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Reciprocal influence of fermentations and bile acid excretion on cholesterol-lowering effect of fermentable carbohydrate

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The lipid-lowering capacity of two different polysaccharides, a high viscosity or a low viscosity guar-gum (HV-GG or LV-GG) were investigated in male Wistar rats. Guar gum (GG) was broken down in the large bowel, yielding fermentation rich in propionic acid, up to 50% of total short-chain-fatty-acid (SCFA) with the LV-GG diet. Compared with the LV-GG diet, the HV-GG diet was more potent in enhancing bile acid excretion and producing a potent cholesterol-lowering effect. There was also a marked effect of HV-GG on sterol excretion, whereas LV-GG had a marginal effect on this process. Both LV-GG and HV-GG depressed plasma triglycerides, and lowered high-density lipoprotein 2 (HDL2) cholesterol, whereas only the HV-GG diet depressed low-density lipoprotein (LDL) and high-density lipoprotein 1 (HDL1) cholesterol. The decreased efficiency of the LV-GG diet to produce a hypocholesterolemic response resulted in a decreased stimulation of hydroxymethylglutaryl coenzyme A (HMG CoA) reductase, and this could explain the lesser efficiency of LV-GG diet to develop a cholesterol-lowering effect. Moreover, the induction of hepatic HMG-CoA reductase in the two GG diets was concomittant to a decrease in fatty acid synthase (FAS) activity. Large bowel fermentations do not appear to significantly influence cholesterol metabolism when soluble fibers fail to enhance bile acid secretion. (J. Nutr. Biochem. 8:127–132, 1997.) © *Elsevier Science Inc. 1997*

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Introduction

Guar gum (GG) is a viscous galactomannan that is not digested in the mammalian small intestine, but is highly fermentable in the large intestine. Because of its efficiency in reducing post-prandial glucose or in exerting an hypocholesterolemic effect,[1,2,3,4] it has been used in the dietary treatment of diabetes and to prevent hypercholesterolemia.

Several possibilities have been proposed to explain the hypocholesterolemic effect of viscous fibers: an increased excretion of bile acids,[5,6,7] an impairment of cholesterol absorption,[8,9] a reduction of the hepatic cholesterol synthesis, or an accelerated uptake of lipoproteins by the liver.[4,10] Numerous questions concerning these mechanisms are not yet completely resolved, and especially the synergic effects of fermentations and steroid excretion in exerting a cholesterol-lowering effect. In the absence of fermentations in the large intestine, a high rate of bile acid excretion is not always sufficient to elicit a cholesterol-lowering effect.[11]

When GG is partially hydrolyzed, hence a lowered viscosity, its efficiency in reducing post-prandial glucose or in exerting an hypocholesterolemic effect is greatly diminished,[3] although it has been found that partially hydrolyzed GG was still effective as a lipid-lowering agent with high-fat diets.[12] The comparison of a highly viscous GG (HV-GG) and its hydrolyzed product, low-viscosity GG, (LV-GG) offers the possibility to study the respective role of viscosity in the small intestine and large intestine fermentations in their cholesterol-lowering effect. This study confirms that a soluble polysaccharide that promotes

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Table 1 Effects of dietary indigestible polysaccharides on final body weight and weight of digestive organs[1]

Diet	Final Body Weight (g)	Cecum Weight (g)	Wall Weight (g)	Cecal Content (g)	Cecal pH
Control diet	294 ± 7[b]	2.3 ± 0.1[a]	0.60 ± 0.03[a]	1.71 ± 0.09[a]	7.20 ± 0.09[b]
8% LV-GG	293 ± 3[b]	3.8 ± 0.2[b]	1.00 ± 0.03[b]	2.80 ± 0.17[b]	6.00 ± 0.07[a]
8% HV-GG	270 ± 5[a]	4.2 ± 0.3[b]	1.10 ± 0.04[c]	3.14 ± 0.26[b]	6.15 ± 0.22[a]

[1]Each value is the mean ± SEM, $n = 10$. Values within a column that are not followed by a common letter are significantly different ($P < 0.05$).

large-bowel fermentations may be devoid of significant influence on plasma cholesterol if its viscosity is low, although it still affects some parameters of cholesterol turnover such as hydroxymethylglutaryl coenzyme A (HMG-CoA) reductase activity or fecal bile acid excretion.

Methods and Materials

Animals and diets

Male Wistar rats (IFFA-CREDO, l'Arbresle, France) weighing approximately 150 g, were fed semipurified diets for 21 days distributed as a moistened powder. The control diet contained the following (in g/Kg diet): casein (L. François, Paris, France) 150; corn oil 50; wheat starch 730; mineral and vitamin mixes (Usine d'Alimentation Rationnelle, Villemoisson/Orge, France) 60 and 10, respectively. The two experimental groups contained 80 g GG/Kg diet, at the expense of wheat starch. GG flours were purchased from Meyhall Chemical AG, CH-8280 Kreuzlingen, Switzerland. Two GG flours, with different molecular weight (M_w) and particle size were studied: LV-GG of low M_w (~47 000) and HV-GG of medium M_w (~590 000). Viscosity of the polysaccharides in distilled water were as follows: HV-GG (1%) 500–700 mPa/s, LV-GG (10%) 900–1300 mPa/s (Meyhall).

Animals were housed two per cage and maintained in temperature-controlled rooms (22°C), with the dark period from 2000 to 0800 hr. Rats were maintained and handled according to the recommandations of the Institutional Ethics Committee (Clermont-Ferrand University). The body weight of rats was recorded every 48 hr during the experimental period; food intake and fecal excretion were recorded over three 2-day periods throughout the last 10 days.

Sampling procedures

Rats were killed at the end of dark period, namely at time at which cecal fermentations are still very active. They were anesthetized with sodium pentobarbital (40 mg/Kg) and maintained at 37°C. An abdominal incision was made and blood (1 mL) was withdrawn from both the portal vein and from the abdominal aorta. The blood was placed in plastic tube containing heparin and centrifuged at 10,000 × g, for 5 min. After centrifugation, plasma was kept at +4°C for lipid and lipoprotein analysis. A portion of liver was freezed-clamped and stored at −80°C for the measurement of fatty acid synthase (FAS) activity. After blood sampling, the cecum with content was removed and weighed. Two samples of cecal content were transfered to microfuges tubes and immediately frozen at −20°C.

Two grams of liver were homogenized in 4 mL of an ice-cold buffer 1 (50 mmol/L Tris-hydrochloride, 250 mmol/L sucrose, 50 mmol/L EDTA, 2 mmol/L dithiothreitol and 2 μmol/L leupeptin, pH 7.2) with a Potter-Elvejhem homogeneizer (Braun, Melsungen, Germany) at moderate speed. The homogenate was first centrifuged at 10,000 × g (15 min, 4°C); the resulting supernatant was then centrifuged at 100,000 × g (60 min, 4°C). Pellets were resuspended in 2 mL of chilled buffer 1. The centrifugation procedure was repeated and the resulting pellets were homogenized in 1 mL of buffer 2 (sucrose 100 mmol/L; KCL 50 mmol/L; K phosphate 40 mmol/L; EDTA 30 mmol/L; dithiothreitol 1 mmol/L; pH 7.2). The microsomal preparation was stored at −80°C until measurement of enzyme activities. The microsomal content of proteins was determined using the Pierce BCA reagent kit (Interchim, Montluçon, France).

Analytical procedures

Short-chain-fatty-acids (SCFA) concentrations were measured by gas-liquid chromatography, after ethanolic extraction of plasma samples as described by Rémésy and Demigné[13] and on supernatants (8,000 × g., 5 min at 4°C) of cecal contents. Bile acids and sterols were extracted from cecal content or feces by 10 volumes ethanolic KOH (0.5 M) and quantified using the reaction catalyzed by the 3 α-hydroxysteroid dehydrogenase[14] (EC 1.1.1.50; Sigma, L'isle D'abeau Chesnes, France) or cholesterol oxidase (EC 1.1.3.6; Boehringer, Meylan, France) respectively. The soluble bile acids levels were determined on supernatants of cecal contents. Triglycerides (Biotrol, Paris, France), total cholesterol and phospholipids (BioMerieux, Charbonnières-les-Bains, France) were determined in plasma by enzymatic procedures.

Plasma lipoproteins were separated on a density gradient by preparative ultracentrifugation as described by Sérougne et al.[15] After centrifugation in a TST 41.14 swinging-bucket rotor (Kontron, Zürich, Switzerland) at 100,000 × g for 36 hr (15°C); the gradient was fractionated (500 μL fractions), the cholesterol and triglycerides contents of each fraction were determined by the method described previously.

The activity of HMG-CoA reductase (EC 1.1.1.34) was determined on microsomal fractions as described by Wilce and Kroone.[16] Labeled mevanololactone was separated from unreacted HMG-CoA by column chromatography using AG1-X8 resin (200 to 400 mesh, formate form, BioRad, Paris, France). Specific radioactivity of the enzyme was expressed in pmoles of [3-^{14}C]HMG-CoA transformed in [^{14}C]mevalonolactone per min per mg of microsomal protein, after correcting for recovery of [^{3}H]mevalonolactone from the column. The FAS activity was measured according to the method described by Hsu et al.[17] and expressed in nmol of [2-^{14}C]malonyl CoA incorporated into fatty acids per minute per gram of liver.

Calculation and data analysis

The cecal pool was calculated as: cecal concentration (mmol/L) × cecal content volume (mL). Values are given as the means ± SEM and, where appropriate, significance of differences ($P < 0.05$) between mean values was determined by analysis of variance (ANOVA) coupled with the student Newman-Keuls' test.

Results

Body weight, cecal development, and cecal pH

As shown in *Table 1*, no significant differences in body weight were detected between the control and LV-GG diets,

Table 2 Effects of dietary indigestible polysaccharides on cecal fermentations[1]

	Cecum					
Diet	Acetate (mmol/L)	Propionate (mmol/L)	Butyrate (mmol/L)	Total SCFA concentration (mmol/L)	SCFA Molar Ratio % (Ac/Pr/Bu)	Total SCFA Pool (μmol/cecum)
Control diet	42 ± 7[a]	18 ± 2[a]	7 ± 1[a]	67 ± 6[a]	(63/27/10)	111 ± 11[a]
8% LV-GG	64 ± 5[b]	80 ± 8[c]	15 ± 2[b]	159 ± 10[c]	(40/50/10)	439 ± 14[c]
8% HV-GG	65 ± 5[b]	51 ± 7[b]	14 ± 2[b]	130 ± 9[b]	(50/39/11)	410 ± 12[b]

[1]Each value is the mean ± SEM, $n = 10$. Values within a column that are not followed by a common letter are significantly different ($P < 0.05$).

whereas there was a slight but significant decrease in rats fed 8% of HV-GG diet. Rats fed the LV-GG and HV-GG diets showed a significant enlargement of the cecum (+65% and +83%, respectively), with a parallel increase of the cecal wall weight. The presence of galactomannan in the diet caused an acidification of the cecal content (pH 6.0 and 6.15 for LV-GG and HV-GG diets, respectively).

Cecal fermentations and SCFA absorption

As shown in *Table 2* the total SCFA concentration of LV-GG and HV-GG groups was very high (159 mmol/L for LV-GG and 130 mmol/L for HV-GG groups). The concentration and the pool of acetic acid were similar with the two types of GG, whereas the molar ratio of propionic acid was significantly enhanced in rats fed the LV-GG diet (+23% against control). The cecal pool of propionate, which reflects the intensity of its production was also enhanced (+39%) with the LV-GG diet. The cecal fermentations of rats fed GG were relatively low in butyrate (15 mmol/L), which represented about 10% of total SCFA, on a molar ratio basis, like in control rats.

The portal vein concentrations of SCFA were relatively proportional to their cecal pool. *Figure 1* shows that the portal concentration of propionate was significantly higher in rats fed GG compared with controls, and no detectable concentration of propionate was found in arterial blood (data not shown); in keeping with the fact that propionate is completely metabolized by the liver. The concentrations of acetate in the portal vein were significantly enhanced in rats fed the LV-GG or HV-GG diets, compared with control and, in parallel, the arterial concentration of acetate was strongly increased in the two experimentals groups (data not shown).

Cecal bile acids and fecal excretion of neutral and acidic steroids

The influence of GG on bile acid accumulation in the cecum and on their fecal excretion is presented in *Table 3*. Rats fed the HV-GG diet showed a significant increase in the cecal concentration of bile acids (+29% compared to control), and in the cecal pool (+137%). In rats fed the LV-GG diet, the cecal concentration of bile acids was the same as in control rats, nevertheless the cecal pool was significantly enhanced (+38%) because of a marked enlargement of the cecum. The bile acid solubility was strongly depressed in acidic pH conditions in the cecum, independently of the initial viscosity of GG. In contrast, the fecal excretion of bile acids was markedly influenced by the type of GG, and the maximal excretion (23.1 μmol/d) was observed with the HV-GG diet. It is noteworthy that LV-GG still enhanced the fecal bile acid excretion (12.5 μmol/d against 8.9 μmol/d for control), in spite of its low viscosity.

The daily fecal excretion of sterols (chiefly coprostanol) was higher than that of bile acids (*Table 3*); it was markedly increased in rats adapted to the HV-GG diet, up to 48 μmol/d compared to 27.5 μmol/d in control rats. As a result, total steroid excretion was doubled in rats fed the HV-GG diet, compared to controls (*Figure 2*). There was a small, but significant, increase in fecal sterol excretion in rats fed the LV-GG diet.

Lipid and lipoprotein metabolism

In response to the enhanced fecal excretion of bile acids, there was an induction of the activity of the microsomal HMG-CoA reductase (*Figure 2*), the rate-limiting enzyme in the cholesterol biosynthesis, in the two group of rats fed GG (+57% and +119% with the LV-GG and HV-GG diet, respectively). On the other hand, the activity of FAS

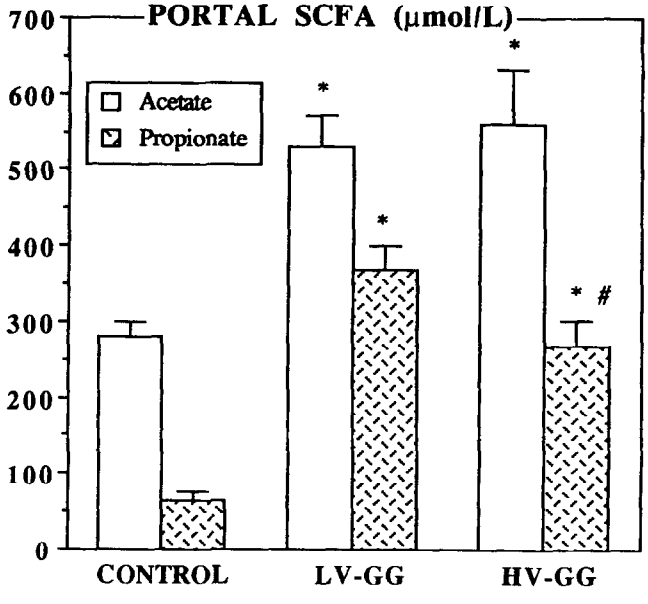

Figure 1 Effect of dietary fermentable carbohydrate on digestive balance of SCFA. *$P < 0.05$: significantly different from the controls diet. #$P < 0.05$: significant difference between rats fed 8% LV-GG and 8% HV-GG diet.

Table 3 Effects of dietary indigestible polysaccharides on cecal concentration and fecal excretion of bile acids[1]

Diet	Total Cecal Bile Acids (mmol/g)	Bile Acids Cecal Pool (μmol/cecum)	Cecal Soluble Bile Acids (%)	Fecal Ecretion (μmol/d)		
				Bile Acids	Cholesterol	Coprostanol
Control Diet	3.8 ± 0.5[a]	6.5 ± 0.1[a]	43 ± 7[b]	8.9 ± 0.7[a]	5.60 ± 0.35[a]	21.9 ± 1.1[a]
8% LV-GG	3.2 ± 0.4[a]	9.0 ± 0.2[b]	15 ± 2[a]	12.5 ± 0.9[b]	5.90 ± 0.15[a]	28.1 ± 1.5[b]
8% HV-GG	4.9 ± 0.4[b]	15.4 ± 0.4[c]	13 ± 2[a]	23.1 ± 1.2[c]	6.70 ± 0.22[b]	41.2 ± 3.1[c]

[1]Each value is the mean ± SEM, n = 10. Values within a column that are not followed by a common letter are significantly different ($P < 0.05$).

(*Figure 3*) in liver cytosol was lowered by GG: −38% and −26% for HV-GG and LV-GG diet, respectively, compared with the control conditions. *Figure 4* shows that only the HV-GG diet, had a significant plasma cholesterol-lowering effect (−25%), together with a potent plasma triglyceride-lowering effect (−48%), moreover LV-GG diet caused only a moderate (−22%) plasma triglyceride-lowering effect (*Figure 3*).

Plasma lipoproteins were fractionated by gradient ultracentrifugation and further analysed for lipids content (*Figure 4*). With the HV-GG diet, cholesterol was significantly depressed in all the lipoprotein fractions, but especially in the high density lipoprotein fractions (HDL1 and HDL2), which represents the major transport lipoprotein for plasma cholesterol in the rat. In contrast, in rats fed the LV-GG diet, a significant decrease of the cholesterol was observed only in HDL2. The triglyceride content of triglyceride-rich-lipoprotein (TGRLP) fraction was significantly depressed in the two groups fed GG, but the effect was more pronounced with the HV-GG diet (−40%, versus −27% for the LV-GG diet, data not shown).

Discussion

Numerous studies have demonstrated that soluble dietary fibers such as GG, pectins, or oat gum, exert a cholesterol- and triglyceride-lowering effect, in experimental animals as well as in humans.[1,2,3,18,19] In accordance with these results, the present work indicates that the capacity of fermentable polysaccharides to exert a cholesterol-lowering effect is strongly dependent on their viscosity. It has been shown that GG is effective to depress the absorption of exogenous cholesterol,[8,9] probably by affecting the rate of cholesterol absorption from micelles.[20] The present results suggest that reabsorption of endogenous cholesterol is also altered by GG, provided that it presents a high viscosity. This could result from a greater resistance to diffusion in the intestine lumen, or from bile acid binding and disturbances of bile acid micelles formation.[20,21,22] In absence of cholesterol supplementation, HV-GG diet increased the quantities of bile acids reaching the cecum, and with LV-GG the efficiency of this drainage was lesser. Another possibility to enhance fecal bile acid excretion is linked to the effects of the large intestine fermentations, which insolubilize bile acids and prevent their subsequent reabsorption.[5,23,24] Thus, in spite of its low viscosity, LV-GG, apparently retains a significant capacity to increase bile acid excretion. The rise of the cecal bile acids pool is a reflect of the enhanced transfer of bile acids from the small intestine to the large bowel. This should result in a greater excretion of bile acids if passive reabsorption in the large intestine fails to compensate for impaired absorption in the ileum. It has been reported that cecal bile acids reabsorption may be enhanced by fermentable polysaccharides[25] because of a rise in the surface area of exchange of cecal mucosa and to a higher cecal blood flow. However, more active fermentations also yield conditions unfavorable for bile acid reabsorption such as reduced solubility, binding to bacteria,[26] or to insoluble Ca salts.[24] These last effects are probably prevailing because both LV-GG and HV-GG did enhance bile acids excretion, the greater potency of HV-GG was probably connected to its capacity to elevate the large intestinal pool as a consequence of impaired reabsorption in the small intestine.

It is noteworthy that both types of GG promoted high-propionic acid fermentations, but LV-GG was more effective in this respect, which could be explained because bacterial fermentation of the galactomannan are facilitated by partial hydrolysis of GG. A role of propionic acid to mediate the hypocholesterolemic effect of fibers has been

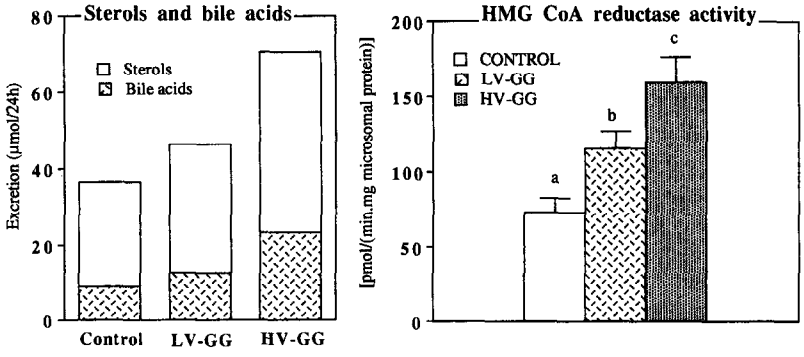

Figure 2 Histograms showing the respective contribution of bile acids and sterols in the overall elimination of steroids and the hepatic activity of HMG CoA reductase in rats adapted to the control diet or diets containing the different fermentable polysaccharides. Each value is a mean ± SEM for 10 rats, values not sharing a common letter are significantly different ($P < 0.05$).

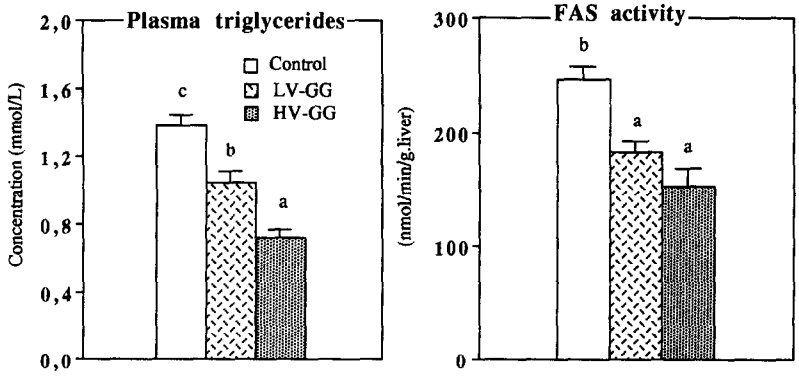

Figure 3 Plasma triglycerides concentration (mmol/L) in relation with the hepatic activity of FAS in rats adapted to the control diet or diets containing the different fermentable polysaccharides. Each value is a mean ± SEM for 10 rats, values not sharing a common letter are significantly different ($P < 0.05$).

proposed.[27,28] Propionic acid has been reported to inhibit hepatic lipogenesis from acetate.[29] However, the concentrations of propionate in portal vein were not very different (340 and 270 μmol/L for the LV-GG and HV-GG respectively, compared to 65 μmol/L for control rats), so the difference between the hypocholesterolemic responses could hardly be explained by the propionic acid availability alone.

FAS activity and plasma triglycerides were more depressed with the HV-GG diet, compared with either the control or LV-GG diet. The mechanism by which a highly viscous hydrocolloid could be more effective to depress the intensity of lipogenesis than a less viscous polysaccharide is probably because of its effectiveness to lower insulin secretion,[30,31] and the effect of fermentations on hepatic lipogenesis could be potentialized by changes in the endocrine status.[32] Reciprocally, the presence of active fermentations seems necessary to maximize a cholesterol-lowering effect. For example, a resistant starch, with a lesser effect on fecal steroid excretion than cholestyramine, was found more potent to decrease plasma cholesterol.[11,33] Fermentations could also contribute to attenuate the induction of HMG-CoA reductase in response to bile acid losses, via a general lowering of liver lipid synthesis. In such conditions, the induction of HMG-CoA reductase might be insufficient to counteract the effects of an enhanced steroid fecal excretion, but the role of SCFA and particularly of propionate in this process, is still disputed.[34] Nevertheless, propionate seems efficient to lower the cholesterogenesis by rat isolated hepatocytes from acetate and from ^3H_2O.[29]

Because the experimental diet was low-fat, it is unlikely that HV-GG would modify the lipoprotein profile by interfering with triglyceride digestion and absorption. In contrast, the possibility to slow down lipid digestion and to shift the intestinal absorption toward a distal site has been considered.[35,36] In the present study, the hypocholesterolemic and hypotriglyceridemic effects were chiefly the results of a modification of bile acid excretion and hepatic lipogenesis.

In contrast to humans, most of the plasma cholesterol is associated with HDL in rats; this fraction is heterogeneous and has been separated into two major subclasses compositionally distinct with regard to the apoE content, lipid and protein composition and particle size. In rats, the hypocholesterolemic effect of HV-GG is accompanied by a characteristic modification of the lipoprotein profile, especially a decreased abundance of cholesterol in the HDL fractions. A decrease of HDL1 apoE-containing fraction could be explained by its accelerated removal (via the apoB/E receptor).[37,38,39] A decrease in HDL1 could also be explained by a reduced rate of formation, corresponding to a depressed supply of the surface components of TGRLP (phospholipids, cholesterol, apos C[40]) a fraction severely depressed in rats fed the HV-GG diet.

In conclusion, this study has shown the reciprocal influence of fermentations and steroids excretion on cholesterol-lowering effect of fermentable carbohydrate.[3] In the digestive tract, the fermentations play a role to insolubilize bile acids and favor their fecal elimination.[11,33] On the other hand, fermentable carbohydrates, by way of SCFA could depress hepatic lipogenesis. However, in the absence of an enhanced fecal steroids excretion, this effect was not sufficient to disturb the cholesterol homeostasis. Viscous fermentable carbohydrates could thus exert their hypocholesterolemic effect by the synergic effects of intestinal fermentations and of enhanced steroid excretion.

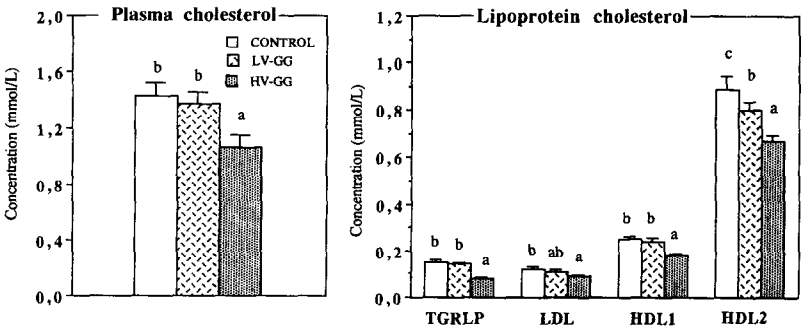

Figure 4 Changes in concentration of cholesterol in plasma and in the various lipoprotein fractions in rats adapted to the control diet or diets containing the different fermentable polysaccharides. Each value is a mean ± SEM of three pools of rat plasma representing an average of 10–12 animals per pool. The LDL to the fractions ranging between 1.006 and 1.040 Kg/L, high density lipoprotein 1 (HDL1) to the fractions ranging between 1.040 and 1.080 Kg/L, and HDL2 to the fractions ranging between 1.080 and 1.210 Kg/L. Value not sharing by a common letter are significantly different ($P < 0.05$).

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