

## Dietary myristic acid modifies the HDL-cholesterol concentration and liver scavenger receptor BI expression in the hamster\*

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The influence of myristic acid in a narrow physiological range (0.5 to 2.4% of total dietary energy) on the plasma and hepatic cholesterol metabolism was investigated in the hamster. The hamsters were fed on a diet containing 12.5 g fat/100 g and 0.05 g cholesterol/100 g with 0.5% myristic acid (LA diet) for 3 weeks (pre-period). During the following 3 weeks (test period), they were divided into four dietary groups with 0.5% (LA), 1.2% (LM), 1.8% (ML) or 2.4% (M) myristic acid. Finally, half the hamsters in each group were again fed the LA diet for another 3 weeks (post-period). At the end of the test period, the hepatic expression of the scavenger receptor BI (SR-BI) was lower in the LM, ML and M groups than in the LA group whereas the hepatic cholesteryl ester concentration was higher. Cholesterol 7 $\alpha$  hydroxylase activity was lower in the ML and M groups than in the LA and LM groups while the sterol 27 hydroxylase and 3-hydroxy-3-methyl glutaryl coenzyme A reductase activities were not modulated by dietary myristic acid. This is the first time a negative correlation has been observed between the HDL-cholesterol concentration and the hepatic mass of SR-BI ( $r = -0.69$ ;  $P < 0.0001$ ) under physiological conditions. An inverse linear regression was also shown between SR-BI and the percentage of myristic acid in the diet ( $r = -0.75$ ;  $P < 0.0001$ ). The hepatic mass of SR-BI in the M group had increased at the end of the post-period compared with the test-period values. The present investigation shows that myristic acid modulates HDL-cholesterol via a regulation of the SR-BI expression.

### Myristic acid: HDL-cholesterol: Scavenger receptor class B type I: Hamster

Myristic acid is considered as the saturated fatty acid which induces the most important increase in plasma total cholesterol especially in LDL-cholesterol levels in human subjects or in animals (Hegsted *et al.* 1965; Hayes & Khosla, 1992). In most of the studies which led to this conclusion, myristic acid represented a very high percentage of the total dietary energy: 16% in human subjects (Kris-Etherton & Yu, 1997) or 16% (Salter *et al.* 1998) to 20% (Woolett *et al.* 1992) in hamsters. Moreover, in these experimental diets, cholesterol is often present in inadequate doses (either none or too high) as reported by Nicolosi (1997). Under these conditions (far from physiological doses), myristic acid could alter cholesterol metabolism. However, myristic acid seems to be an important cell component since numerous proteins need to be

myristoylated in order to play their biological role in the transduction pathway, vesicular trafficking and structural positioning (Boutin, 1997). Myristic acid is also found in significant levels in most mammalian milk and is mainly in the *sn*-2 position on the triacylglycerol (TG) molecule (Jensen *et al.* 1990). As suggested a few years ago (Berner, 1993), the impact of myristic acid (milk fat) on serum lipids needed to be re-evaluated using a more realistic mixed-fat diet. In human milk, myristic acid represents 9% of total fatty acids (about 3.4% of total energy) (Jensen, 1996). Since maternal milk is a well-balanced food, the hypothesis of the present study is that when myristic acid makes up to 9% of total fatty acids in the lipid part of the diet, it would have no undesirable effects on cholesterol metabolism.

**Abbreviations:** CYP27A1, sterol 27 hydroxylase; CYP7A1, cholesterol 7 $\alpha$  hydroxylase; HDL-C, HDL-cholesterol; HMGCoA-R, 3-hydroxy-3-methyl glutaryl coenzyme A reductase; LA, lard diet; LM, lard and milk fat diet; M, milk fat diet; ML, milk fat and lard diet; non-HDL-C, non-HDL-cholesterol; SR-BI, scavenger receptor class B type I; SREBP, sterol regulatory element binding protein; TG, triacylglycerol.

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Male golden Syrian hamsters were chosen to test this hypothesis because they have a well-established similarity with human cholesterol metabolism (Spady & Dietschy, 1983) and are sensitive to changes in the composition of dietary fats (Spady & Dietschy, 1988; Lindsey *et al.* 1990; Kris-Etherton & Dietschy, 1997). They received semi-purified diets with low concentrations of cholesterol (0.05%) and a lipid content (12.5%), which represented 27% of the total dietary energy (i.e. generally advised for human nutrition by Grundy (1997)). Under these nutritional conditions, the percentage of myristic acid varies between 0.5% (i.e. generally found in human nutrition) and 2.4% (about 9% of total fatty acids in the diet) of total energy.

Since the liver is the main organ concerned with the regulation of cholesterol homeostasis, the major hepatic activities implicated in this process have to be assayed in order to better understand the role of myristic acid. The scavenger receptor class B type I (SR-BI) was shown to be an HDL receptor (Acton *et al.* 1996). This receptor, like the LDL receptor in man, plays a major role in cholesterolaemia regulation, especially in rodents (Combettes-Souverain *et al.* 1999). However, only one study (Spady *et al.* 1999) has reported that saturated fatty acids modify the expression of SR-BI compared with polyunsaturated fatty acids. Dietary myristic acid could also modulate bile acid biosynthesis which is the major process of cholesterol degradation in man and animals and can occur via two important pathways (neutral and alternative; Vlahcevic *et al.* 1996). Some studies in other species than hamsters (mice and gerbils) have shown that dietary fatty acids are able to regulate bile acid biosynthesis (Cheema *et al.* 1997; Hajri *et al.* 1998b).

The purpose of the present study was to test the hypothesis that myristic acid (0.5 to 2.4% of total energy and mostly in the *sn*-2 position, as in maternal milk) has no undesirable effects on plasma cholesterol (particularly in the LDL fraction). The effects of dietary myristic acid on the lipid concentrations, masses of lipoprotein receptors (LDL-receptor, SR-BI) and activities of certain key enzymes of cholesterol and bile acid metabolism (3-hydroxy-3-methyl glutaryl coenzyme A reductase (HMGCoA-R), cholesterol 7 $\alpha$  hydroxylase (CYP7A1), sterol 27 hydroxylase (CYP27A1)) in the liver were also observed.

## Materials and methods

### Chemicals and isotopes

Kits for cholesterol, triacylglycerol and phospholipid assays were purchased from Boehringer-Mannheim (Meylan, France) (CHOD-PAP and GPO-PAP methods), and from Wako Unipath (Dardilly, France). A rabbit polyclonal antibody against a peptide containing residues 495–509 from murine SR-BI (kindly prepared by André Mazur, Theix, France) was used to detect SR-BI as described by Acton *et al.* (1996). Hydroxymethylglutaryl coenzyme A, [5-<sup>3</sup>H]mevalonolactone, [4-<sup>14</sup>C]cholesterol and [24-<sup>14</sup>C]chenodeoxycholic acid were obtained from Dupont-NEN Products (Les Ulis, France). Emulsifier-safe

was purchased from Packard Instrument Company (Meriden, CT, USA). Hydroxypropyl- $\beta$ -cyclodextrin was kindly provided by Société Roquette frères (62136 Lestrem, France).

### Animals and diets

Five-week-old male golden Syrian hamsters (*Mesocricetus auratus*) from our breeding unit designated LPN (Laboratoire de Physiologie de la Nutrition) were randomly assigned to four groups fed diets (Table 1) differing only in their lipid component (natural fat): LA (lard), M (milk fat), ML (milk fat+lard; 7:3) or LM (lard+milk fat; 6:4) containing myristic acid (C14:0) as 0.5, 1.2, 1.8 or 2.4% of the total dietary energy respectively. The basal composition of these diets (g/100 g total dry weight) was as follows: maize starch 34.95, sucrose 20, casein 20, vitamin mix (see below) 2.5, salt mix (see below) 5, cellulose 5, cholesterol 0.05, lipid 12.5 (natural fat 10 + rapeseed oil–high oleic sunflower seed oil mix 2.5 (1:1)) which brought the minimum essential fatty acids.

The vitamin mix prepared on cellulose support contained (kg diet): retinol 27 mg; ergocalciferol 2 mg; thiamin 50 mg; riboflavin 37.5 mg; calcium pantothenate 175 mg; pyridoxine 25 mg; meso-inositol 375 mg; vitamin B<sub>12</sub> 0.125 mg; vitamin C 2000 mg; DL  $\alpha$  tocopherol 425 mg; menadione 100 mg; nicotinic acid 250 mg; choline 3400 mg; folic acid 12.5 mg; biotin 0.75 mg; para-amino benzoic acid 125 mg; cellulose 17.37 mg.

The salt mix contained (g/kg diet): NaCl 5; KCl 5; CaHPO<sub>4</sub> 21.5; MgCl<sub>2</sub> 2.5; MgSO<sub>4</sub> 2.5; Fe<sub>2</sub>O<sub>3</sub> 0.15; FeSO<sub>4</sub>.7H<sub>2</sub>O 0.25; MnSO<sub>4</sub>.H<sub>2</sub>O 0.1225; CuSO<sub>4</sub>.5H<sub>2</sub>O 0.025; ZnSO<sub>4</sub>.7H<sub>2</sub>O 0.1004; CoSO<sub>4</sub>.7H<sub>2</sub>O 0.0002; KI 0.0004, maize starch 12.851.

The hamsters were individually caged and had free access to food and water. Lighting conditions were controlled according to a 12 h light–12 h dark cycle (7.00–19.00 hours). The temperature was maintained at 25°C.

The standard diet, vitamin mix and salt mix were purchased from UAR (Villemoisson, 91360 Epinay/orge France); lard (Orsay France); and milk fat ‘huile de beurre fractionnée’ were given by BESNIER BRIDEL (355240 Retiers France). All the experiments were conducted according to the French Regulations for Animal Experimentation (Art 19. Oct 1987, Ministry of Agriculture).

### Experimental design

After weaning, the animals were fed on a standard diet (containing 5% lipids) for 2 weeks in order to homogenize their body weight before the experiment. The experiment was separated into three periods.

During the first 3-week experimental period (pre-period), the hamsters were fed on LA which contained 0.5% of total dietary energy as myristic acid. At the end of this period, the animals were slightly anaesthetized with an intramuscular injection of Tiletamine and Zolazepam (Zoletil 50, Virbac, Carros, France) at a dose of 50 mg/kg body weight and blood samples (500  $\mu$ l) were obtained by cardiac puncture, after an overnight fast. The plasma was separated from the blood cells by centrifugation

**Table 1.** Fatty acid composition of the test diets\*  
(g/100g fatty acids)

Test diets . .	LA		LM		ML		M	
	g/100g	%†	g/100g	%†	g/100g	%†	g/100g	%†
<C12:0	0.064	0.02	4.34	1.2	7.6	2.1	10.8	3
C12:0	0.11	0.03	1.44	0.4	2.4	0.7	3.4	0.9
C14:0	1.81	0.5	4.4	1.2	6.5	1.8	8.54	2.4
C16:0	21.6	6	19.6	5.4	18	5	16.6	4.6
C18:0	14	3.9	10.6	2.9	8.05	2.2	5.5	1.5
C18:1	46.4	12.9	43.2	12	41.1	11.4	39	10.8
C18:2	10.2	2.8	9.6	2.65	9.1	2.5	8.75	2.4
C18:3	1.45	0.40	1.45	0.40	1.45	0.40	1.45	0.40
SFA	38.7	10.7	41.7	11.6	44	12.2	46.3	12.9
MUFA	49.6	13.8	46.5	12.9	44.2	12.3	41.9	11.6
PUFA	11.6	3.2	11.1	3.1	10.6	2.9	10.2	2.8
P:S	0.49		0.43		0.39		0.36	

LA, 10% lard + 2.5% rapeseed oil–high oleic sunflower seed oil mix (1:1); LM, 6% lard + 4% milk fat + 2.5% rapeseed oil–high oleic sunflower seed oil mix (1:1); ML, 7% milk fat + 3% lard + 2.5% rapeseed oil–high oleic sunflower seed oil mix (1:1); M, 10% milk fat + 2.5% rapeseed oil–high oleic sunflower seed oil mix (1:1); SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; P:S, ratio of polyunsaturated fatty acids to saturated (C12:0 + C14:0 + C16:0) fatty acids.

\* For details of the test diets, see p. 200.

† Values represent the part of individual fatty acid expressed as the percentage of total energy.

(10 min at 2600g, at 4°C) and stored at –20°C for further analysis (plasma lipids). During the second 3-week period (test period), each group received its corresponding diet: LA, LM, ML or M, and cardiac punctures were performed as above. Each group was then divided into two subgroups. The animals from the first subgroup were anaesthetized with an intramuscular injection of Tiletamine and Zolazepam (Zoletil 50, Virbac, Carros, France) at a dose of 100 mg/kg body weight and killed by heart blood puncture. The abdomen was opened by a midline incision and the liver was excised, weighed and samples were taken for lipid measurement, SR-BI receptor mass and enzymic assays. Hamsters from the second subgroup were again fed on LA (post-period) for 3 weeks and the same procedure used for the first subgroup was applied.

#### Plasma and lipoproteins

Plasma cholesterol, phospholipids and TG concentrations were measured by enzymic procedures using commercial kits. Apolipoprotein B containing lipoproteins VLDL and LDL was precipitated with phosphotungstate reagent (Weigand & Daggy, 1990). The supernatant fraction was assayed for HDL-cholesterol (HDL-C) with commercial kits. The quantity of VLDL- and LDL-cholesterol (non-HDL-C) was calculated from the difference between plasma total cholesterol and HDL-C.

#### Liver lipids

Frozen liver samples (0.5 g) were thawed and homogenized in 5 ml isopropanol, using an Ultra-Turrax apparatus (Janke & Kunkel Gmb & Co., Staufen, Germany). After incubation at 60°C for 1 h and centrifugation for 5 min at 3000g, the supernatant fraction was collected and the pellet was re-extracted with 5 ml isopropanol. TG and

total cholesterol were measured enzymically on pooled isopropanolic extracts, using appropriate kits. Free and esterified cholesterol were separated by TLC on silica gel plates eluted with diethyl ether, dried and dissolved in isopropanol before enzymic cholesterol determination.

#### Hepatic enzyme activities

The microsomal and mitochondrial fractions were isolated according to the procedure described by Einarsson *et al.* (1986) and Souidi *et al.* (1999). HMGCoA-R activity was determined in the microsomal fractions in the presence of alkaline phosphatase using Phillip & Shapiro's (1979) radioisotopic technique. CYP7A1 was assayed in the microsomal fractions according to a radioisotopic method using [4-<sup>14</sup>C]cholesterol, solubilized and carried by hydroxypropyl-β-cyclodextrin (Souidi *et al.* 1998). CYP27A1 was assayed in the mitochondrial fractions according to a radioisotopic method using [4-<sup>14</sup>C]cholesterol, solubilized and carried by hydroxypropyl-β-cyclodextrin (Souidi *et al.* 1999).

#### Scavenger receptor class B type I receptor binding

Total membranes from frozen liver samples stored at –80°C (1 g) were prepared according to Kovanen *et al.* (1979). Membrane proteins were solubilized in a buffer containing Triton-X100 2% (Schneider *et al.* 1982). They were then assayed by Lowry's method using bovine serum albumin as a standard. (Lowry *et al.* 1951)

Immunodetection was then carried out for SR-BI. Liver protein membranes were diluted in a dilution buffer (Tris-maleate 125 mM; CaCl<sub>2</sub> 2 mM, aprotinin 200 IU/ml; DTT 15 mM; pH 6). Diluted samples (2 μg in 50 μl) were boiled for 5 min at 90°C and spotted onto a nitrocellulose membrane using a dot-blot apparatus (Bio-Rad, Richmond,

**Table 2.** Body weights, dietary intake and liver weights at the end of the different dietary periods from hamsters fed on semi-purified diets containing different dietary fatty acids\*

(Mean values and standard errors for sixteen, eighteen, sixteen or eighteen hamsters per group (pre- and test periods) and for eight, nine, seven or nine hamsters per group (post-period))

Test diets...	LA		LM		ML		M	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Body weight (g)								
Pre-period	69.3	1.8	69.7	1.2	68.3	1.6	67.3	1.1
Test period	79.5	1.5	79.5	1.4	79.5	1.2	78.3	1
Post-period	84.7	2	84	1.6	83.9	1.6	84.3	2
Dietary intake (g/d)								
Pre-period	5.8	0.2	5.6	0.1	5.3	0.2	5.6	0.2
Test period	5.8	0.2	5.6	0.1	5.7	0.2	6	0.3
Post-period	6.4	0.4	5.3	0.2	5.9	0.4	6.2	0.3
Liver Weight (g)†								
Test period	3.08	0.09	3.2	0.1	3.3	0.1	3.08	0.06
Post-period	3.1	0.1	3.2	0.05	3.4	0.1	3.4	0.1

\* For details of diets and procedures, see Table 1 and p. 200.

† For this parameter, there were eight, nine, nine or nine hamsters per group (test period) and eight, nine, seven or nine hamsters per group (post-period).

CA). The nitrocellulose membranes were incubated in a quenching buffer containing 5% fat-free milk (Tris-HCl 25 mM; NaCl 25 mM; CaCl<sub>2</sub> 2 mM, pH 8). The membranes were washed with Tween Tris buffered saline (NaCl 500 mM; Tris-base 250 mM; Tween 20, 0.05%; pH 7.5) buffer and incubated for 90 min in the presence of the antibody against SR-BI diluted 1:2000 in an incubation buffer containing 0.1% fat-free milk (Tris-HCl 60 mM; NaCl 25 mM; CaCl<sub>2</sub> 2 mM, pH 8). The membrane was washed three times with Tween Tris buffered saline and was incubated for 90 min with anti-immunoglobulin antibodies conjugated with horseradish peroxidase diluted at 1:2000. The membranes were washed three times with Tween Tris buffered saline buffer, and incubated for 1 min with a chemiluminescence reagent (ECL, Amersham). The membranes were protected with a plastic film and the sensitive film was revealed in a dark room. The intensity of the spots was measured with a laser densitometer (ultrascan 2222 LKB, Sweden) and the relative SR-BI content in each spot was estimated by the peak height of the scan.

The linearity of the response as a function of the protein quantity spotted was checked. The specific antibodies raised against SR-BI gave a unique band in Western blots with apparent molecular weights of about 82 kD (Milliat *et al.* 2000).

#### Statistical analysis

Results were given as mean values and their SEM. Statistical differences among the groups were determined by ANOVA followed by a Student–Newman–Keuls test. Differences between pre-period and test-period values (for cholesterol, phospholipid and TG concentrations) were analysed by a paired *t* test in which individual animals served as their own controls. Differences between test-period and post-period values were analysed by a *t* test. A value of *P* < 0.05 was considered significant. The Spearman method (software package Statview 4.5 for Windows) was used to determine the correlations.

## Results

### Physiological status

The hamsters remained in good health throughout the experiment. Daily food intake, body weights and liver weights during the three experimental periods are reported in Table 2. There were no significant differences in these parameters between the four groups at the end of each experimental period.

### Plasma lipid concentrations

Plasma lipid concentrations measured at the end of each dietary period are shown in Table 3.

*Test period.* At the end of this period, plasma cholesterol levels increased in the LM, ML and M groups compared with the pre-period values. However, there were no significant differences between the four groups.

The plasma phospholipid levels increased in the LM, ML and M groups compared with the pre-period values but were lower in the LA group than in the other groups.

The plasma TG levels in the M and ML groups increased compared with the pre-period values. There were no significant differences between the four groups.

*Post-period.* Plasma cholesterol levels in the LM group decreased compared with the test-period values. Plasma cholesterol levels in the M group were higher than in the LA and LM groups. Plasma phospholipid levels in the LM group decreased compared with the test-period values. Plasma phospholipid levels in the M group were higher than in the LA and LM groups. The plasma TG concentrations in the four groups were similar to those of the test period. No differences were observed between the four groups.

### Lipoprotein cholesterol concentrations

Plasma HDL-C and non HDL-C concentrations measured at the end of each dietary period are shown in Table 4.

*Test period.* The plasma HDL-C concentrations

**Table 3.** Plasma lipid concentrations at the end of the different dietary periods from hamsters fed on semi-purified diets containing different dietary fatty acids\*

(Mean values and standard errors for thirteen, sixteen, thirteen or seventeen hamsters per group (pre- and test periods) and for eight, nine, six or nine hamsters per group (post-period))

Test diets...	LA		LM		ML		M	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
<b>Cholesterol (mmol/l)</b>								
Pre-period	3.59	0.10	3.20	0.10	3.69	0.13	3.88	0.10
Test period	3.90	0.23	4.32†	0.15	4.42†	0.21	4.49†	0.18
Post-period	3.67 <sup>a</sup>	0.10	3.75 <sup>a,†</sup>	0.18	3.95 <sup>a,b</sup>	0.15	4.45 <sup>b</sup>	0.23
<b>Phospholipids (mmol/l)</b>								
Pre-period	4.26	0.13	3.79	0.09	3.97	0.10	4.02	0.09
Test period	3.79 <sup>a</sup>	0.14	4.56 <sup>b,†</sup>	0.14	4.60 <sup>b,†</sup>	0.24	4.69 <sup>b,†</sup>	0.21
Post-period	3.97 <sup>a</sup>	0.09	3.88 <sup>a,†</sup>	0.07	4.17 <sup>b</sup>	0.13	4.39 <sup>b</sup>	0.15
<b>Triacylglycerols (mmol/l)</b>								
Pre-period	1.48	0.27	1.22	0.11	0.85	0.13	0.81	0.08
Test period	1.24	0.20	1.55†	0.19	1.14	0.10	1.40†	0.15
Post-period	1.15	0.13	1.38	0.17	0.95	0.11	1.25	0.18

<sup>a,b</sup>Mean values within a row with unlike superscript letters were significantly different as determined by ANOVA followed by a Student–Newman–Keuls test ( $P < 0.05$ ).

\* For details of diets and procedures, see Table 1 and p. 200.

† Mean values for the test period were significantly different from those of the pre-period values ( $P < 0.05$ ).

‡ Mean values for the post period were significantly different from those of the test period values ( $P < 0.05$ ).

increased in the LM, ML and M groups compared with the pre-period values. No significant difference in the HDL-C could be observed between the four groups. However, a linear regression ( $r = 0.49$ ,  $P = 0.01$ ,  $n = 23$ ,  $y = 2.57 + 0.51x$ ) was found between the percentage of myristic acid in the diets and the plasma HDL-C concentrations.

Non-HDL-C decreased in the M group compared with the pre-period values. There was no significant difference in non-HDL-C between the four groups at the end of the test period. There was no significant regression between the percentage of myristic acid in the diets and the plasma non-HDL-C concentrations.

*Post-period.* The plasma HDL-C concentrations decreased only in the ML group compared with the test-period values. However, there were no significant differences

between the four groups. Non-HDL-C decreased in the LA group compared with the test-period values. Non-HDL-C was lower in the LA group than in the ML and M groups.

#### Hepatic cholesterol concentrations

The hepatic free, ester, and total cholesterol concentrations measured at the end of each dietary period are shown in Table 5.

*Test-period.* Total and cholesteryl ester concentrations in the LM, ML and M groups were higher than in the LA group. Moreover, a linear regression ( $r = 0.60$ ,  $P < 0.0001$ ,  $n = 35$ ,  $y = 9.2 + 7.8x$ ) was found between the percentage of myristic acid in the diets and the hepatic cholesteryl ester concentrations. Free cholesterol concentrations in the M group were higher than in the LA group.

**Table 4.** Cholesterol lipoprotein concentrations at the end of the different dietary periods from hamsters fed on semi-purified diets containing different dietary fatty acids\*

(Mean values and standard errors for nine, seven, five or eight hamsters per group (pre-period) and for five, seven, six or five hamsters per group (test period) and for five, seven, five or seven hamsters per group (post-period))

Test diets...	LA		LM		ML		M	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
<b>HDL-cholesterol (mmol/l)</b>								
Pre-period	2.23	0.16	1.96	0.13	2.17	0.20	2.31	0.10
Test period	2.77	0.31	3.22†	0.15	3.58†	0.32	3.70†	0.26
Post-period	2.87	0.21	2.82	0.18	2.64‡	0.13	3.18	0.15
<b>Non-HDL-cholesterol (mmol/l)</b>								
Pre-period	1.36	0.05	1.06	0.10	1.63	0.15	1.50	0.07
Test period	1.32	0.21	1.33	0.10	1.37	0.36	1.06†	0.13
Post-period	0.72 <sup>a,†</sup>	0.05	1.03 <sup>a,b</sup>	0.15	1.33 <sup>b</sup>	0.05	1.43 <sup>b</sup>	0.13

<sup>a,b</sup>Mean values within a row with unlike superscript letters were significantly different as determined by ANOVA followed by a Student–Newman–Keuls test ( $P < 0.05$ ).

\* For details of diets and procedures, see Table 1 and p. 200.

† Mean values for the test period were significantly different from those of the pre-period values ( $P < 0.05$ ).

‡ Mean values for the post-period were significantly different from those of the test-period values ( $P < 0.05$ ).

**Table 5.** Hepatic cholesterol concentrations at the end of the different dietary periods from hamsters fed on semi-purified diets containing different dietary fatty acids\*

(Mean values and standard errors for eight, nine, nine or nine hamsters per group (test period) and for eight, nine, seven or nine hamsters per group (post-period))

Test diets...	LA		LM		ML		M	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Total cholesterol (mg/g liver)								
Test period	15.2 <sup>a</sup>	3.2	23.9 <sup>b</sup>	2.6	25.5 <sup>b</sup>	1.8	32.6 <sup>b</sup>	2.5
Post-period	17.4	2.4	22.7	3.6	22.2	3.3	28.4	3.7
Free cholesterol (mg/g liver)								
Test period	2.6 <sup>a</sup>	0.4	3.2 <sup>a,b</sup>	0.2	3.7 <sup>a,b</sup>	0.3	4.2 <sup>b</sup>	0.4
Post-period	2.9	0.1	3.4	0.3	3.4	0.5	3.6	0.2
Esterified cholesterol (mg/g liver)								
Test period	12.3 <sup>a</sup>	2.9	20.8 <sup>b</sup>	2.4	21.7 <sup>b</sup>	1.7	28.4 <sup>b</sup>	2.2
Post-period	14.3	2.3	19.3	3.3	18.8	2.9	24.8	3.5

<sup>a,b</sup>Mean values within a row with unlike superscript letters were significantly different as determined by ANOVA followed by a Student–Newman–Keuls test ( $P < 0.05$ ).

\* For details of diets and procedures, see Table 1 and p. 200.

*Post-period.* Hepatic cholesterol concentrations measured at the end of this period were similar to those obtained at the end of the test period. The cholesteryl ester, total or free cholesterol concentrations were the same in the four groups.

#### Hepatic enzyme activities

Hepatic CYP7A1 and CYP27A1 activities assayed at the end of each dietary period are shown in Fig. 1.

*Test period.* At the end of this period, no significant differences in the hepatic enzyme HMGCoA-R activity were found between the four groups (LA: 259 (SEM 26) ( $n$  6), LM: 275 (SEM 20) ( $n$  9), ML: 287 (SEM 22) ( $n$  9), M: 258 (SEM 16) ( $n$  9) pmol/min per whole liver).

Hepatic CYP7A1 activity in the LA and LM groups was higher than in the ML and M groups and a negative linear regression ( $r -0.53$ ,  $P=0.001$ ,  $n$  33,  $y = 37.4 - 8.7x$ ) was found between the percentage of myristic acid in the diets and the hepatic CYP7A1 activities.

No significant difference in the CYP27A1 activity was found between the four groups. No significant regression between the percentage of myristic acid in the diets and the hepatic CYP27A1 activities was observed.

*Post-period.* CYP7A1 activity in the LA and LM groups decreased compared with the test-period values. There were no significant differences in the CYP7A1 and CYP27A1 activities between the four groups.

#### Scavenger receptor class B type I receptor mass

The hepatic SR-BI receptor mass assayed at the end of each dietary period is shown in Fig. 2.

*Test-period.* The SR-BI mass was higher in the LA group than in the other groups. The SR-BI mass in the LM and ML groups was higher than in the M group.

*Post-period.* The SR-BI mass increased in the M group compared with the test-period values. No significant difference was found between the four groups.

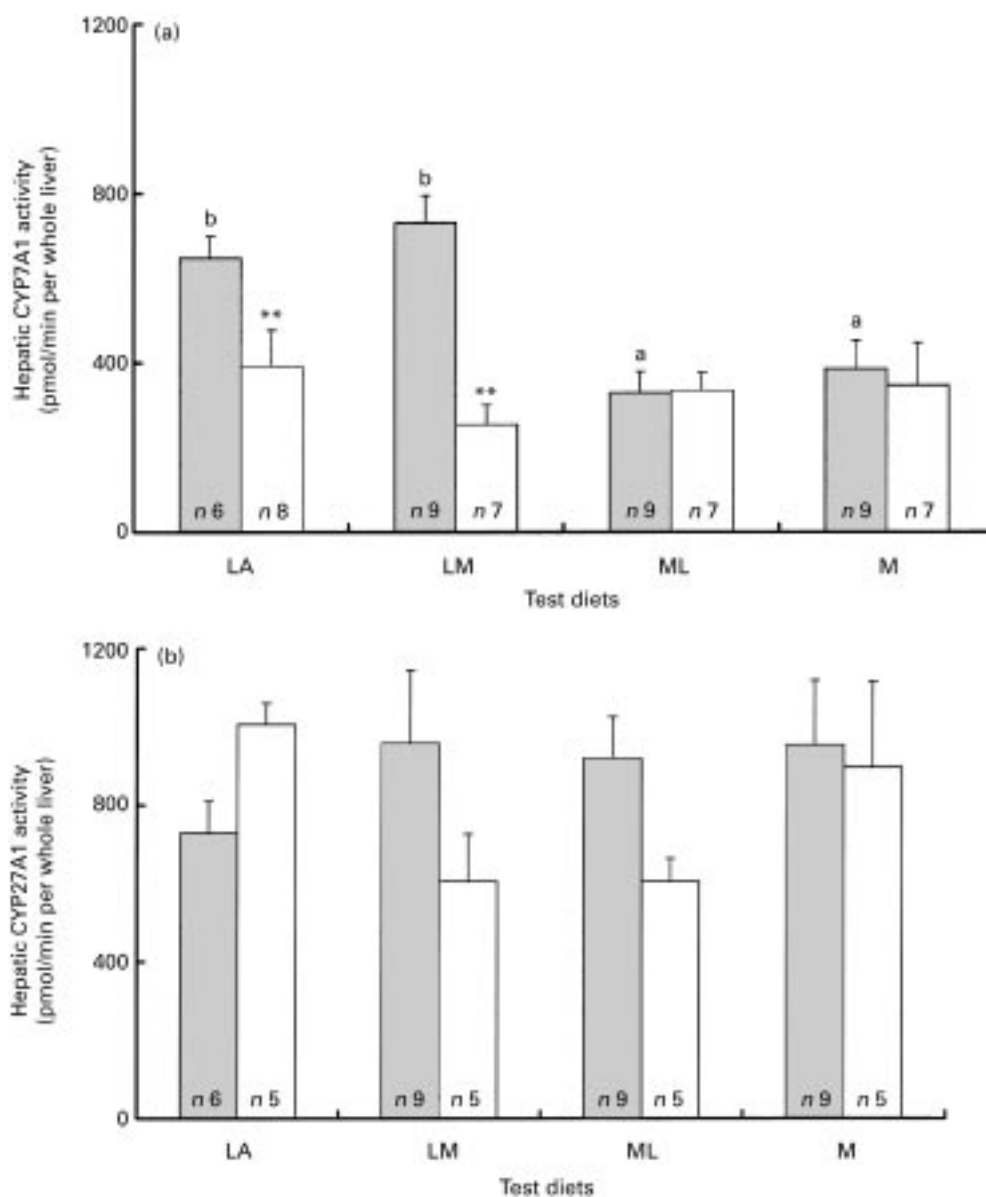
Two correlations were found with the SR-BI mass. The first correlation between the hepatic SR-BI mass and

plasma HDL-C ( $r -0.69$ ,  $P < 0.0001$ ,  $n$  38) is represented in Fig 3(a). The second correlation between the hepatic SR-BI mass and hepatic cholesteryl ester concentration ( $r -0.56$ ,  $P < 0.0001$ ,  $n$  55) is represented in Fig. 3(b).

A linear regression between the hepatic SR-BI mass and the percentage of myristic acid in the diet was found at the end of the test period ( $r -0.75$ ,  $P < 0.0001$ ,  $n$  35) and is represented in Fig. 3(c).

#### Discussion

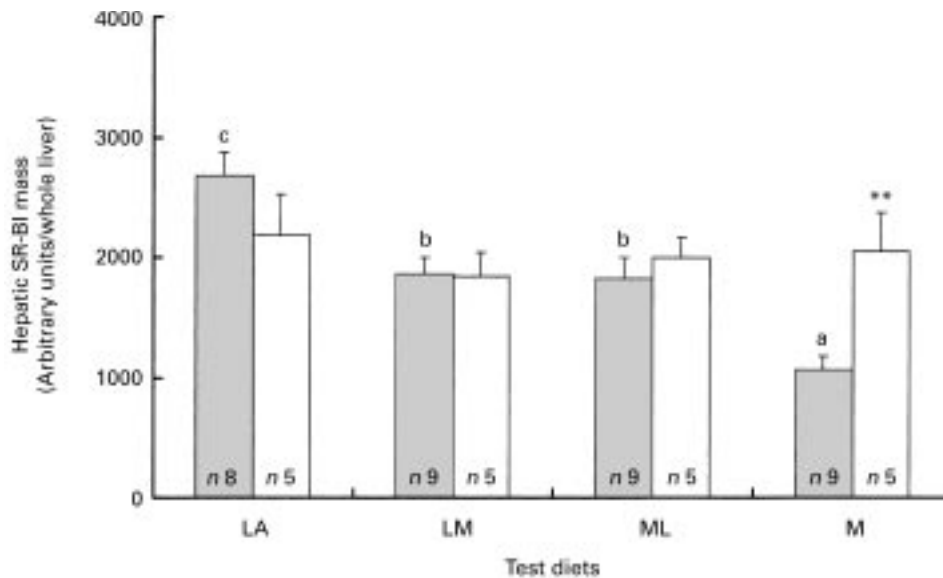
At the beginning of the experiment, the hamsters were fed on a semi-purified diet (12.5% lipid) which contained a low myristic acid level (0.5% of the total dietary energy). This period, called the pre-period, allowed the young animals which are usually fed on a chow diet (5% lipid) to adapt their cholesterol metabolism. Blood samples collected at the end of this period enabled certain plasma parameters to have individual control values as is currently carried out in other species such as monkeys (Khosla *et al.* 1997) or man (Snook *et al.* 1999). After this adaptation period, the hamsters were fed on experimental diets for 3 weeks: LA (0.5% myristic acid), LM (1.2% myristic acid), ML (1.8% myristic acid) or M (2.4% myristic acid). At the end of this test period, plasma cholesterol concentrations increased in the LM, ML and M groups compared with control values. The increase observed in these groups probably reflected the influence of higher percentages of myristic acid in the diet. However, this increase in the plasma cholesterol level observed in the M, ML and LM groups at the end of the test period was not sufficient to observe significant differences in this parameter between the four dietary groups. Our data show that the changes in plasma cholesterol concentration in response to dietary myristic acid concentration are smaller than those reported earlier in man (Hegsted *et al.* 1965; Keys & Parlin, 1966; Mensink, 1993) or in animals for example, (hamsters and guinea-pigs; Nicolosi, 1997). The distribution of cholesterol among the different lipoprotein classes was also determined in the four dietary groups of our experiment. Although the important difference between the animals



**Fig. 1.** Hepatic enzyme activities from hamsters fed on semi-purified diets containing different dietary fatty acids: LA (lard), LM (lard and milk fat, 6:4), ML (milk fat and lard, 7:3), M (milk fat) during the test period (■) and the post-period (□). Vertical bars represent standard errors of the mean. Mean values within each period with unlike superscript letters were significantly different as determined by ANOVA followed by a Student–Newman–Keuls test ( $P < 0.05$ ). Mean values for the post-period were significantly different from those of the test-period values: \*\* $P < 0.05$ . CYP7A1, cholesterol  $7\alpha$  hydroxylase; CYP27A1, cholesterol 27 hydroxylase.

fed a low myristic acid level diet (LA: HDL-C = 2.77 (SEM 0.31) mmol/l) and those fed a higher one (M: HDL-C = 3.70 (SEM 0.26) mmol/l) was not statistically significant, a linear regression between plasma HDL-C and the percentage of myristic acid in the diet ( $r = 0.49$ ,  $P = 0.01$ ,  $n = 23$ ) was clearly established. This type of effect was not observed on the plasma non-HDL-C concentration. On the contrary, this latter parameter decreased in the hamsters fed the M diet (higher level of myristic acid) compared with their pre-period values. These results are noteworthy since in previous animal studies (Hajri *et al.* 1998a; Salter *et al.* 1998; Spady *et al.* 1999) or in man (Temme *et al.* 1997), dietary myristic acid increased not

only the plasma HDL-C concentration but also those of plasma LDL-cholesterol. In some of the studies where natural fats were used, a disturbing effect of other saturated fatty acids present in the diet such as lauric acid in the coconut oil or palmitic acid in the palm oil cannot be excluded. As speculated by Tholstrup *et al.* (1994), the effects of myristic acid on plasma cholesterol should be added to those of lauric or palmitic acids (considered as hypercholesterolaemic). In this case, it became difficult to evaluate the specific action of myristic acid. Previous studies (Horton *et al.* 1993; Tsai *et al.* 1999) have demonstrated that a strong variation in the lauric acid composition of the diets, which is not the case in the present



**Fig. 2.** Hepatic scavenger receptor class B type I (SR-BI) receptor mass from hamsters fed on semi-purified diets containing different dietary fatty acids: LA (lard), LM (lard and milk fat, 6:4), ML (milk fat and lard, 7:3), M (milk fat) during the test period (■) and the post-period (□). Vertical bars represent standard errors of the mean. Mean values within each period with unlike superscript letters were significantly different as determined by ANOVA followed by a Student–Newman–Keuls test ( $P < 0.05$ ). Mean values for the post-period were significantly different from those of the test-period values: \*\* $P < 0.05$ ).

study (Table 1), is necessary to induce a modification in the plasma cholesterol concentration. Moreover, in the present study, the increase in HDL-C in the M group compared with the LA group can not be attributed to variations in the palmitic acid level (Table 1), since the proportion of this fatty acid is lower in the M group than in the LA group. Table 1 also shows that the proportions of the other fatty acids (<C12:0, C18:0 or C18:1) vary between the four test diets. However, it has already been demonstrated that these fatty acids have no real effects on the plasma cholesterol concentrations (Grundy, 1994; Kris-Etherton & Yu, 1997; Nicolosi, 1997). Finally, in the present study the proportion of linoleic acid which along with myristic acid governs the plasma cholesterol response (Hayes & Khosla, 1992) did not vary excessively and less drastically than the proportion of myristic acid between the test diets.

In the light of these observations, the effects on cholesterolaemia (increase in the HDL-C) can mainly be attributed to the action of myristic acid.

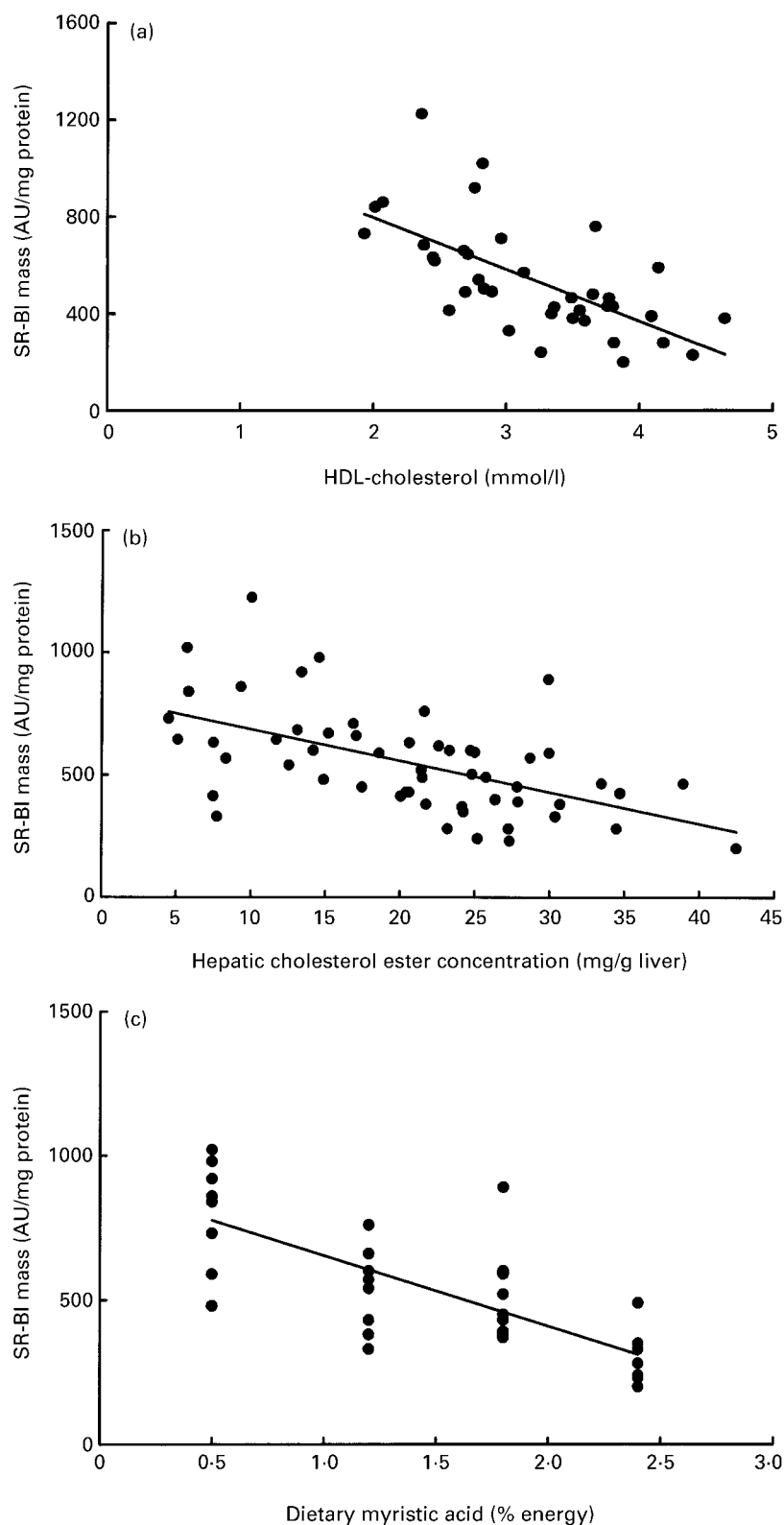
Previous studies have shown that the position of palmitic acid on the TG molecule (*sn* position) influences lipoprotein metabolism (Innis *et al.* 1993; Zock *et al.* 1995; Nelson & Innis, 1999). The majority of the myristic acid present in the milk-fat diets was in the *sn*-2 position (Jensen *et al.* 1990). By contrast, in previous studies using coconut oil (Spady & Dietsch, 1988; Hajri *et al.* 1998a; Spady *et al.* 1999), there was a low proportion of myristic acid in the *sn*-2 position and a high level of lauric acid in this position (Small, 1991). This could further explain the differences observed between the previous studies (increase in LDL-cholesterol and HDL-C) and the present one (increase in HDL-C alone). These observations suggest that the environment of myristic acid in the diet (absence or presence of other saturated

fatty acids), the position of myristic acid on the TG molecule and the percentage of this fatty acid in the diet could modulate its effects on lipoprotein metabolism.

It is generally accepted that variations in the plasma HDL-cholesterol and apolipoprotein AI levels are controlled not by the rate of their production but by the rate of their elimination (Rader & Maugeais, 2000). This latter mechanism is controlled by two major proteins. The first, ATP-binding cassette 1 facilitates the transport of cholesterol between the peripheral cells and the nascent HDL particles (Fielding & Young, 1999). The second, SR-BI mediates the selective hepatic uptake of the cholesteryl ester of mature HDL particles (Acton *et al.* 1996). A negative correlation was observed between the hepatic mass of SR-BI and the plasma HDL-C concentration (Fig. 3(a)). These data were consistent with those observed in mice which overexpressed SR-BI (decrease of HDL-C) (Kosarsky *et al.* 1997; Wang *et al.* 1998) or in transgenic mice in which the expression of SR-BI was attenuated by 50% (increase of HDL-C) (Rigotti *et al.* 1997; Varban *et al.* 1998). However, to our knowledge, it is the first time that such a correlation was obtained under physiological conditions, with genetically unmodified animals. Another interesting linear inverse regression between the hepatic SR-BI mass and the percentage of myristic acid in the diet was also established (Fig. 3(c)). Spady *et al.* (1999) have recently shown that in hamsters a diet rich in polyunsaturated fatty acids increased the SR-BI expression compared with a diet rich in saturated fatty acids (8% myristic acid). The present study shows that increasing the amount of myristic acid in the diet is the most important factor in the increase of HDL-C concentration and that this effect is linked to a decrease in the amount of SR-BI in the liver.

Numerous nuclear factors are involved in the regulation





**Fig. 3.** Correlation between the hepatic scavenger receptor class B type I (SR-BI) mass and plasma HDL-cholesterol (a), and the hepatic cholesteryl ester concentration (b) during the test and post-periods. Linear regression between the hepatic SR-BI mass and the percentage of myristic acid in the four diets (LA, lard; LM, lard and milk fat (6:4); ML, milk fat and lard (7:3); M, milk fat) during the test-period (c). Each point represents the value obtained from a single animal. For (a), the correlation was characterized by  $r = -0.69$ ;  $P < 0.0001$ ,  $n = 38$ ,  $y = 1221.3 - 213.54x$ . For (b), the correlation was characterized by  $r = -0.56$ ;  $P < 0.0001$ ,  $n = 55$ ,  $y = 814.3 - 12.85x$ . For (c), the regression was characterized by  $r = -0.75$ ;  $P < 0.0001$ ;  $n = 35$ ,  $y = 898.7 - 244.6x$ . AU, arbitrary units.

of the SR-BI gene (Lopez & McLean, 1999; Mizutani *et al.* 2000). More specifically, three forms of sterol regulatory element binding protein (SREBP), SREBP1-a, SREBP1-c, and SREBP2 are expressed in the liver and their truncated forms activate the transcription of the sterol-regulated gene (Brendel *et al.* 1998). Lopez & McLean (1999) have observed that SREBP1a bind to the promoter of the rat SR-BI gene. Worgall *et al.* (1998) have shown *in vitro* that some fatty acids decreased the level of mature SREBP. These authors also suggested that this effect of fatty acid is linked to an increase in the intracellular cholesterol pool. The present study supports this hypothesis since a negative correlation between the hepatic mass of SR-BI and the hepatic cholesteryl ester concentration (Fig 3(b)) was observed.

Newly synthesized cholesterol or that brought to the liver by the lipoproteins is mainly eliminated by its transformation into bile acids (Lutton, 1990). The hepatic CYP7A1 activity (rate-limiting enzyme of the neutral biosynthesis pathway) is higher in the LM and LA groups (low in myristic acid) than in the ML and M groups (high in myristic acid). These results agree with those of Hajri *et al.* (1998b) in gerbils. The authors observed a decrease in the CYP7A1 activity in gerbils fed a coconut-oil diet (8% myristic acid) compared with those fed a safflower-seed-oil diet (0% myristic acid). The mechanism by which fatty acids are able to regulate CYP7A1 activity *in vivo* is unknown. Nevertheless, a recent *in vitro* study (Cheema & Agellon, 2000) has shown that the CYP7A1 gene can be regulated by fatty acids via the peroxisome proliferator receptor  $\alpha$ . However, fatty acids influenced the CYP7A1 gene differently according to *in vitro* (Cheema & Agellon, 2000) or in *in vivo* conditions (Cheema & Agellon, 1999). Therefore, Cheema *et al.* (2000) suggested as others have (Marrapodi & Chiang, 2000; Patel *et al.* 2000) that other nuclear factors and/or cofactors could compete with peroxisome proliferator receptor  $\alpha$  for the regulation of the CYP7A1 gene. In contrast to the CYP7A1 activity, there was no difference in the CYP27A1 activity (main enzyme of the alternative pathway of bile acid biosynthesis) between the four dietary groups. Some studies (Kushawa *et al.* 1995; Chen *et al.* 1998) have observed the effects of a high-fat high-cholesterol diet on the CYP27A1 expression in monkeys. However there are no data concerning the eventual effect of a specific fatty acid. There is no significant difference in the activity of HMGCoA-R (the rate-limiting enzyme of cholesterol biosynthesis) between the four groups in our experiment. This effect was also shown in other studies with diets containing cholesterol and different fatty acid (myristic+lauric *v.* linoleic acids or trimyristin *v.* triolein) (Spady & Dietsch, 1988; Bennet *et al.* 1995).

At the end of the test period, the remaining hamsters in each group returned to a low-myristic diet (LA) for 3 weeks. This post-period was required to test an eventual reversal in the major effects of dietary myristic acid observed at the end of the test period. At the end of this post-period, the plasma HDL-C concentration in the M group (higher in myristic acid) decreased but not significantly compared with the test-period values. However, this decrease is sufficient to abolish the difference between

this group and the LA group (lower in myristic acid) observed at the end of the test period. It is noteworthy that, with regards to HDL-C, the mass of hepatic SR-BI in the M group was restored to a value similar to the one observed in the LA group (group in which myristic acid (0.5%) did not vary during the three experimental periods). At the end of the post-period, the hepatic cholesteryl ester concentration in the M group did not completely return to a value similar to the one measured in the LA group. However, this decrease is sufficient to abolish the difference observed between these two groups at the end of the test-period. These observations support our hypothesis that myristic acid is the main modulator of SR-BI expression and that the hepatic cholesteryl ester concentration is involved in this regulation via a mechanism implicating SREBP or other transcription factors.

In conclusion, the results obtained in the present study show for the first time that myristic acid increases HDL-C alone. Given the atheroprotective role of HDL (Stein & Stein, 1999; Von Eckardstein & Assmann, 2000), it will be interesting to confirm this effect in human subjects. The present paper also demonstrates the importance of myristic acid in the regulation of HDL-C via SR-BI in the hamster.

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