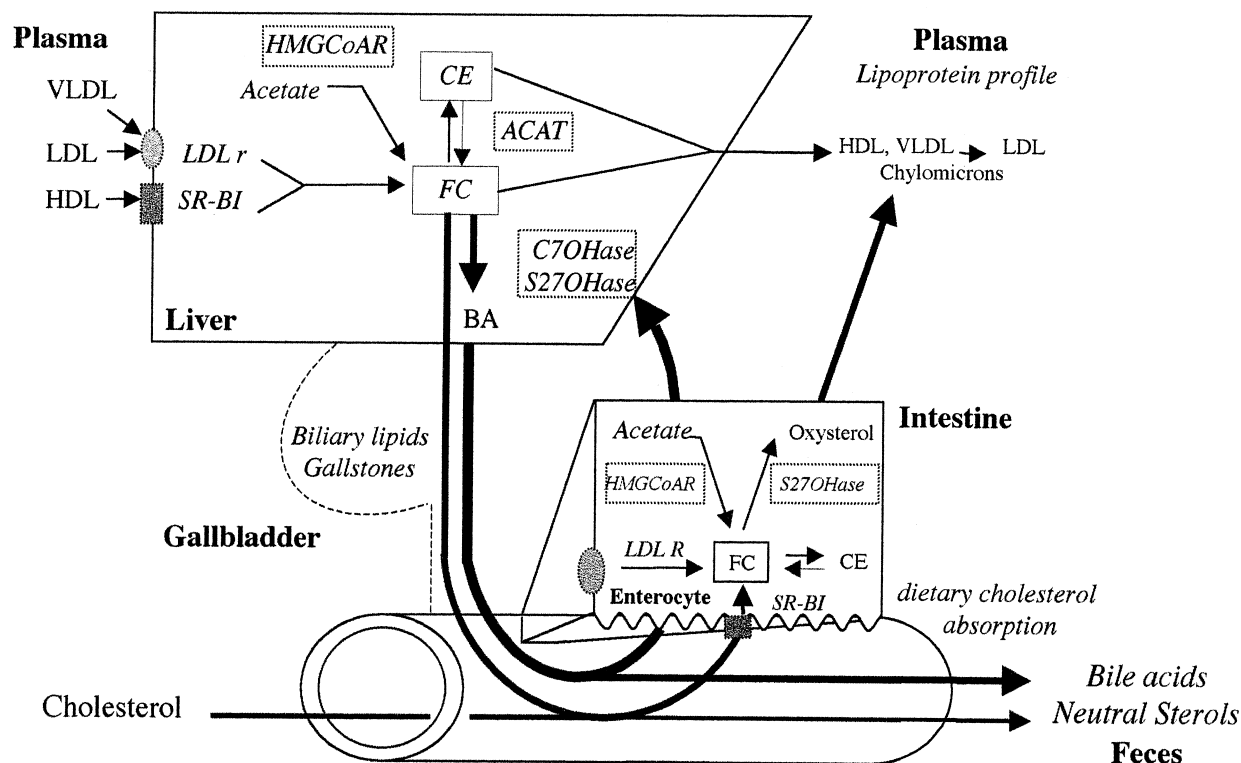

Fig. 1. Scheme for hepatic, biliary, and intestinal cholesterol metabolism. Italicized parameters were investigated in the present study (ACAT, acyl-coenzyme A:cholesterol acyltransferase; BA, bile acids; CE, cholesteryl esters; FC, free cholesterol; HMG-CoAR, 3-hydroxy-3-methylglutaryl-coenzyme A reductase; LDLr, LDL receptor; SR-BI, scavenger receptor class B type I; C7OHase, cholesterol 7α-hydroxylase; S27OHase, sterol 27-hydroxylase).

cholesterol gallstones (13, 14). During periods of limited availability of LPN hamsters, we observed that a more readily available commercial strain of hamster (termed Janvier) developed pigment gallstones, and never cholesterol gallstones, when fed the same 5% lipid sucrose-rich diet. This marked difference in cholesterol gallstone susceptibility provided us with an opportunity to establish the major factors responsible for the development of cholesterol gallstones in LPN hamsters. Thus we undertook a detailed investigation of lipid metabolism in these two strains of hamster under both the basal and gallstone-inducing dietary conditions. Many of the important regulatory elements of lipid metabolism, as they relate to pathogenesis of cholesterol gallstones, are shown in **Fig. 1** and those italicized were determined in the present study.

MATERIALS AND METHODS

Chemicals

Hydroxypropyl-β-cyclodextrin was a gift from Roquette Frères (Lestrem, France). Cholesterol (>99% pure, thin-layer chromatography grade), essentially fatty acid-free bovine serum albumin, dithiothreitol (DTT), alkaline phosphatase, and other chemicals of the highest purity were purchased from Sigma-Aldrich (St-Quentin Fallavier, France). Intralipid 20% was purchased from Pharmacia-Upjohn (St-Quentin-en-Yvelines, France) and heparin from Sanofi-Winthrop (Ambarès, France). Anion-exchange AG1-X8 resin was purchased from Bio-Rad (Ivry/Seine, France). Solvents were purchased

from Prolabo (Fontenay sous Bois, France), the Emulsifier-Safe cocktail for radioactivity counting from Packard Instrument (Meriden, CT), and silica gel G-precoated plastic sheets from Macherey-Nagel (Hoerdt, France). A polyclonal antibody raised against the low density lipoprotein (LDL) receptor purified from bovine adrenal cortex was kindly provided by P. Roach (Adelaide, Australia). Antibody raised against 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMG-CoAR) was a gift from Gene Ness (Tampa, FL). A rabbit polyclonal antipeptide antibody, raised against amino acid residues 495–509 of the murine scavenger receptor class B, type I (SR-BI) (15) was kindly prepared by A. Mazur (INRA, Theix, France). [14C]oleoyl-CoA and enhanced chemiluminescence reagent were purchased from Amersham France (Les Ulis, France); [14C]cholesterol, [3H]cholesterol, [3H]cholesterol oleate, [14C]HMG-CoA, [3H]mevalonolactone, [3H]25-cholesterol, and sodium [14C]acetate were purchased from Dupont-NEN Products (Les Ulis, France).

Diets

The semipurified sucrose (S)-based diet was composed of 67.5% sucrose, 20% casein, 5% lard (28% 16:0, 20% 18:0, 41% 18:1, 12% 18:2), 5% mineral salts (formula 205 from UAR, Epinay-sur-Orge, France), and 2.5% vitamin-cellulose (formula 200 from UAR). The commercial (P) diet (autoclavable pellets CRF from UAR) contained 12% moisture, 53% carbohydrates, 20% proteins, 5% lipids (20% 16:0, 10% 18:0, 36% 18:1, 33% 18:2), 4% cellulose, and 6% mixture of mineral salts and vitamins. These two diets contained approximately 11 mg of sterols per 100 g, consisting of 10.2 mg of cholesterol and 0.3 mg of phytosterols in the S diet, and 2.3 mg of cholesterol and 9.0 mg of phytosterols in the P diet, as assessed by gas-liquid chromatography analysis of the unsaponifiable material (16).

Animals

All experiments used 4-week-old male golden hamsters weighing 30–40 g, born in our breeding unit (LPN hamsters) or purchased from a commercial source (Centre d'Elevage Janvier, Le Genest-St Isle, France) and we labeled them as Janvier. The animals were caged in pairs and fed the commercial (P) diet for 1 week to allow for adaptation. They were then housed individually and fed the sucrose (S) or the commercial (P) diet for the next 5 weeks. Food and water were given ad libitum. The room temperature was maintained at $23 \pm 1°C$ and lighting conditions were controlled (lights on from 7:00 AM to 9:00 PM). Body weight was monitored throughout the experiments and body growth was calculated for the last 5 weeks. In all experiments, animals were killed in a postprandial state, between 9:00 and 12:00 AM, approximately 30 min after withdrawing food. All hamsters were 10 weeks old at the end of the experiment. The care and use of the animals for experimental purposes were in accordance with the ethical standards of French decree 87-849 (October 19, 1989).

Plasma, bile, and organ sampling

The animals were anesthetized by intramuscular injection of Tiletamine and Zolazepan (Zoletil 50; Reading, Nice, France) at a dose of 250 mg/kg body weight and killed by intra-aortic puncture, using a 2-ml syringe (Becton Dickinson Europe, Meylan, France) containing 200 IU of heparin. The abdomen was opened, the gallbladder examined for the presence of gallstones, and the bile was collected with a 0.5-ml syringe (Becton Dickinson Europe) and stored at $-20°C$ until required. The gallbladder was removed, opened, and rinsed with saline and stored at $-20°C$ for subsequent tissue analysis. The liver was rapidly excised, weighed, and apportioned for preparing cellular fractions for enzyme assays or stored at -20 or $-80°C$ (for mRNA assays). The small intestine was excised, its content removed by washing with physiological saline, weighed, and stored at $-20°C$ or placed in ethanol for subsequent sterol extraction. Blood was centrifuged at 3,500 g at $4°C$ for 15 min and the plasma was collected and kept at $-20°C$ for subsequent lipid measurements and lipoprotein analysis. In one selected experiment carried out in the two groups of LPN and Janvier hamsters fed the commercial diet (P), hepatic bile was collected after gallbladder bile puncture. The hole left in the gallbladder wall served to introduce a silastic catheter, which was gently pushed into the cystic bile duct and ligated. The condition of the catheter and bile flow was checked prior to bile being collected for 1 h in order to measure the rate of bile secretion.

In vivo isotopic studies

Dietary cholesterol absorption experiments. Intestinal dietary cholesterol absorption was assessed in another group of hamsters according to a single-isotope procedure adapted from that described in hamsters (17). For each animal, a 200-μl aliquot of ethanol containing a known dose (2 μCi) of [³H]cholesterol was added to a sample (0.8 g) of the powdered diet and gently dried under nitrogen. After an overnight fast, the labeled food was made available to the hamsters between 10:00 AM and 2:00 PM, and then replaced by the regular unlabeled food. The hamsters were killed, as described above, approximately 24 h after the ingestion of the radioactive bolus, that is, between 1:00 and 3:00 PM. After extraction of the unsaponifiable material (18) from a liver sample (0.8 g), the radioactivity was measured and expressed for the entire liver. Plasma samples (200 μl) were mixed with 10 ml of scintillation fluid for radioactive counting. To obtain the whole plasma radioactivity, an assumption was made that each 100 g of body weight is equivalent to 4.0 ml of plasma (17). To calculate the radioactivity ingested by each animal, the non-ingested labeled food was extracted twice with 5 ml diethylether and the radioactivity of the dried extract was measured. The degree of dietary cholesterol absorption was expressed as the fraction of the ingested radioactivity recovered in the liver and plasma 24 h after the bolus administration of labeled cholesterol.

Incorporation of Na [¹⁴C]acetate into sterols. The activity of cholesterogenesis was assessed from the in vivo incorporation of sodium [¹⁴C]acetate into sterols of liver and intestine (19). Briefly, the animals were anesthetized 60 min after a subcutaneous injection of 20 μCi of sodium [¹⁴C]acetate and killed 10 min afterward by intracardiac blood puncture. The liver and the intestine were rapidly excised, weighed, and saponified. The sterol-containing unsaponifiable fraction was extracted three times with petroleum ether and washed three times with a mixture of ethanol–water 1:1 (v/v). A fraction of the unsaponifiable material was counted and the remaining fraction was used for sterol digitonin precipitation. The sterol-digitonin complex was assessed for radioactivity. The results were expressed as disintegrations per minute per 20 μCi injected for both liver and intestine.

Fecal elimination of endogenous sterols. The fecal output of endogenous cholesterol was calculated in another series of hamsters by a single-isotope procedure adapted from a clinical method (20). The animals received a prior subcutaneous injection of 4 μCi of [³H]cholesterol in 0.2 ml of 20% Intralipid. Eight days later, a blood puncture (0.5 ml) was performed from the retro-orbital sinus under light diethyl ether anesthetization to obtain plasma. Feces were collected between day 8 and day 14, and the animals were killed by intra-aortic puncture under anesthesia on day 14. The two plasma samples (from intermediate and final blood punctures) were assayed for radioactivity and the specific activity of plasma cholesterol was calculated for both time points. As the two values were similar, the mean was used for all subsequent calculations, without correction for the transit time time (21). Feces were extracted with boiling ethanol for 24 h, using a Soxhlet apparatus, and the ethanolic lipid extract was assessed for radioactivity. A fraction of the extract was saponified and the unsaponifiable material was assessed for radioactivity. The daily fecal output of endogenous sterols (mg/day) was calculated as follows: total radioactivity of the ethanolic extract (dpm) divided by the mean specific activity of plasma cholesterol (dpm/mg) and then divided by 6, being the number of days in which feces were collected. In the same way, the fecal output of endogenous cholesterol excreted but not converted to bile acids was calculated by dividing the radioactivity of the unsaponifiable extract by the mean specific activity of plasma cholesterol, divided by 6. The daily fecal output of cholesterol excreted as bile acids was calculated as the difference between the endogenous output of total sterols and that of neutral sterols.

Chemical and biochemical assays

Plasma, bile, and tissue analyses. Plasma lipids were measured by enzymatic procedures, using commercial kits, by means of an automatic analyzer (Abbott VP, Rungis, France): total cholesterol (CHOD-PAP method; Boehringer Mannheim, Meylan, France), triglycerides, and phospholipids (Wako method; Oxoid, Rungis, France). Plasma glucose was assayed enzymatically (Boehringer Mannheim) and insulin was assayed by radioimmunoassay (rat insulin radioimmunoassay kit; Linco Research, St. Louis, MO). Bile samples were diluted (1:20) with physiological saline and analyzed for cholesterol and phospholipids as previously described (14). The total bile acid concentrations were assayed by the 3α-hydroxysteroid dehydrogenase method (22) and the cholesterol saturation index (lithogenic index) was calculated (23). The determination of bile salt species was performed in bile samples by isocratic high performance liquid chromatography analysis (24). Lipids were extracted from homogenates of liver samples (0.5 g) and assayed enzymatically, as described previously

(14). The technique was adapted for gallbladder wall samples by homogenizing them in 1 ml of isopropanol with an Ultra-Turrax apparatus (Janke & Kunkel, Staufen, Germany), followed by incubation at 60°C for 1 h and 30 min and centrifugation for 10 min at 3,000 *g*. The supernatant was taken to dryness and dissolved in 200 µl OF isopropanol before enzymatic cholesterol assay. The pellet was dried and dissolved in 1 ml OF 0.1 N NaOH for protein assay by the Lowry method (25), using bovine serum albumin as a standard.

Plasma lipoproteins. Lipoproteins were fractionated by ultracentrifugation of plasma samples (0.4 ml) in a saline density gradient, using an SW41 rotor in an L8-70 apparatus (Beckman Instruments, Gagny, France) as previously described (26). Twenty-two fractions (0.5 ml) were collected and analyzed for cholesterol as described above.

Cellular fractions. Fresh liver samples (1 g) or the entire small intestine was homogenized in 7 ml of buffer (50 mM KH_2PO_4, 300 mM sucrose, 0.5 mM DTT, 10 mM ethylenediaminetetraacetic acid, 50 mM NaCl; pH 7.4) at 4°C by 25 strokes of a Teflon pestle, as detailed (13). Microsomal and mitochondrial fractions were prepared according to a procedure already described (27), adapted from Einarsson et al. (28). The protein content was assayed by the Lowry method (25), using bovine serum albumin as a standard, and aliquots were stored at $-80°C$. Total RNA was extracted from frozen ($-80°C$) liver samples (100 mg), using a commercial kit (Quickprep total RNA extraction; Pharmacia Biotech, Piscataway, NJ) and stored at $-80°C$. The RNA concentration was determined by measuring the absorbance at 260 nm. All samples had an A_{260}/A_{280} ratio greater than 1.8–2.0.

Enzyme activities. Acyl-coenzyme A:cholesterol acyltransferase (ACAT) activity was determined in the absence of exogenous cholesterol, as previously described (13).

HMG-CoA reductase (HMG-CoAR) activity was assessed in microsomal fractions from liver and intestine, using a radioisotopic technique adapted from Philipp and Shapiro (29). Each assay tube (80–300 µg of protein) was preincubated for 60 min at 37°C in the presence of 0.5 IU of phosphatase, cooled in an ice bath, and then incubated for 10 min at 37°C after adding [^{14}C]HMG-CoA (20 nmol, 100,000 dpm) and NADPH (0.7 mg). The reaction was stopped by the addition of 30 µl of 5 N HCl in order to convert the reaction product into [^{14}C]mevalonolactone. After adding [^{3}H]mevalonolactone (10,000 dpm) as an internal marker, each sample was passed through a minicolumn (0.9 g) of anion-exchange resin (AG1-X8; Bio-Rad) eluted with 1.5 ml of distilled water and the radioactivity of the eluate was measured.

The improved assays for cholesterol 7α-hydroxylase (C7OHase) and mitochondrial sterol 27-hydroxylase (S27OHase) activities in hamster liver and small intestine have been described in detail (27, 30).

All assays were performed in duplicate and 4 tubes containing boiled microsomal or mitochondrial fractions were treated in parallel, as controls. Enzymatic activities were expressed in pmol/min/mg protein and total activities were calculated per each entire organ.

Immunoassays. The protein level of hepatic receptors was determined by Western blot from microsomal fractions from liver or intestine (31). Briefly, proteins were fractioned by 7.5% sodium dodecyl sulfate-glycerol polyacrylamide electrophoresis, using a Mini Protean II apparatus (Bio-Rad), and electrotransferred onto 0.45-µm nitrocellulose membranes for immunoblotting, as previously described (32). After incubation with antibodies against HMG-CoAR, LDL receptor, or SR-BI, the antibody binding was visualized with a rabbit IgG labeled with horseradish peroxidase and the enhanced chemiluminescence method (Amersham). The films (Hyperfilm; Amersham) were scanned by a laser densitometer (LKB 2222 Ultroscan XL; LKB, Bromma, Sweden) interfaced with a microcomputer. Results were expressed in arbitrary units (AU) per milligram of protein, using the peak area of each band.

Liver mRNA levels of HMG-CoAR and C7Ohase. Quantification of HMG-CoAR and C7OHase mRNA levels was performed by reverse trancription-polymerase chain reaction (RT-PCR), using a Retroscript™ kit (Ambion, Clinisciences, France). Reverse transcription was carried out with 2 µg of total cellular RNA, 42 mM Tris-HCl (pH 8.3), 62.5 mM KCl, 2.5 mM $MgCl_2$, 4.2 mM DTT, 10 U of RNase inhibitor, 4.2 µM random decamer primers, 0.41 mM dNTPs, and 100 U of Moloney murine leukemia virus reverse transcriptase in a total volume of 24 µl at 42°C for 1 h. Reverse transcription was terminated by a 10-min incubation at 92°C. PCR was performed with a Retroscript™ kit (Ambion) and *Thermophilus aquaticus* (*Taq*) DNA polymerase (Qiagen, Les Ulis, France). For purposes of semiquantitation, glyceraldehyde-3-phosphate deshydrogenase (G3PDH) was coamplified with either HMG-CoAR or C7OHase. The choice of the sequences of oligonucleotides primers (Oligo-express, Paris, France) was determined from the hamster cDNA sequences of C7OHase (33), HMG-CoAR (34), and G3PDH (35), as follows: HMG-CoAR, 5′-AGGAAGAGGAAAGAC-3′/3′-CGGTAGGAGGTTGGT-5′; C7OHase, 5′-TTTGGACACAGAAGCATT-3′/3′-GCCATGTCATCAAAGGTA-5′; G3PDH, 5′-GGCTCTCTGCTCCTC-3′/3′-CAGCCCCAGCAT CAA-5′.

For HMG-CoAR and C7OHase, 3.6 µl (300 ng) of reverse transcription product was amplified in a reaction mix consisting of 1× PCR buffer (Qiagen), 2.5 U of *Taq* DNA polymerase (Qiagen), and a 125 µM concentration of each dNTP (Ambion). This mixture was supplemented with 1.5 mM $MgCl_2$ (Qiagen), 0.5 µM HMG-CoAR and G3PDH primer, 5 µl of Q-Solution (Qiagen) for G3PDH/HMG-CoAR coamplification, or with 4.5 mM $MgCl_2$, 0.2 µM C7OHase primer, and 0.7 µM G3PDH primer for G3PDH/C7OHase coamplification. Sterilized distilled water was added to give a final reaction volume of 50 µl. PCR was performed with a cycler (TRIO-Thermoblock; Biometra, Gottingen, Germany). After denaturation at 94°C for 5 min, the procedure consisted of either 26 cycles at 94°C for 30 s, annealing step at 49°C for 30 s and 72°C for 30 s, ending with 10 min of elongation at 72°C, with a simplified hot start system, for G3PDH/HMG-CoAR coamplification, or 30 cycles at 94°C for 30 s, annealing step at 51°C for 3 s and 72°C for 30 s, ending with 10 min of elongation at 72°C, with a simplified hot start system (adding the PCR tubes in the cycler when it has reached the initial 94°C), for G3PDH/C7OHase. The expected sizes of amplification products were 951 bp for G3PDH, 325 bp for HMG-CoAR, and 496 bp for C7OHase. Products were run in ethidium bromide-stained 1% agarose gels, and the fluorescence associated with DNA bands was measured with a laser densitometer (LKB Ultroscan XL). The mRNA levels were assessed from the HMG-CoAR or C7OHase/G3PDH cDNA signal ratio.

Statistical analysis

Results are given as mean values $\pm$ SEM. Statistical analyses were performed by a Student's *t*-test: Janvier versus LPN strain (strain effect), sucrose-rich (S) versus commercial (P) diet (diet effect). Spearman coefficients were computed to determine the correlations between the parameters.

RESULTS

Physiological characteristics of hamsters from the two strains are shown in **Table 1** for the two dietary conditions studied: sucrose-based diet (S) or commercial pellets (P).

TABLE 1. Final body weight, body growth, liver and intestine weights, and incidence of biliary gallstones[a] in LPN and Janvier hamsters fed a sucrose-rich diet or commercial pellets[b]

| | Sucrose Rich | | Commercial | |
	LPN	Janvier	LPN	Janvier
n[c]	24	24	33	29
Final body weight, g	70.9 ± 0.8[+++]	91.5 ± 2.7***	76.8 ± 1.1	89.4 ± 3.6**
Growth, g/day	0.82 ± 0.05[+]	1.33 ± 0.07***	0.97 ± 0.03	1.29 ± 0.03***
Liver				
g	3.61 ± 0.09[+++]	4.54 ± 0.17*	2.94 ± 0.06	3.91 ± 0.27***
% Body weight	5.11 ± 0.10[+++]	4.95 ± 0.09	3.84 ± 0.07	4.42 ± 0.22*
n	12	12	12	12
Intestine				
g	1.92 ± 0.03	1.84 ± 0.05[+]	1.93 ± 0.03	1.67 ± 0.03***
% Body weight	2.74 ± 0.08	1.81 ± 0.03***,[+++]	2.55 ± 0.04	1.63 ± 0.02***
Gallstones	18/24 (white)	19/24 (black)	0/33	10/29 (black)

[a] White; cholesterol gallstones; black, pigment stones.
[b] Significantly different: Strain effect for each diet, Janvier versus LPN: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$
                  Diet effect for each strain, S versus P: [+] $P < 0.05$, [++] $P < 0.01$, [+++] $P < 0.001$
[c] Number of animals in each group.

Regardless of diet, Janvier hamsters had higher final body weights than LPN hamsters because of higher body growth. As compared with the commercial chow diet, the sucrose-based diet lowered the body growth and the final body weight of LPN but not of Janvier hamsters. For the commercial diet, the weight of the liver was higher, while that of the small intestine was lower in Janvier than in LPN hamsters. As compared with the commercial diet, the sucrose-based diet increased the relative liver weight in LPN hamsters only, and had no effect on the relative intestine weight. Examination of the gallbladder showed that about 30% of Janvier hamsters developed black gallstones when fed the commercial diet, a proportion, which increased to 79% when fed the sucrose-rich diet. In contrast, LPN hamsters did not develop pigmented stones on the chow diet and had a high incidence (75%) of typically white cholesterol gallstones on the sucrose-rich diet.

Plasma parameters and liver cholesterol content

Regardless of diet, LPN differed from Janvier hamsters by lower plasma levels of cholesterol and phospholipids (**Table 2**). Comparisons of the lipoprotein cholesterol profiles (**Fig. 2**) indicate that there is a strain effect for both LDL and HDL when hamsters are fed the sucrose-rich diet and for HDL only when animals are fed the standard chow diet. As compared with the chow diet, the sucrose-rich diet increased all plasma lipids in the two strains. Despite the significant differences in plasma lipid concentrations between the two strains, the percent cholesterol contained in LDL (Fig. 2, fractions 4–9) and HDL (Fig. 2, fractions 11–14) was similar in hamsters fed the chow diet, being 28% for LDL and 60% for HDL. When fed the sucrose-rich diet, the percent cholesterol decreased to 16% for LDL and increased to 70% for HDL in both LPN and Janvier hamsters. The proportion of choles-

TABLE 2. Plasma lipid, glucose, and insulin concentrations and liver cholesterol content in nonfasted LPN and Janvier hamsters fed a sucrose-rich diet or commercial pellets[a]

| | Sucrose Rich | | Commercial | |
	LPN	Janvier	LPN	Janvier
n[b]	24	24	33	29
Plasma, mg/L				
Cholesterol	1,308 ± 54[+++]	1,835 ± 53***,[+++]	759 ± 15	1,171 ± 48***
Triglycerides	1,677 ± 137[+++]	2,020 ± 163***,[+++]	879 ± 62	956 ± 87
Phospholipids	2,950 ± 97[+++]	4,027 ± 148***,[+++]	1,809 ± 43	2,463 ± 95***
n	6	6	6	6
Glucose	1,180 ± 113	1,010 ± 62	1,086 ± 123	956 ± 83
Insulin	4.5 ± 0.9	4.1 ± 0.1	4.5 ± 0.7	2.6 ± 0.6*
n	6	6	15	12
Liver, mg/g				
Total cholesterol	3.67 ± 0.37[+]	4.34 ± 0.35[+++]	2.09 ± 0.07	2.02 ± 0.17
Esterified cholesterol	1.88 ± 0.34[+++]	2.62 ± 0.32[+++]	0.44 ± 0.05	0.51 ± 0.11

[a] Significantly different: Strain effect for each diet, Janvier versus LPN: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$
                  Diet effect for each strain, S versus P: [+] $P < 0.05$, [++] $P < 0.01$, [+++] $P < 0.001$
[b] Number of animals in each group.

A Sucrose-rich diet
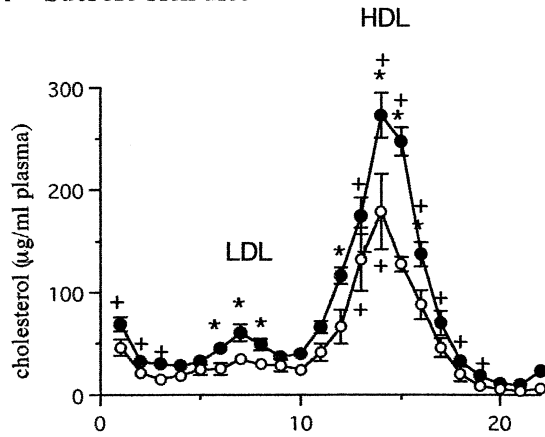
B Commercial pellets
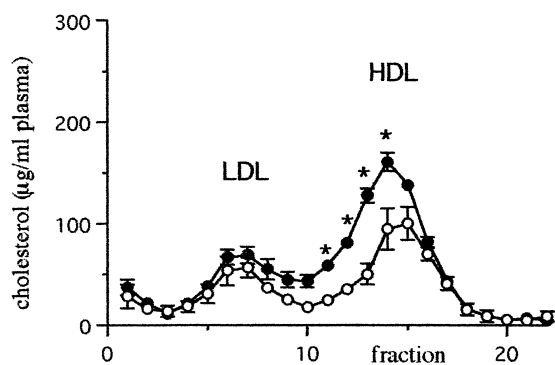
Fig. 2. Distribution of plasma cholesterol in lipoprotein fractions separated by density-gradient ultracentrifugation from plasma samples (mean ± SEM, n = 6 animals per group) obtained from LPN or Janvier hamsters fed commercial pellets or a sucrose-rich diet.

terol contained in triglyceride-rich lipoproteins (Fig. 2, fractions 1 and 2) remained unchanged at 5–7% for both LPN and Janvier animals fed either the commercial or the sucrose diet. Whereas glycemia was unaffected by the strain or the diet, the insulin concentration was reduced in Janvier compared with LPN hamsters in the basal state, and increased in the Janvier hamsters by the sucrose-rich diet to be similar to LPN hamster levels. The liver cholesterol content, similar in the two strains under the basal condition of chow diet, was increased by the sucrose-rich diet in both strains. This effect was due mainly to the increased concentration of esterified cholesterol (4-fold for LPN, 5-fold for Janvier hamsters).

Gallbladder wall and biliary lipid concentrations

The gallbladder wall showed a similar cholesterol and protein content in LPN and Janvier hamsters under basal dietary conditions; however, when fed the sucrose-rich diet, the cholesterol and protein content doubled in LPN hamsters, resulting in a thicker gallbladder wall, while these parameters remained essentially unchanged in Janvier hamsters (**Table 3**).

Regardless of diet, LPN displayed higher biliary lipid concentrations than Janvier hamsters. As compared with the commercial diet, the sucrose-rich diet increased the lipid concentrations in the gallbladder bile: the strongest effect was observed for cholesterol in the LPN strain (4-fold vs. 3-fold in Janvier), whereas phospholipid and bile acid concentrations increased similarly in the two strains (3-fold and 2-fold, respectively). The lithogenic index, identical in both strains under the basal condition, increased slightly in Janvier, but markedly in LPN hamsters when fed the sucrose-based diet. For each dietary condition, no major strain difference appeared in the bile acid composition or in the hydrophobic index. Under basal conditions, however, the glyco/tauroconjugated bile acid ratio was 3-fold higher in LPN than in Janvier hamsters. When hamsters were fed the sucrose-rich diet, this ratio increased in both strains, the proportions of glycochenodeoxycholic and glycodeoxycholic acids increased (by about 3-fold) at the expense of taurocholic acid, leading to an increase in the hydrophobic index by about 2-fold in both strains. The mean rate of bile secretion under the basal condition (n = 6) was about 2-fold higher in LPN than in Janvier hamsters (220 and 120 µl/h, respectively).

Cholesterol absorption, synthesis, excretion, and transformation to bile acids

The dietary cholesterol absorption experiments indicated that 3% of the radioactivity was present in plasma and 7–8% in the liver, 24 hours after the oral bolus of labeled cholesterol, in the two strains of hamsters fed the commercial diet (**Table 4**). As compared with this basal condition, the sucrose-rich diet had little effect on the plasma radioactivity, but it significantly increased the radioactivity in the liver, according to the strain (3-fold in Janvier, 2-fold in LPN hamsters). This indicates that the absorption of dietary cholesterol was more efficient in Janvier than in LPN hamsters.

After prior radiolabeling of the mobile pool of body cholesterol, the daily fecal outputs (calculated) indicate that the excretion of endogenous neutral sterols in LPN is two to three times greater than that of Janvier hamsters, regardless of diet. Conversely, that of endogenous cholesterol converted to bile acids was 3-fold lower in LPN than in Janvier hamsters fed the commercial diet and similar for both strains fed the sucrose-rich diet (Table 4). In LPN hamsters, the fecal excretion of endogenous cholesterol (as total steroids) was not influenced by the diet, whereas in Janvier hamsters this rate was decreased by 50% by the sucrose-rich diet.

Cholesterol synthesis, as assessed in vivo by sodium [^{14}C]acetate injection, demonstrated that LPN differed from Janvier hamsters in both dietary conditions, by a greater incorporation of radioactivity into unsaponifiable material in liver (12-fold or 4-fold, according to the commercial or sucrose-rich diet, respectively) and in intestine (2- to 3-fold, regardless the diet). A strain difference was

TABLE 3. Cholesterol and protein content of gallbladder wall, lipid concentrations, lithogenic index, distribution of major bile acids, glyco/tauroconjugation ratio, and hydrophocity index of the gallbladder bile, in LPN and Janvier hamsters fed a sucrose-rich diet or commercial pellets[a]

	Sucrose Rich		Commercial	
	LPN	Janvier	LPN	Janvier
n[b]	6	6	6	6
Gallbladder wall content, µg				
Cholesterol	15.5 ± 2.5[+++]	6.9 ± 0.5***	6.6 ± 0.4	7.5 ± 0.5
Proteins	614 ± 95[+++]	325 ± 25***	280 ± 18	355 ± 19
n	11	13	17	12
Biliary lipids, mg/ml				
Cholesterol	2.90 ± 0.53[+++]	1.27 ± 0.15**,[+++]	0.69 ± 0.06	0.43 ± 0.06**
Phospholipids	18.33 ± 4.12[++]	9.06 ± 1.09*,[+++]	5.98 ± 0.66	2.65 ± 0.42***
Bile acids	153.00 ± 24.68[+++]	90.02 ± 8.82*,[+++]	74.64 ± 6.84	45.26 ± 2.99**
Lithogenic index	0.31 ± 0.03[+++]	0.23 ± 0.01*	0.18 ± 0.01	0.18 ± 0.02
n	11	13	10	7
Bile acids, % Mol[c]				
Taurocholic	14.68 ± 1.45[+++]	12.90 ± 1.03[+++]	33.72 ± 3.15	48.49 ± 4.17**
Glycocholic	36.08 ± 2.45	39.35 ± 0.68[+++]	43.43 ± 3.42	24.56 ± 3.60**
Taurochenodeoxycholic	10.88 ± 2.59	7.67 ± 0.56[+++]	9.46 ± 1.34	14.60 ± 1.60**
Taurodeoxycholic	4.36 ± 0.28[+++]	4.95 ± 0.34[+]	1.41 ± 0.15	2.71 ± 0.7*
Glycochenodeoxycholic	29.5 ± 2.18[+++]	26.42 ± 1.75[+]	11.04 ± 0.99	7.45 ± 1.33*
Glycodeoxycholic	3.63 ± 0.60[+++]	7.34 ± 0.66***,[+++]	0.60 ± 0.16	1.47 ± 0.45*
Glyco/tauroconjugation ratio	2.89 ± 0.3[+]	3.25 ± 0.37[+++]	1.44 ± 0.27	0.57 ± 0.14*
Hydrophobicity index	0.28 ± 0.01[+++]	0.29 ± 0.01[+++]	0.15 ± 0.01	0.16 ± 0.01

[a] Significantly different: Strain effect for each diet, Janvier versus LPN: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$
Diet effect for each strain, S versus P: [+] $P < 0.05$, [++] $P < 0.01$, [+++] $P < 0.001$
[b] Number of animals in each group.
[c] The small proportions of conjugated ursodeoxycholic and lithocholic acids are not shown.

evident in the ratio of sterol/unsaponifiable radioactivity measured in the liver, being >0.8 for LPN compared with 0.5 for Janvier hamsters regardless of diet. This ratio was unchanged (>0.9) in the intestine for both strains.

Rate-limiting enzymes of cholesterol metabolism and lipoprotein receptors

In the basal state, ACAT activity (expressed as per milligram of microsomal protein) was 2-fold higher in Janvier

TABLE 4. Efficiency of dietary cholesterol absorption calculated from radioactivity in plasma and liver, fecal output of endogenous cholesterol, and cholesterogenesis[a]

	Sucrose Rich		Commercial	
	LPN	Janvier	LPN	Janvier
n[b]	6	6	6	6
Radioactivity, %				
Plasma	3.95 ± 0.16[+]	5.32 ± 0.26**,[+++]	3.15 ± 0.19	2.80 ± 0.17
Liver	14.96 ± 0.99[+++]	22.65 ± 0.42***,[+++]	7.54 ± 0.45	7.72 ± 0.47
Sum	18.91 ± 1.11[+++]	27.97 ± 0.22***,[+++]	10.87 ± 0.79	10.59 ± 0.72
Fecal output, mg/day				
Total steroids	3.06 ± 0.59	2.11 ± 0.07[+++]	3.19 ± 0.16	4.12 ± 0.22**
Neutral sterols	1.51 ± 0.53	0.51 ± 0.04**,[+++]	2.10 ± 0.12	0.93 ± 0.12***
Bile acids	1.55 ± 0.09	1.60 ± 0.06[+++]	1.09 ± 0.13	3.19 ± 0.22***
Radioactivity, dpm				
Liver				
Unsaponifiable	22,720 ± 4,260	5,360 ± 600***	17,420 ± 3,420	1,500 ± 150***
Sterols	18,320 ± 4,000	2,880 ± 360***,[+++]	14,700 ± 3,000	860 ± 160***
Intestine				
Unsaponifiable	29,420 ± 3,800[++]	14,600 ± 1,100***,[+++]	14,940 ± 580	5,480 ± 320***
Sterols	26,620 ± 3,200[++]	13,020 ± 1,060***,[+++]	14,620 ± 1,120	5,300 ± 260***

Efficiency of dietary cholesterol absorption was calculated from the radioactivity in plasma, liver, and their sum, expressed as a percentage of the cholesterol radioactivity ingested 24 h before; fecal outputs of endogenous cholesterol were measured as total steroids, neutral sterols, and bile acids calculated after prior radiolabeling of the mobile pool of cholesterol; cholesterogenesis was assessed from the radioactivity measured in the unsaponifiable fraction and purified sterols of liver and intestine 70 min after labeled acetate (20 µCi) injection. Isotopic methods are detailed in text (see Materials and Methods).
[a] Significantly different: Strain effect for each diet, Janvier versus LPN: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$
Diet effect for each strain, S versus P: [+] $P < 0.05$, [++] $P < 0.01$, [+++] $P < 0.001$
[b] Number of animals in each group.

TABLE 5. Hepatic activities[a] and total activities[b] of ACAT, HMG-CoAR, C7OHase, and S27OHase, and mRNA levels of HMG-CoAR and C7OHase, in LPN or Janvier hamsters fed a sucrose-rich diet or commercial pellets[c]

	Sucrose Rich		Commercial	
	LPN	Janvier	LPN	Janvier
n[d]			9	5
ACAT activity	ND	ND	5.3 ± 0.4	12.3 ± 2.3**
Total activity			98 ± 9	268 ± 48***
n	6	6	15	17
HMG-CoAR activity	168 ± 38+	55 ± 11*	291 ± 32	77 ± 17***
Total activity	3,267 ± 775	1,611 ± 413	4,959 ± 571	2,074 ± 469***
n	6	6	9	9
mRNA	0.98 ± 0.27+	0.83 ± 0.23	3.24 ± 0.76	0.75 ± 0.17**
n	6	6	12	16
C7OHase activity	30 ± 3+++	15 ± 1*,++	119 ± 11	76 ± 11*
Total activity	569 ± 66++	416 ± 86++	1,850 ± 80	1,504 ± 195
n	6	6	8	9
mRNA	1.67 ± 0.21++	0.77 ± 0.20*,+++	3.09 ± 0.31	2.32 ± 0.20*
n	6	6	12	12
S27OHase activity	55 ± 21	7 ± 4*,+++	91 ± 12	59 ± 6*
Total activity	478 ± 154+++	68 ± 41*,+++	1,769 ± 183	1,200 ± 138*

Abbreviation: ND, not determined.
[a] Picomoles per minute per milligram protein.
[b] Expressed per entire liver.
[c] Significantly different: Strain effect for each diet, Janvier versus LPN: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$
Diet effect for each strain, S versus P: + $P < 0.05$, ++ $P < 0.01$, +++ $P < 0.001$
[d] Number of animals per group.

than in LPN hamsters, and the total activity (expressed for the entire liver) was 3-fold higher (**Table 5**). Conversely, HMG-CoAR activity was markedly higher (4-fold under basal conditions, 3-fold with the sucrose-rich diet) and the total activity was still higher (about 2-fold) in LPN than in Janvier hamsters. In comparison with the basal condition, the sucrose-rich diet induced a reduction in HMG-CoAR activity in the two strains, but the effect was significant only in the LPN strain. The HMG-CoAR mRNA levels paralleled the changes found for activities. The detection of the HMG-CoAR protein (100-kDa band) by Western blotting showed that its amount was positively correlated ($r = 0.906$, $P < 0.001$; n = 15) with HMG-CoAR activity.

Regardless of diet, the specific activities of C7OHase and S27OHase were about 2-fold greater in LPN than in Janvier hamsters, but the strain difference was not significant for total C7OHase activities because of the higher liver weight in Janvier hamsters. As compared with the commercial diet, the sucrose-rich diet induced a profound fall in the C7OHase specific activity in both strains (4- to 5-fold). A similar effect appeared for S27OHase, with the greatest effect for Janvier hamsters (8-fold decrease). As for HMG-CoAR, the liver C7OHase mRNA levels paralleled C7OHase activities.

In both dietary conditions, the liver amounts of LDL receptor (expressed per milligram of protein) were slightly lower in LPN than in Janvier hamsters, the difference being more significant when the results were calculated for the entire liver. As compared with the commercial diet, the sucrose-rich diet significantly increased the amount of

TABLE 6. Immunoreactive amounts expressed in arbitrary units[a] and total amounts[b] of LDL receptor and SR-BI in the liver of LPN and Janvier hamsters fed a sucrose-rich diet or commercial pellets[c]

	Sucrose Rich		Commercial	
	LPN	Janvier	LPN	Janvier
n[d]	4	4	4	4
LDL receptor				
AU	116 ± 2	143 ± 12	100 ± 9	121 ± 5
Total amount	135 ± 7+	263 ± 27**,+	100 ± 11	172 ± 17*
SR-BI				
AU	7 ± 11	138 ± 50	100 ± 5	130 ± 11*
Total amount	90 ± 13	254 ± 92	100 ± 9	185 ± 15**

[a] AU per milligram of protein.
[b] Au per whole organ.
[c] Significantly different: Strain effect for each diet, Janvier versus LPN: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$
Diet effect for each strain, S versus P: + $P < 0.05$, ++ $P < 0.01$, +++ $P < 0.001$
[d] Number of animals in each group.

TABLE 7. HMG-CoAR and S27OHase activities[a] and total activities [b] in small intestine of LPN
and Janvier hamsters fed a sucrose-rich diet or commercial pellets[c]

| | Sucrose Rich | | Commercial | |
	LPN	Janvier	LPN	Janvier
n[d]	6	6	6	6
HMG-CoAR				
Activity	200 ± 26	$176 \pm 49^+$	205 ± 45	$62 \pm 15^*$
Total activity	$1,351 \pm 213$	$829 \pm 279^+$	736 ± 233	$146 \pm 40^*$
n	6	6	12	12
S27OHase				
Activity	ND	ND	8 ± 1	$52 \pm 16^{**}$
Total activity			60 ± 9	$360 \pm 110^*$

Abbreviation: ND, not detectable.
[a] Picomoles per minute milligram of protein.
[b] Expressed per entire organ.
[c] Significantly different: Strain effect for each diet, Janvier versus LPN: $* P < 0.05$, $** P < 0.01$, $*** P < 0.001$
                      Diet effect for each strain, S versus P: $^+ P < 0.05$, $^{++} P < 0.01$, $^{+++} P < 0.001$
[d] Number of animals in each group.

LDL receptor protein in both strains. For SR-BI, the only significant effect was the strain difference observed in the total liver amount under the basal condition (**Table 6**).

As shown in **Table 7**, HMG-CoAR activity in small intestine was higher (3-fold) in LPN than in Janvier hamsters fed the commercial diet, a difference that was accentuated (5-fold) when expressed for whole intestine because of the larger intestinal mass for LPN animals (see Table 1). When fed the sucrose lithogenic diet, there was essentially no effect on reductase activity in LPN hamsters, whereas it was increased significantly in Janvier hamsters. Assays of mitochondrial S27OHase activity indicate a major strain effect in hamsters fed the commercial diet, with a 6-fold higher activity in Janvier compared with LPN hamsters. When fed the sucrose-based diet, S27OHase activity was not detectable in both strains of hamsters.

In the small intestine (**Table 8**), the LDL receptor protein level was the lowest in Janvier hamsters fed the commercial diet (only 10–17% of the level found in LPN hamsters). When fed the sucrose-rich diet, Janvier hamsters produced a 50-fold increase over the basal level, whereas for LPN the change was much less marked, at a 4- to 6-fold increase. Qualitatively, the variations of SR-BI protein levels were similar to those of LDL receptor but the differences in magnitude were lower.

DISCUSSION

To establish the important metabolic and physiological events responsible for the pathogenesis of cholesterol gallstones in hamsters, a comprehensive study of lipid metabolism (Fig. 1) was undertaken in two strains of hamsters that differed markedly in their response to a sucrose-rich/ low fat diet. While the LPN strain readily developed cholesterol gallstones with high incidence, the Janvier strain developed only pigment gallstones and was totally resistant to cholesterol gallstone induction. Hamsters were studied under both basal and gallstone-inducing dietary conditions to, first, identify their inherent biochemical and physiological differences and, second, determine what compensatory mechanisms are operative to favor cholesterol gallstone development or their prevention. Special attention was paid to strain differences in activities of the

TABLE 8. Immunoreactive amounts[a] and total amounts[b] of LDL receptor and SR-BI in small intestine
of LPN and Janvier hamsters fed a sucrose-rich diet or commercial pellets[c]

| | Sucrose Rich | | Commercial | |
	LPN	Janvier	LPN	Janvier
n[d]	4	4	4	4
LDL receptor				
AU	$439 \pm 53^{+++}$	$3,456 \pm 105^{***,+++}$	100 ± 7	$17 \pm 1^{***}$
Total amount	$698 \pm 143^{++}$	$5,215 \pm 568^{***,+++}$	100 ± 20	$10 \pm 1^{**}$
SR-BI				
AU	$156 \pm 20^+$	$637 \pm 124^{++}$	100 ± 9	72 ± 4
Total amount	$249 \pm 32^{++}$	$1,020 \pm 199^{**,++}$	100 ± 9	$47 \pm 3^{**}$

[a] AU per milligram of protein.
[b] AU per whole organ.
[c] Significantly different: Strain effect for each diet, Janvier versus LPN: $* P < 0.05$, $** P < 0.01$, $*** P < 0.001$
                      Diet effect for each strain, S versus P: $^+ P < 0.05$, $^{++} P < 0.01$, $^{+++} P < 0.001$
[d] Number of animals in each group.

major regulatory enzymes HMG-CoAR, C7OHase, S27OHase and ACAT, and lipoprotein receptors to account for the differences in plasma and biliary lipid concentrations. While LPN and Janvier hamsters had a similar external appearance at all stages of development, adult LPN animals differed from those of Janvier in that they had a lower body weight and a relatively higher intestinal mass. These differences were maintained when fed the high-sucrose rich lithogenic diet.

Differences between LPN and Janvier hamsters under basal conditions

When fed the commercial diet, LPN animals differed from Janvier hamsters by an increased bile secretion rate associated with higher lipid concentrations in gallbladder bile and no pigment gallstones, indicating that the factors controlling bile secretion are different between the two strains. The lithogenic and hydrophobicity indices were, however, similarly low in both strains. In LPN hamsters, the relatively high glyco/tauroconjugation ratio of bile acids probably reflects a limitation of the taurine pool, which could alter bile acid production (36). The lower plasma cholesterol level in LPN hamsters, combined with the increased bile secretion rate, strongly suggests that the cholesterol turnover is more rapid in this strain than in Janvier animals. This hypothesis is supported by the striking strain differences observed in cholesterogenesis. In the in vivo experiments, the radioactivity incorporated into sterols after the labeled acetate injection was markedly higher in LPN than in Janvier hamsters for both liver and small intestine (20-fold and 3-fold, respectively). Moreover, comparing the sterol/unsaponifiable radioactivity ratios indicates that the process of cholesterol synthesis is more efficient in the LPN strain. Qualitatively, the same strain differences appear for hepatic HMG-CoAR activity and expression (4-fold for both activity and mRNA level; 2.5-fold for total liver activity) and intestinal HMG-CoAR activity (3-fold, and 5-fold when expressed for the entire organ). Taken together, these results indicate that whole body cholesterol synthesis is much more active in LPN than in Janvier hamsters.

With major differences in cholesterol synthesis, it was surprising that a strain difference was not evident in the fecal outputs of endogenous cholesterol, as determined by total steroids (neutral sterols plus bile acids) after prior radiolabeling of the mobile pool of cholesterol. The underestimated value in LPN hamsters could be explained by a strain difference in the transit time, which was not taken into account in the calculation of the fecal output of steroids because it is unknown. Nevertheless, the fraction of endogenous cholesterol converted into bile acids, before fecal excretion, is obviously smaller in LPN than in Janvier hamsters (0.34 vs. 0.77, respectively), indicating a weaker capacity to convert cholesterol into bile acids. As LPN hamsters have only slightly higher hepatic activities of C7OHase (and mRNA amounts) and S27OHase than Janvier animals, it can be deduced that these two bile acid-synthesizing enzymes do not compensate for the high rate of cholesterogenesis. Moreover, the relatively low hepatic

ACAT activity indicates a reduced capacity by the liver to store cholesterol. As the liver cholesterol concentration and the efficiency of dietary cholesterol absorption are similar in both strains, the net result of these enzymatic changes would be to increase the availability of hepatic free cholesterol for efflux into bile in LPN hamsters compared with Janvier animals. This combined with the relatively low capacity to metabolize cholesterol to bile acids is probably, in part, responsible for the sensitivity of LPN hamsters to the dietary induction of cholesterol gallstones.

The control of glucose metabolism is another important factor in gallstone development (37–39). In this respect, both LPN and Janvier hamsters have similar plasma glucose concentrations when fed the basal diet, although LPN animals have a higher postprandial insulinemia than Janvier animals. These data suggest that the regulation of glucose metabolism differs between the two strains and that LPN hamsters are more insulin resistant. What response would be expected in animals challenged with a sucrose-enriched diet such as the lithogenic diet used in the present study? Postprandial insulinemia is likely to increase to maintain plasma glucose levels. This normal response occurred in the Janvier hamsters but did not in LPN hamsters, which supports the hypothesis of insulin resistance in LPN hamsters and could explain, in part, their predisposition to biliary cholesterol gallstones.

Differences between LPN and Janvier hamsters fed the lithogenic diet

Feeding the sucrose-rich diet in place of commercial chow increases the plasma lipid concentration by the same percentage in both strains, with LPN hamsters still having significantly lower levels of cholesterol and phospholipids than Janvier hamsters. The HDL cholesterol concentration is predominantly increased in both strains; however, for LPN hamsters LDL cholesterol is reduced. As to be expected, dietary cholesterol absorption is enhanced for both strains because the semisynthetic sucrose-rich diet contains much less fiber and phytosterols than the chow diet and these are known to prolong the transit time and improve cholesterol absorption. The increase in dietary cholesterol absorption is, however, less marked in LPN than in Janvier hamsters. In response to an increased flux of exogenous cholesterol, the liver neutralizes the additional cholesterol by esterifying it. On the basis of the hepatic concentration of cholesteryl esters and basal ACAT activities, the storage capacity tends to be lower in LPN than in Janvier hamsters. The massive cholesterogenesis activity, which characterizes LPN hamsters under basal conditions, is also present when hamsters are fed the sucrose-rich diet. Taking into account that the local dilution of the exogenous labeled acetyl-CoA in the endogenous pool varies according to the organ and the physiological conditions (40), sterol radioactivities cannot be used to study the diet effect on cholesterogenesis. However, they mirror the strain differences in local cholesterogenesis, which is still significantly greater in LPN than in Janvier hamsters. On the basis of HMG-CoAR activities and mRNA levels, it may be concluded that hepatic cho-

lesterogenesis is decreased in both strains, with the effect being more prominent in LPN hamsters. In contrast, intestinal cholesterogenesis is unaffected in LPN hamsters, but surprisingly is stimulated in Janvier animals. These data indicate that cholesterogenesis may be independently regulated in liver and intestine. When fed the sucrose-rich diet, the decrease in hepatic cholesterol synthesis may be related to a change in insulin secretion, as this is known to influence cholesterol biodynamics and/or to enhance dietary cholesterol absorption. Under this condition, the fecal output of endogenous cholesterol is higher in LPN than in Janvier hamsters, which is in agreement with the strain difference in cholesterogenesis. Moreover, the activity and mRNA of C7OHase and the S27OHase activity are dramatically reduced by feeding the sucrose-rich diet, the effect being more pronounced in Janvier hamsters, without any major imbalance in bile acid composition (glycodeoxycholic was different) between the two strains. The reduction in the activities of enzymes of bile acid metabolism could also be due to an increased insulin secretion induced by sucrose, as demonstrated by in vitro studies (41). The fraction of endogenous cholesterol transformed to bile acids before fecal excretion still remained lower in LPN than in Janvier hamsters (0.5 vs. 0.75); a condition that also favors cholesterol gallstone development.

When the data obtained from both dietary conditions are combined, it is remarkable that a positive correlation between C7OHase and HMG-CoAR activities is found for Janvier ($r = 0.93$, $P < 0.001$, n = 11) but not for LPN hamsters ($r = 0.52$, $P = 0.118$, n = 10). Such a relationship is well known in rats, apart from when fed excess cholesterol (42), and most experimental data support the concept that the regulation of HMG-CoAR activity is secondary to that of C7OHase in rats (43, 44). The same strain difference occurs for the linkage between S27OHase and HMG-CoAR activities, which are positively correlated in Janvier hamsters ($r = 0.75$, $P = 0.006$, n = 11) but not in LPN hamsters ($r = 0.02$, $P = 0.99$, n = 11). The absence of such a coordinate regulation in LPN hamsters partly explains why bile acid synthesis is not greatly increased to compensate for a very active cholesterol synthesis, as already discussed (11). Consequently, the excess cholesterol is eliminated via the biliary route without prior conversion to bile acids, even under basal conditions.

The pathophysiology of cholesterol gallstones in the LPN strain is further supported by the marked differences found in the gallbladder. When challenged with the lithogenic diet, both hamster strains had increased lipid concentrations in gallbladder bile, an elevated bile acid hydrophobic index reflecting a greater proportion of chenodeoxycholic acid (plus its derivatives). Despite these changes, none of the Janvier hamsters developed cholesterol gallstones whereas three-quarters of the LPN hamsters did. This strain effect corresponds to the different change induced by the sucrose diet on the lithogenic index, which increases significantly only in the LPN strain. In addition to the alterations in biliary lipids, LPN hamsters also had a dramatic thickening of the gallbladder wall, an effect that is likely to affect the motility of this organ. However, the biliary secretion rate for LPN hamsters, twice that of Janvier animals in the basal state, is further increased when fed the lithogenic diet [(14) and J. Férézou, M. Combettes-Souverain, M. Souidi, J. L. Smith, N. Boehler, F. Milliat, E. Eckhardt, G. Blanchard, M. Riottot, C. Sérougne, and C. Lutton, unpublished observations]. Therefore, cholesterol gallstone formation in LPN hamsters cannot be explained by extrahepatic bile stasis, in contrast with observations in prairie dogs (45) or in patients with cholesterol gallstones (46).

Other important strain differences also exist in the small intestine, which could impact on the enterohepatic circulation of steroids. S27OHase is not only expressed in the liver, but also in extrahepatic tissues such as lungs, vascular endothelium, ileum, and small intestine (47). This enzyme contributes to the elimination of excess cholesterol by an oxidative process, the oxysterol product being carried in the circulation mainly by HDL and rapidly removed by the liver, where it is converted into bile acids (48). As 27-OH cholesterol is described to be a strong in vivo inhibitor of HMG-CoAR (49), we hypothesize that the 27-OH cholesterol formed in extrahepatic tissues, such as small intestine, is inhibitory for local cholesterogenesis. Indeed, the intestinal activity of S27OHase varied inversely with that of cholesterogenesis in all four animal and dietary groups examined in the present study. Under basal conditions, S27OHase activity was lower in LPN than in Janvier hamsters, whereas cholesterol synthesis was greater. In hamsters fed the lithogenic diet, S27OHase activity was undetectable in intestinal samples from both strains. These interesting preliminary data raise the question of an involvement of intestinal macrophages in this oxidative process. A similar pattern of inhibition was not observed for cholesterol synthesis in liver and this is probably due to the rapid conversion of the oxysterol, both from endogenous and exogenous sources, into bile acids.

Further insight into the predisposition of LPN hamsters to cholelithiasis can be related to their low plasma cholesterol level together with the associated hepatic lipoprotein receptors. Surprisingly, LPN hamsters have a lower hepatic expression of LDL receptor, even when the sucrose-rich diet induces a significant fall in LDL cholesterol in this strain. In a similar vein, LPN animals also have reduced plasma HDL cholesterol levels and SR-BI expression. The importance of SR-BI in cholesterol metabolism has been established only recently. This multifunctional protein has been identified as an HDL receptor involved in the selective uptake of HDL cholesterol, in both liver and steroidogenic tissues, and participates in the export of cholesterol into bile by the hepatocyte (15). Much of the understanding of its physiological role has been deduced from genetic manipulations in mice, which produce spectacular alterations in plasma and biliary lipid parameters (50). Under more physiological conditions in hamsters, an inverse relationship between HDL cholesterol level and hepatic SR-BI expression has been reported (51), but other results are consistent with no association (52), as found in the present study. We observed only minor changes in SR-BI or LDL receptor levels in the

face of increased plasma HDL and LDL cholesterol concentrations for both strains of hamsters when challenged for the sucrose-rich diet. There is also preliminary evidence demonstrating that SR-BI mediates intestinal absorption of dietary lipids, including cholesterol (53). Our results in this regard are confounded by the fact that SR-BI concentration varies in parallel with the efficiency of dietary cholesterol absorption; the highest of which is found in Janvier hamsters fed the sucrose-rich diet. Surprisingly, the amounts of LDL receptor measured in intestine show even greater variations depending on the strain and diet. Similar to liver, the highest expression is found again in Janvier hamsters fed the sucrose diet, which also have the lowest intestinal activity of cholesterogenesis. Although the physiological significance of these changes are not yet fully understood, it is clear that these two lipoprotein receptors may be regulated independently in the liver and intestine, as reported for LDL receptor in rats (54). In FB1 hamsters fed a high fat atherogenic diet, differential variations of the HMG-CoAR and LDL receptor message levels between liver and intestine have been also recently reported (55).

In summary, the present study underlines the importance of cholesterol homeostasis in the pathogenesis of cholesterol gallstones and further highlights the usefulness of animal models to elucidate the mechanisms involved. Our two-strain hamster model offers an uncanny analogy with the well-accepted notion of an inverse association between plasma cholesterol levels and cholesterol gallstones in humans often reported in epidemiological studies (56–58). In our experimental model, the etiology of cholesterol gallstone formation is basically related to a strain difference in both lipid and glucose metabolism that is evident in hamsters fed either commercial pellets or the semisynthetic diet consisting of a high proportion of sucrose. This contrasts with other studies using lithogenic diets containing significant quantities of added cholesterol (6–8). In this respect, a gene named *Lith1* has been identified among inbred strains of mice to have a significant influence in cholesterol gallstone formation induced by dietary cholesterol (59). In the present study, the striking strain differences found in the major liver parameters controlling cholesterol turnover allow us to be more certain as to why LPN hamsters are genetically predisposed to sucrose-induced cholelithiasis: they are characterized, especially under basal conditions, by a more active synthesis of cholesterol associated with a limited capacity of the liver to store and transform cholesterol into bile acids, as compared with Janvier hamsters, which are resistant to cholesterol gallstone induction. The variations observed in SR-BI and LDL receptor expression, together with the differences in intestinal activities of the regulatory enzymes of lipid metabolism, indicate that further studies are required to fully understand the role of the gut in lithogenesis.

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