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Cholesterol-lowering effect of non-viscous soluble dietary fiber NUTRIOSE®6 in moderately hypercholesterolemic hamsters

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NUTRIOSE®6 is a new wheat starch-based low-digestible carbohydrate. This study investigated the effect of this soluble non-viscous fiber on cholesterol metabolism. Hamsters fed with 0.25% cholesterol-enriched diet (CHO) were given graded amounts of NUTRIOSE®6, i.e. 0% (cellulose, CHO), 3% (N3), 6% (N6) or 9% (N9) (w:w). As compared to CHO diet, 9% NUTRIOSE®6 significantly lowered plasma and LDL cholesterol by 14.5 and 23.8%, respectively. The LDL-cholesterol lowering effect was also significant with the 6% dose (-21.4%). NUTRIOSE®6 diets prevented hepatic cholesterol accumulation (-10 to -20%) and significantly decreased bile cholesterol (-47 to -68%) and phospholipids (-30 to -45%) concentrations. The 9% NUTRIOSE®6 diet significantly decreased the rate of dietary cholesterol absorption (-25%) and markedly stimulated faecal neutral sterol (+81%) and bile salts (+220%) excretion. No significant change in cholesterol 7-α-hydroxylase or LDL-receptor activities was observed whereas 3-hydroxy-3-methylglutaryl-coenzyme A reductase activity was reduced by 29%. Reduced cholesterol and bile salt absorptions and lowered cholesterol synthesis are likely mechanisms underlying the cholesterol lowering effect of NUTRIOSE®6. Results suggest the use of NUTRIOSE®6 as a new dietary cholesterol-lowering agent that should be tested in humans as treatment and evenly prevention of mild hypercholesterolemia.

Keywords: Cholesterol absorption, Dietary fiber, Hypercholesterolemia, Lipid, NUTRIOSE®6

The main physicochemical properties of dietary fiber such as solubility, viscosity and fermentability, are thought to predict their physiological effects. Clinical studies have mainly focused on evaluating the ability of psyllium, pectin, oat bran β-glucan or guar gum to lower LDL-cholesterol concentration. A meta-analysis that combined the results of 67 controlled trials in humans showed that a modest 10 g/day increase in viscous fiber intake, such pectin, psyllium, oat bran and guar gum, resulted in reduction in LDL-cholesterol averaging 0.57 mM and reduction in total cholesterol averaging 0.45 mM[1]. Moreover, a recent cross-sectional study involving 2532 men and 3429 women showed that the total dietary fiber intake was inversely correlated with several cardiovascular risk factors, with a minor contribution of the soluble fiber fraction[2].

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It is generally considered that the common chemical property of the cholesterol-lowering soluble dietary fiber is a high viscosity and, except for hydroxypropyl methylcellulose, high fermentability. Data from animal[3] and human[4] studies support the hypothesis that viscosity plays a major role in the cholesterol-lowering effect and resulting physiological responses rather than fermentability[5,7]. In fact, it has been shown that the increase in bile salt excretion is related to either direct binding of bile acids or entrapment of bile acids within viscous medium, as many water-soluble fiber form a viscous matrix within the small intestine[8]. Nevertheless, Carr *et al.*[3] that did not show greater faecal bile salt excretion induced by highly viscous non-fermentable hydroxypropyl methylcellulose, suggesting that the role of viscosity should be further investigated. In that line, other data point that fiber from cereal flours eliciting different viscosities but similar caecal fermentations have cholesterol-lowering effects independently of their ability to increase intestinal

content viscosities or to promote faecal steroid excretion[9]. Finally, it is noteworthy that some non-viscous resistant starch preparations, inulin (poorly viscous soluble fiber) or insoluble fiber from carob pulp can display a noticeable cholesterol-lowering effect[10-12].

Thus, the specific mechanisms by which dietary fiber reduces plasma and liver cholesterol concentrations still remain uncertain. NUTRIOSE®6 is a newly manufactured dextrin, processed from wheat starch. This glucose polymer is non-viscous and characterized by several α-1,6 and other (e.g. α-1,2 and α-1,3) non-digestible glucoside linkages[13]. *In vitro* and animal studies have shown that NUTRIOSE®6 is poorly hydrolysed and absorbed in the small intestine, and well fermented in the human gastrointestinal tract[14]. In human, a noticeable effect on stool output[15] suggested its use as "bulking agent". The fermentability and solubility of NUTRIOSE®6 suggest that this poorly digestible carbohydrate could have dietary fiber characteristics and thus exert other biological activities through its effect within the gastrointestinal tract.

In the present study, it is hypothesized that the poorly digestible carbohydrate NUTRIOSE®6, displaying soluble but no viscous property could be effective in lowering cholesterol. The golden Syrian hamster was selected as an appropriate animal model regarding its response to hypercholesterolemic diet and its extensive use in comparable nutritional investigations[16]. A first set of experiment is aimed to determine the efficient dose of NUTRIOSE®6 necessary to induce a significant cholesterol-lowering effect and to limit cholesterol accumulation in liver. In a second set of experiment, the rate of cholesterol absorption along with the faecal excretion of total steroids (neutral sterols and bile salts) and hepatic metabolism have been investigated, to highlight the potent mechanisms of action.

Materials and Methods

Chemicals products—NUTRIOSE®6 is a glucose polysaccharide produced from maize, wheat or other edible starch heated at high temperature and adjusted to a low moisture level in the presence of an acid catalyst. The dextrin obtained is purified with activated carbon and demineralised by exchange resins. Afterwards, the product is subjected to chromatographic partitioning which removes glucose and lower molecular weight oligosaccharides. The final product, NUTRIOSE®6, is a mixture of glucose polymers with a fairly narrow range of molecular weight (number average Mol. Wt., Mn = 2,600 g/Mol; weight average Mol. Wt., Mw = 5,000 g/Mol). The degree of polymerization is 12-25. In comparison, starch may contain up to a million glucose units. During the heating step, hydrolysis and repolymerization occur. In addition to the typical starch α-1,4 and α-1,6 glucosidic linkages, the recombination mainly results in other specific glucosidic linkages that are not found in starch. These can include both linear and branched linkages: (α-1,6 and/or β-1,6), (α-1,2 and /or β-1,2), (α -1,3 and /or β-1,2), and β-1,4. This point confers to NUTRIOSE®6 a resistance against the action of endogenous glucidolytic enzymes and permits classification of the product among the soluble dietary fiber with a total fiber content nearly 85.0%.

Animals— Experiments were conducted according to the French Regulations for Animal Experimentation (Art. 19 Oct. 1987, Ministry of Agriculture) and after approval by the Committee of Animal Care at Faculty of Medecine,"Université de la Méditerranée -Marseille". Male golden Syrian hamsters (60), 8 weeks old and weighting 84-98 g, were purchased from the Janvier Breeding Center (Saint-Genest-St-Ile, France). They were housed in colony cages (4/cage) and fed a commercial chow diet (U.A.R.04, Usine d'Alimentation Rationnelle, Villemoison-sur-orge, France) for a 10 day adaptation period before experiment. Hamsters were kept in a controlled environement (22°C, 14:10-h light-dark cycle, 70% RH). They were fed *ad libitum*. Body weight and food intake were measured weekly.

Experimental diets—All experimental diets were semi-purified pellet diets purchased from Mucedola (Settimo Milanese, Italy). To induce moderate hypercholesterolemia, atherogenic diets were enriched with 0.25 g cholesterol/100 g diet (Groups CHO, N3, N6 and N9) and compared with a cholesterol-free control diet (Group C). Given its neutral dietary fiber property on lipid metabolism, pure crystalline cellulose was chosen as the control dietary fiber to assess the cholesterol-lowering effects of NUTRIOSE®6. The diets provided either 0 g/100 g (Groups C and CHO), 3 g/100g (Group N3), 6 g/100 g (Group N6) or 9 g/100g (Group N9) NUTRIOSE®6 and were adjusted to 9 g/100 g total dietary fiber with cellulose, when necessary. The compositions of the experimental diets are given in Table 1.

Table 1—Composition of the 5 experimental diets[a]

Diet ingredient (g/kg)	C (8)	CHO (8)	N3 (6)	N6 (8)	N9 (8)
Cholesterol	-	2.5	2.5	2.5	2.5
Lipid	40	40	40	40	40
Sucrose	205	205	205	205	205
Starch	265	265	265	265	265
NUTRIOSE®6	-	-	30	60	90
Cellulose	90	90	60	30	-
Protein	230	230	230	230	230
Mineral mix	50	50	50	50	50
Vitamin mix	12	12	12	12	12
Water	108	105.5	105.5	105.5	105.5

C = cholesterol free control diet
CHO = cholesterol-cellulose control diet
N3, N6, N9 = cholesterol diet containing NUTRIOSE®6

Experiment 1: Effective cholesterol-lowering dose of NUTRIOSE®6—In a first set of experiment, the cholesterol-lowering effect of various doses of the non-viscous soluble dietary fiber was investigated in the hamster showing dietary-induced moderate hypercholesterolemia. Hamsters (40) were assigned into 5 groups and fed for 21 days either the diet C (n = 8), CHO (n = 8), N3 (n = 8), N6 (n = 8) or N9 (n = 8). At the end of this experimental period, hamsters were food deprived for 18 h. Hamsters were anesthetized between 07:00 and 09:00 hrs by inhalation of isofluorane (Abbott, Wiesbaden, Germany). Blood (2-3 ml) for lipid analyses was carefully collected by cardiac puncture in EDTA/K_3 tubes. The liver was removed, rinsed in cold saline and weighted. Bile was collected by aspiration from the gallbladder in a 1 ml syringe and stored at -80°C.

After plasma separation, a protease inhibitor cocktail[17] was added (10 µl /ml) and 0.5 ml of plasma was used for lipoprotein isolation. Lipoproteins were isolated using a sequential ultracentrifuge method[18]. Briefly, plasma was overlayed by a 1.006 g m/L KBr solution and ultracentrifuged for 160 min at 15°C and 415,000 *g* (TL-100, 100.2 rotor, Beckman, Palo Alto, CA). The VLDL fraction was removed from the top and the infranatant was adjusted to 1.080 g/mL and overlayed by a 1.063 g/ml KBr solution. After the second run (160 min at 15°C and 415,000 *g*), LDL were removed from the top and the infranatant was adjusted to 1.034 g/ml and overlayed by a 1.021 g/ml KBr solution. After the third run, the HDL (320 min at 15°C and 415,000 *g*) were removed from the top. Cholesterol concentrations in plasma, LDL and HDL were assessed using commercial kits (Biomérieux, Craponne, France).

Livers were blended in isopropanol with an Ultra-Turrax T18 (power 5, 1 min, 4°C) and heated at 60°C for 1 h. Then, homogenates were centrifuged for 5 min at 3500 *g* at 4°C. The supernatant and the pellet were re-extracted following the same procedure and the overall resulting supernatants were stored at -20°C. Cholesterol concentrations were assessed using commercial kits (Biomérieux, Craponne, France).

Concentrations of bile cholesterol and phospholipids were assessed using specific commercial kits (Biomérieux, Craponne, France and Dyasis, Bouffemon, France). Bile salts were assessed according to an indirect colorimetric method[19].

Experiment 2: Mechanisms involved in the cholesterol-lowering of NUTRIOSE®6—In a second set of experiment, the cholesterol-lowering effect was investigated on intestinal, hepatic and bile metabolisms in 30 hamsters fed the C diet (n =10), the 0% (CHO, n =10) and the 9% NUTRIOSE®6 (N9, n =10) diets for 24 days. To proceed to faecal measurement and extent of cholesterol absorption, hamsters were placed in individual metabolic cages from day 19 to day 24. From day 19 to 21, stools were collected for faecal bile salts and neutral sterol measurements. On day 22, hamsters were forced fed, after 18 h-food-deprivation, with a lipid emulsion containing radiolabelled sterols (Dupont-NEN, les Ulis, France). Animals were killed as described in experiment 1.

The 72 h-stool samples were weighted and placed in cold distilled water for 1 h at 4°C. Faecal samples were blended using an Ultra-Turrax T18 and freeze-dried. The lyophilized extracts were incubated with an ethylene-glycol/KOH 0.1 M solution for 2 h at 220°C in a sand bath. Lipids were extracted with ether, dried under nitrogen and mixed into methanol.

Faecal neutral sterols were extracted according to the Folch method[20]. Lipid extracts were added with 5-alpha-cholestane as recovery standard. Lipid extracts were hydrolyzed with 1 *M* NaOH solution in 90% ethanol for 1 h at 67°C in a water bath. After cooling under water, neutral sterols were extracted by hexane and dried under nitrogen. The dried extract was dissolved in methanol and dried again under nitrogen. Neutral sterols were sylylated with Hydroxy-Sil (Interchim, HMDS:TMCS:Pyridine 2:1:10) for 60 min at room temperature. After the sylylation step, neutral sterols were dissolved in hexane and separated and quantified by gas-liquid chromatography (Autosystem XL, Perkin-Elmer using a capillary

column SPB-5, 30 m, 0.25 mm ID, 0.25 μm film thickness). Samples were analyzed under the following conditions: 3 μl were injected into the column at 300°C, and with an oven temperature of 285°C. The carrier gas (hydrogen) flow was 10 psi with the inlet splitter set at 100:1.

Faecal bile acids were assessed from the aqueous phase of the Folch extracts. The aqueous phase was acidified to *p*H 2 with HCl and extracted 3 times by diethylether. Ether phases were collected, evaporated to dryness under nitrogen and dissolved into methanol. Total bile acid concentration was measured using an enzymatic colorimetric method[19].

The rate of cholesterol absorption was measured by the faecal dual-isotope ratio method described by Wang and Carey[21], with minor modification. Hamsters were food-deprived for 18 h and given a single intragastric dose (0.5 ml) of a labelled-lipid emulsion and returned immediately to their own diet. Lipid emulsion contained ^{14}C-cholesterol (Sp. activity = 110 mCi/mmol) and ^{3}H-β-sitostanol used as non-absorbable marker (Sp. activity = 30-60 mCi/mmol). The radiolabelled isotopes from 72 hours-pooled faecal samples were saponified, extracted and counted according to the method of Turley *et al.*[22] and Miettinen *et al.*[23]. The ratios of the two radiolabels in the faecal extracts and the dosing mixture were used for the following calculation of the percent of cholesterol absorption:

Percent cholesterol absorption = 100 – (faecal ^{14}C-cholesterol/^{3}H-β-sitostanol)/(Dietary ^{14}C-cholesterol/^{3}H-β-sitostanol) × 100)

The activities of the cholesterol 7-α-hydroxylase (CYP7A) and 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMG-CoA reductase) were determined in liver microsomes. Hepatic microsomes were prepared from fresh liver samples (1-2 g) by homogenization in 5 ml buffer (KH_2PO_4 50 m*M*, EDTA 10 *M*, NaCl 50 m*M*, DTT 0.5 m*M*, Sucrose 300 m*M*, *p*H 7.4) at 4°C with an Ultra-Turrax. Microsomal fraction was isolated according to the procedure of Einarsson *et al.*[24].

The level of cholesterol CYP7A activity in the hepatic microsomes was determined according to the radioisotopic method of Souidi *et al.*[25] using ^{14}C-cholesterol solubilised and carried by hydroxypropyl-β-cyclodextrin. The cholesterol 7-α-hydroxylase activity is expressed as picomoles of 7-α-OH-4-cholest-3-one formed/min/mg microsomal protein.

HMG-CoA reductase activity was determined in hepatic microsomes, in the presence of alkaline phosphatase using Philipp and Shapiro's radioisotopic technique[26]. The HMG-CoA reductase activity is expressed as picomoles mevalonate formed/min/mg microsomal protein.

The hepatic level of LDL receptor protein was assessed from plasmic membranes[27,28]. Aliquot of liver (1 g) were thawed at 4°C in a 7 ml buffer (20 m*M* Tris-HCl, 150 m*M* NaCl, 1 m*M* $CaCl_2$, *p*H 8) using an Ultra-Turrax. Homogenates were centrifuged for 15 min at 4°C and 64,666 *g* (L7, 60Ti rotor, Beckman). The supernatant was filtered and ultra-centrifuged at 4°C and 436000 *g* (TL100, 100.2 rotor, Beckman) to collect plasmic membrane proteins. The proteins were solubilized from the pellet in 0.4 ml buffer (125 m*M* Tris-Maleate, 2 m*M* $CaCl_2$, 140 μl Trasylol/Apoprotinine, 2% Triton X 100, *p*H 6). Protein levels of LDL receptor were determined by immunoassay. Nitrocellulose membranes containing proteins spotted using dot-blot apparatus (Bio-rad) were incubated with specific antibodies after incubation with anti-IgG antibody to horse-radish peroxidase and detection was performed using enhanced chemiluminescence's reagents (ECL, Amersham, les Ulis, France).

Statistical analysis— Values are given as means ± SE. The one-way ANOVA and Fischer tests were used to examine the effect of treatment on the different parameters measured. Differences were considered significant at $P < 0.05$. Linear regressions were calculated between the plasma, LDL and HDL total cholesterol for hamsters fed the four cholesterol-enriched diets and the increasing dose (0 to 9%) of NUTRIOSE®6. All statistical analyses were performed using the Stat View V software (Abacus Concepts, Berkeley, CA).

Results

Experiment 1: Effective cholesterol-lowering dose of NUTRIOSE®6—As compared to the 0% NUTRIOSE®6 (CHO diet), the 3, 6 or 9% NUTRIOSE®6-containing diets did not alter the daily body weight gain (CHO group: 0.90 ± 0.15 g/d; N3 group: 1.18 ± 0.11 g/d; N6 group:1.05 ± 0.07 g/d^{-1}; N9 group: 0.81 ± 0.12 g/d). In this line, no significant difference was shown in mean food consumption (CHO group: 190 ± 2 kcal/kg$^{/}$d; N3 group: 193 ± 2 kcal/kg$^{/}$d; N6 group: 183 ± 2 kcal/kg$^{/}$d; N9 group: 189 ± 2 kcal/kg$^{/}$d).

As compared to a cholesterol-free diet, all the 0.25% cholesterol enriched-diets induced a significant raise in plasma and LDL cholesterol concentrations. Except for the N9 diet, the cholesterol concentrations in HDL fraction were significantly increased as compared to the cholesterol-free diet. The N9 diet significantly decreased dietary induced-hypercholesterolemia (-14%), as well as both LDL-cholesterol (-24%) and HDL-cholesterol (-23%) concentrations as compared to the control cellulose-cholesterol diet (CHO) (Table 2). No significant decrease in plasma and HDL cholesterol concentrations was observed with lower dose of NUTRIOSE®6 (3-6%), while the 6% dose (N6 diet) tended to lower plasma cholesterol and significantly reduced the concentration of LDL-cholesterol (-21%). The cholesterol concentrations in plasma (r^2=0.512, *P*=0.0039), LDL (r^2=0.556, *P*=0.0014), and HD (r^2=0.627, *P*=0.0002) were inversely correlated to the 0-9% NUTRIOSE®6 dose in the atherogenic diet.

As compared to the 0% NUTRIOSE®6 (CHO) diet, the cholesterol accumulation in the liver was significantly lowered in hamsters fed the enriched-NUTRIOSE®6 diets at the dose of 3% (-10%), 6% (-20%) and 9% (-19%) (Table 3).

Compared to the control CHO diet, chronic feeding with NUTRIOSE®6-containing diets prevented change in cholesterol concentrations in bile (Table 3). Indeed, cholesterol concentrations in bile were significantly lower (-47 to -68%) in all NUTRIOSE®6 groups than in the CHO one. Comparable patterns were observed for bile phospholipids concentrations ranging -30% (N9 group) to -45% (N6 group). As compared to the control-cellulose diet, addition of 6 to 9% NUTRIOSE®6 slightly increased the bile salt concentration in bile (6 to 11%, respectively) whereas the addition of 3% NUTRIOSE®6 reduced this concentration by 9%.

Experiment 2: Mechanisms involved in the cholesterol-lowering of NUTRIOSE®6—As shown in Table 4, hepatic HMG-CoAR activity, the rate-limiting enzyme of cholesterol synthesis, was slightly but significantly affected by the NUTRIOSE®6-containing diet as compared to the control CHO diet

Table 2— Cholesterol concentrations (m*M*) in plasma and lipoproteins in hamsters fed the experimental diets
[Values are mean±SE. Figures in parentheses are the number of animals]

Diets	C (8)	CHO (8)	N3 (6)	N6 (8)	N9 (8)
Plasma	4.76 ± 0.20	7.43 ± 0.28*	7.38 ± 0.47*^{u}	6.87 ± 0.21*^{u,v}	6.24 ± 0.26*$^{\dagger\ v}$
LDL	0.68 ± 0.04	1.26 ± 0.07*	1.28 ± 0.11*^{u}	0.99 ± 0.06*^{u†}	0.96 ± 0.07$^{\dagger\ v}$
HDL	3.82 ± 0.22	5.43 ± 0.20	4.96 ± 0.33*^{u}	4.90 ± 0.13*^{u}	4.20 ± 0.17$^{\dagger\ v}$

C = cholesterol free control diet
CHO = cholesterol-cellulose control diet
N3, N6, N9 = cholesterol diet containing NUTRIOSE®6
P<0.05; * significant between CHO, N3, N6, N9 and C diet
† indicates a significant difference (*P* < 0.05) between the N3, N6 or N9 diets and the CHO diet
u and v letters indicate a significant difference (*P* < 0.05) between the N3, N6 or N9 diets

Table 3—Lipid parameters measured in liver and bile of the hamsters fed the experimental diets
[Values are mean±SE. Figures in parentheses are the number of animals]

Dietary group	C (8)	CHO (8)	N3 (6)	N6 (8)	N9 (8)
			Hepatic parameters		
Cholesterol (mg/g)	3.22 ± 0.47	15.61 ± 0.53*	14.03 ± 0.46*$^{\dagger u}$	12.44 ± 0.40*$^{\dagger v}$	12.66 ± 0.24*$^{\dagger\ v}$
			Bile parameters		
Cholesterol (g/l)	1.28 ± 0.17	2.79 ± 0.57*	1.48 ± 0.16†	0.88 ± 0.08†	1.44 ± 0.19†
Phospholipids (g/l)	8.58 ± 1.31	10.75± 0.75	6.93 ± 1.14†	5.87 ± 0.85*†	7.51 ± 0.69†
Bile salts (g/l)	18.45 ± 0.47	17.94 ± 0.34	16.34 ± 0.53*$^{\dagger\ u}$	19.09 ± 0.22$^{\dagger\ v}$	19.93 ± 0.22*$^{\dagger\ v}$

C = cholesterol free control diet
CHO = cholesterol-cellulose control diet
N3, N6, N9 = cholesterol diet containing NUTRIOSE®6
P<0.05; * significant between CHO, N3, N6, N9 and C diet
† indicates a significant difference (*P* < 0.05) between the N3, N6 or N9 diets and the CHO diet
u and v letters indicate a significant difference (*P* < 0.05) between the N3, N6 or N9 diets

Table 4—Hepatic activities of HMG-CoAR, Cyp7a and LDL-R binding, faecal output of steroids and efficiency of dietary cholesterol absorption in hamsters fed with experimental diet

[Values are mean±SE. Figures in parentheses are the number of animals]

Dietary group	C (10)	CHO (10)	N9 (10)
	Hepatic parameters		
HMG-CoAR (picomol/min/mg protein)	9.3 ± 0.3	9.9 ± 0.4	7.0 ± 0.6*†
CYP7A (picomol/min/mg protein)	40.4 ± 4.7	44.5 ± 4.3	38.5 ± 3.0
LDL-R (Arbitrary Unit)	100.0 ± 7.3	92.2 ± 6.6	83.0 ± 6.0
	Faecal parameters		
Bile salts (mmol/g)	4.76 ± 0.94	6.31 ± 0.11	20.18 ± 1.11†
Total neutral sterol (µg/g)	120.7 ± 19.2	443.3 ± 17.1*†	804.9 ± 79.6*†
	Cholesterol absorption		
Rate (%)	71.7 ± 5.3	62.4 ± 2.2	46.8 ± 4.4*†

C = cholesterol free control diet
CHO = cholesterol-cellulose control diet
N9 = cholesterol diet containing NUTRIOSE®6
$P<0.05$; * significant between CHO, N3, N6, N9 and C diet
† indicates a significant difference ($P < 0.05$) between the N3, N6 or N9 diets and the CHO diet

(29% decrease in the N9 group). In contrast, no significant change in hepatic activity of CYP7A, the initial and rate-limiting enzyme in bile synthesis, was observed. The level of hepatic binding activity of the LDL-R, the rate-limiting receptor involved in plasma LDL uptake, was found decreased by 10% with 9% NUTRIOSE®6.

As compared to the control diet (CHO), the N9 diet induced a significantly greater faecal excretion of total neutral sterols (81%) and bile acids (220%) (Table 4).

Dietary cholesterol absorption was measured in cholesterol-fed groups CHO and N9, as well as in non-cholesterol fed group (C). In the later group, the calculated rate of dietary cholesterol absorption was higher (71.7±5.3%) than those of the two cholesterol-fed group. Data show that the replacement of 9% cellulose by 9% NUTRIOSE®6 led to a 25% significant decrease in the rate of dietary cholesterol absorption (Table 4).

Discussion

In the present study, for the first time the effect of a dietary fiber source manufactured from wheat starch was examined on cholesterol metabolism in moderately hypercholesterolemic hamster. The originality of the present work was to investigate the potential effect of soluble fiber showing high fermentability but low viscosity. Both physiochemical properties are usually specific to functional soluble fibers with cholesterol-lowering effect.

The dietary-induced mild hypercholesterolemia agreed with other published data in this animal model[29] and strain[30]. As already observed by others, cholesterol is mainly carried in HDL fraction in the hamster model[31,32]. When challenged with a semi-synthetic cholesterol-rich diet, the subsequent response is an overproduction of HDL and LDL, even cholesterol concentration in the latest is still less than in HDL one[31,32]. As a consequence, the increase in HDL-cholesterol concentration can neither be considered as a beneficial effect of the fiber, nor the LDL/HDL ratio improvement regarding its weak significance in the hamster model[31,33]. The higher dose of NUTRIOSE®6 (9%) leads to significant decrease in cholesterolemia conversely to the two lower doses (3 or 6%). Nevertheless, an inverse correlation was found between the amount of NUTRIOSE®6 and hypercholesterolemia development (r = 0.512 and P: 0.0039) suggesting that the cholesterol-lowering effect of NUTRIOSE®6 could be dose-dependant. In the same way, the 6 and 9% NUTRIOSE®6 diet, but not 3% concentration, significantly prevented total cholesterol accumulation in LDL. An inverse correlation was also observed (r = 0.556 and P: 0.0014), that provides evidence that NUTRIOSE®6 could have cholesterol-lowering dose/effect.

In agreement with the observed results, Fernandez *et al.*[32] reported that pectin-enriched diets (5.4 to 10.7%) induced a significant decrease of plasma total cholesterol (-21%) and, to greater extent, LDL-cholesterol (-32%). Purified barley β-glucan significantly reduced total plasma cholesterol (-12%) and non-HDL cholesterol without affecting HDL-cholesterol in hamsters fed with a 10.5% fiber diet,

providing 4.7% β-glucan[34] . The most relevant effect was observed with a 6% psyllium-containing diet that induced a cholesterolemia reduction amounting 61%[31]. This marked cholesterol-lowering effect of psyllium was also observed by other investigators[35] in hamsters fed 7.5% psyllium diets; i.e. cholesterolemia decreased by 69% and LDL-cholesterol concentrations by 68%. This drastic effect could be related to higher dietary-induced hypercholesterolemia (0.4% cholesterol-enriched diet). Indeed, in hamsters fed with 0.08 and 0.12% cholesterol enriched-diets, the cholesterol-lowering effect reached 47% with 8% psyllium diet[36] and 26% with a 7.5% one[37]. As compared to the cholesterol-celllulose diet, the 9% NUTRIOSE®6 diet significantly decreased hypercholesterolemia by 14% where as both the 6 and 9% NUTRIOSE®6 diets lowered LDL-cholesterol concentrations by 21 and 24%, respectively. So, in the hypercholesterolemic hamster model, NUTRIOSE®6 has a cholesterol-lowering effect as efficient as other soluble dietary fiber except psyllium.

The mechanism of action probably differs among the various dietary fiber sources and the contribution of several complementary mechanisms is possible. Numerous hypotheses have been proposed, including binding or sequestration of bile acids leading to an increase in bile salt excretion, subsequent hepatic sterol metabolism adaptation leading to lower hepatic cholesterol storage, disruption of mixed micelles leading to fat malabsorption, and suppression of hepatic sterol synthesis by short-chain fatty acid, an end-product of fiber fermentation in caecum.

To explore the possible mechanisms involved in the observed hypocholesterolemic effect of NUTRIOSE®6, biliary and hepatic function were investigated. Gallbladder cholesterol and phospholipid concentrations were lower in hamsters fed with the 6% NUTRIOSE®6 diets than the 3 and 9% dose but not in a significant way, suggesting that NUTRIOSE®6 has probably no dose-effect on these parameters. Conversely, bile salt concentrations increased with the 6 and 9% NUTRIOSE®6 diet suggesting a beneficial reduction in the bile lithogeneicity index under NUTRIOSE®6 supplementation. This enhanced bile secretion of bile salts did not seem to be related to an increased synthesis through the cholesterol 7-alpha-hydroxylase activity. This lack of effect was surprising as a noticeable faecal loss of bile salt was induced by NUTRIOSE®6. It was expected that a greater faecal output of bile salts, by interrupting the enterohepatic circulation of bile salts, leads to a compensatory up-regulation of bile acid synthesis. It is usually assumed that this forward-regulation was one of the mechanisms accounting for the cholesterol-lowering effect of some dietary fiber, such as psyllium[32,37], or barley β-glucan[38], known to up-regulate the activity of the cholesterol 7-alpha-hydroxylase.

It has been demonstrated in hamster fed lithogenic diets that changes in the bile acid pool modified the activity of the enzymes of both bile acid synthesis pathways[30,33]. More specifically, the decrease in chenodesoxycholate and the increase in cholate modulated the bile acid biosynthesis in liver[39]. The profile of bile acids was not established in the present study. Therefore, the hydrophilic-hydrophobic balance could not be linked to the absence of compensatory bile acid synthesis. As no change in cholesterol 7-alpha-hydroxylase activity was found, one could conclude that cholesterol-lowering effect of NUTRIOSE®6 is probably not due to the catabolic pathway of cholesterol excess via its conversion into bile acids. Nevertheless, due to the large amount of faecal bile salts and neutral sterol under NUTRIOSE®6 feeding, a sequestrant effect could be likely. Viscous water-soluble fibers have generally been considered as bile acid sequestrant-like agents due to their high ability in entraping bile salts[31] and/or neutral sterols. Moreover, it has been established that the capacity of viscous fiber to increase viscosity of intestinal content plays a major role in such sequestrant effect[3,5]. Nevertheless, other investigators failed to demonstrate greater cholesterol-lowering effect in guar gum or pectin displaying high versus low viscosity[36]. The consistent faecal loss in bile acids induced by NUTRIOSE®6 intake strongly suggests that this fiber possesses bile salt binding capacity and/or is able to affect the micelle processing in intestinal lumen. Both mechanisms could alter bile salt reabsorption and lead to the subsequent imbalance of the enterohepatic bile salt circulation.

We finally assessed the impact of NUTRIOSE®6 on the bioavailability of exogenous cholesterol. Cholesterol absorption is described as a multistep process, and many factors including biliary lipid output, as well as species and hydrophilic-hydrophobic balance of the bile salt pool could together exert a major influence on the efficiency of intestinal cholesterol absorption[21].

As expected from the data showing an increased sterol excretion, NUTRIOSE®6 was markedly effective in reducing cholesterol absorption. Two co-existing mechanisms could explain this decrease. First, entrapment of cholesterol is likely to take place and secondly, it was observed that NUTRIOSE®6 stimulates faecal output of bile salt that probably reduced the available pool of bile acid that should be recruited into mixed micelles. As a result of this indirect effect, solubilization of cholesterol into micelles could be affected and displaced cholesterol from the micellar phase to the aqueous phase, leading to its precipitation. Another effect could also occur as it has been shown that various dietary soluble fibers such as guar gum or pectin[40] and other cereal fibers can alter lipid emulsification and subsequent lipase activity. In addition to indirect or direct effects on micelle processing, it is not excluded that NUTRIOSE®6, in regards to other water-soluble fibers, has some gel-forming properties involved in cholesterol entrapment. All these physicochemical events that can occur in the intestinal lumen possibly accounted in reducing cholesterol absorption efficiency. Further investigations should be necessary to highlight which are the dominant physicochemical mechanisms.

Finally, the present study provides evidence that NUTRIOSE®6 prevents hepatic cholesterol accumulation. This observation is probably linked to the lower rate of cholesterol absorption. Nevertheless, no significant change was noted in LDL-receptor binding activity. As demonstrated by Millat *et al.*[41], the decrease in hepatic cholesterol storage might not be as large to induce the well-known up-regulation of LDL-receptor expression[42]. The reduced cholesterol accumulation in the liver could also be linked to a minored cholesterol synthesis, as suggested by the lower level of HMG-CoA reductase in NUTRIOSE®6 hamster. Such an effect could be attributed to the short-chain fatty acid produced by NUTRIOSE®6 fermentation by colonic microflora. Indeed, previous studies *in vitro*, or in animal and human have demonstrated that NUTRIOSE®6 was almost completely fermented. It has been recognized that end-product fermentation, and especially propionate could down-regulate the HMG-CoA reductase[43]. Indeed, the soluble dietary fiber NUTRIOSE®6 fermentation leads to production of propionate as compared to other soluble dietary fibers (unpublished data).

In conclusion, the data of the present study demonstrate that NUTRIOSE®6 has a cholesterol-lowering effect in moderately hypercholesterolemic hamster. This effect results in reduced plasma and LDL cholesterol level as well as cholesterol accumulation in the liver. The overall data suggest that NUTRIOSE®6 possibly delays the delivery and the amount of cholesterol to the liver by affecting the rate of cholesterol absorption and by interrupting the enterohepatic circulation of bile salts, despite no compensatory increase in bile acid synthesis was found. This mechanism is probably related to the binding of steroids and/or an altered micellar cholesterol solubilization. In addition, the high fermentability of NUTRIOSE®6 could indirectly act on hepatic cholesterol synthesis by generating short chain fatty acid.

A previous clinical study has shown that large amounts of NUTRIOSE®6 are well-tolerated and have beneficial effect on intestinal time residence[15]. Thus, the results of the present animal study strongly support the concept that NUTRIOSE®6 could display a cholesterol-lowering effect in hypercholesterolemic subjects. This remains to be tested.

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