Effect of Cyclodextrin on Plasma Lipids and Cholesterol Metabolism in the Rat

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β-Cyclodextrin (β-CD) is a bile acid and sterol sequestrant produced by enzymatic modification of starch; this product has the potential to decrease plasma cholesterol. In contrast to the sequestrants having resin- or saponin-like properties, β-CD is rapidly broken down by the large intestine microflora. β-CD effects on cecal fermentations and lipid metabolism were thus investigated in rats adapted to semipurified diets containing 0%, 2.5%, or 5% β-CD. In rats fed β-CD diets, there was an enlargement of the cecum together with a dramatic increase in the cecal concentration of propionic acid (even with the 2.5% level, in moderately acidic pH conditions). Propionic acid produced in the cecum was readily absorbed and entirely taken up by the liver, whereas there was no significant acetic acid uptake. Dietary β-CD was highly effective in enhancing bile acid entry into to the cecum: the cecal bile acids pool was 2.2- and 3.6-fold enlarged in rats fed the 2.5% and 5% β-CD diets, respectively. The solubility percentage of bile acids decreased to approximately 25% in rats fed the β-CD diets (*v* 51% in controls); the cecal concentration of soluble bile acids was thus relatively low in these animals. The fecal excretion of steroids was strongly enhanced by β-CD, and bile acids excretion was practically proportional to the dietary β-CD level. There was a net lipid-lowering effect of β-CD, even at the 2.5% level. The effect was more pronounced on triglycerides than on cholesterol. Hypocholesterolemia corresponded to a decrease of very–low-density lipoprotein (VLDL), low-density lipoprotein (LDL), and high-density lipoprotein 1 (HDL_1) cholesterol, whereas HDL_2 cholesterol was unchanged. In parallel, there was an induction of microsomal hydroxymethyl glutaryl coenzyme A (HMG CoA) reductase, also proportional to the dietary β-CD level. In conclusion, β-CD appears to be an effective lipid-lowering agent due to its bile acid–sequestrant properties. The production of short-chain fatty acids (SCFA) from β-CD by the large intestine microflora probably plays a minor role in its effects on lipid metabolism. Nevertheless, by acidifying the large intestine content and solubilizing Ca, cecal SCFA contribute to inhibit the passive reabsorption of bile acids.

A VARIETY OF PRODUCTS have the potential to decrease blood cholesterol, such as hydroxymethyl glutaryl coenzyme A (HMG CoA) reductase inhibitors, bile acid–binding resins, or cholesterol-binding derivatives of saponin. Some complex carbohydrates such as fibers present in plant foods (for example, pectins, gums, or psyllium fiber) also have the capacity to bind or adsorb bile acids and neutral sterols in the small intestine. This process enhances the quantities of steroids reaching the large intestine and their subsequent excretion in the feces.[1-3] Complex carbohydrates are extensively broken down by the microorganisms in the large intestine. Their fermentation by microorganisms has important consequences in the large intestine: acidification of the digestive content, release of the steroids bound to complex carbohydrates, and production of short-chain fatty acids (SCFA), which are readily absorbed and liable to affect splanchnic metabolism. A portion of the steroids present in the large intestine are subject to metabolic transformations by the microflora[4,5] (generally reductive modifications), and some unbound bile acids are absorbed from the large bowel.[3] It must be noted that carbohydrates devoid of significant steroid-binding capacity in the small intestine but readily fermented in the large intestine in acidic conditions (amylase-resistant starch, oligosaccharides) may also enhance bile acid excretion and alter cholesterol metabolism.[6,7] In this case, an enhanced removal from the enterohepatic circulation probably reflects a reduced solubility of bile acids and an effective binding on various structures (Ca complexes, microorganisms) in the large intestine.

Many studies have concluded that neutral sterol and bile acid excretion are the major mechanisms by which dietary fiber decreases the cholesterol concentration, but it has been proposed that SCFA (especially propionic acid) may play an important role in the effect of certain fiber sources.[8] It thus seems that polysaccharides characterized by a high production of propionic acid during the fermentation process should be effectively hypocholesterolemic. A series of investigations in the laboratory have shown that oligosaccharides (such as inulin or cyclodextrin) are readily fermented by the cecal microflora, and the SCFA produced show a very high proportion of propionic acid[9]; however, their hypocholesterolemic effect (quite limited with inulin) could not be directly connected to the availability of propionic acid in the large intestine. It must be noted that, in contrast to cyclodextrin, inulin is virtually devoid of any steroid-binding capacity.

Cyclodextrins represent oligosaccharides that are produced by an enzymatic modification of starch: β-cyclodextrin (β-CD) contains seven glucopyranosyl units linked in a ring structure in the α(1-4) position, with hydrogen and glycoside oxygen atoms oriented toward the cavity, which is therefore hydrophobic.[10] β-CD has the capacity to form inclusion complexes with a variety of molecules, especially bile acids and sterols, the absorption of which in the small intestine is thus impaired. β-CD is not hydrolyzed by the salivary or pancreatic α-amylase,[11,12] but it is now well established that despite its unusual structure, β-CD is fermented by the large intestine microflora in experimental animals[9,12] and in humans.[13,14]

In view of the potential effectiveness of β-CD in decreasing plasma lipids, it was thus decided to investigate its effects to assess whether bile acid and sterol excretion is a

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major factor in the cholesterol-lowering effect. A relatively low dietary level (2.5% or 5%) was used to limit the role of the fermentation processes and of SCFA metabolism.

MATERIALS AND METHODS

β-CD was purchased from Roquettes (Lestrem, France), casein and wheat starch from L. François (Saint Maur, France), and the mineral and vitamin mixtures from U.A.R. (Villemoisson/Orge, France). Cholesterol oxidase and coenzymes (NADH, NADPH) were obtained from Boehringer (Meylan, France), 3-α steroid dehydrogenase, HMG CoA, dithiothreitol, and leupeptin from Sigma (St Louis, MO), AG1-X8 resin from BioRad (Paris, France), and [^{14}C]HMG CoA and [^{3}H]mevalonate from Amersham (Les Ulis, France).

Experimental Protocol

Male Wistar rats (IFFA-CREDO, L'Arbresle, France) weighing approximately 170 g were housed two per cage and fed for 21 days with semipurified diets distributed as a moistened powder. The basal control diet contained the following (in grams per kilogram): casein, 180; corn oil, 50; wheat starch, 700; minerals, 60; and vitamins, 10. The other experimental diets contained 25 or 50 g β-CD/kg diet, at the expense of wheat starch. The animals were maintained in temperature-controlled rooms (22°C) with a dark period from 9 AM to 9 PM and access to food from 9 AM to 5 PM. The animals were maintained and handled according to the recommendations of the Institutional Ethics Committee.

The body weight was recorded on days 0, 5, 15, and 20 of the experiment; food intake determination and collection of feces was performed on 3 consecutive days at the end of the experimental period. At the time of sampling (namely after 8 hours of access to the food), the rats were anesthetized with sodium pentobarbital and maintained at 37°C. An abdominal incision was made, and blood (0.8 mL) was withdrawn from the hepatic vein, the portal vein, and then the abdominal aorta. Blood flow was determined by an indicator-dilution procedure using a method adapted from Katz and Bergman.[15] After blood sampling, the cecum with contents was removed and weighed. Two samples (~1 g) of cecal content were transferred to microfuge tubes that were immediately frozen at −20°C. Approximately 1 g liver was immediately freeze-clamped and stored at −80°C before extraction of lipids and determination of cholesterol content.[16]

Preparation of Microsomes

Two grams of liver was also homogenized in 3 mL of an ice-cold buffer (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 Teflon-pestle Potter-Elvejhem homogenizer (Braun, Melsungen, Germany; 1-mm clearance, three strokes at moderate speed). The homogenate was first centrifuged at 10,000 × *g* (4°C, 15 minutes); the supernatant was then centrifuged at 100,000 × *g* (4°C, 60 minutes). The pellets were resuspended in 2 mL chilled buffer (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 centrifugation procedure was repeated, and the resulting pellets were homogenized in 1 mL of the latter buffer. The microsomal preparation was stored at −80°C until measurement of enzyme activities. The microsomal protein content was determined using the Pierce BCA Reagent kit (Interchim, Montluçon, France).

Analytical Procedures

SCFA levels were measured by gas-liquid chromatography, after ethanolic extraction of plasma samples[17] and on supernatants (8,000 × *g*, 5 minutes at 4°C) of cecal contents. Total bile acids and sterols were extracted from cecal contents or feces by 10 vol ethanolic KOH (0.5 mol/L) and quantified using the reaction catalyzed by the 3α-hydroxysteroid dehydrogenase (EC 1.1.1.50)[18] and cholesterol oxidase (EC 1.1.3.6), respectively. Soluble bile acids levels were determined on the 8,000 × *g* supernatants of cecal contents. Ca was assayed on the cecal supernatant (soluble), and after mineralization of the untreated cecal content (total) Ca was measured by atomic absorption spectrophotometry (Perkin-Elmer 400, Norwalk, CT).

Triglycerides (Biotrol, Paris, France), phospholipids, and cholesterol (BioMérieux, Charbonnières-les-Bains, France) levels were determined using enzymatic procedures. A polyvalent control serum (Biotrol 33-Plus) was treated in parallel to samples and served as a control for the accuracy of results in triglyceride and cholesterol analysis. Plasma (from arterial blood) lipoproteins were separated on a density gradient by preparative ultracentrifugation as described by Sérougne et al.[19] After centrifugation in a TST 41.14 (Kontron, Zürich, Switzerland) swinging-bucket rotor (100,000 × *g*, 36 hours), the gradient was fractionated (500-μL fractions) and the cholesterol, phospholipid, and protein contents of each fraction were determined by the method described above. The activity of HMG CoA reductase was determined as described by Wilce and Kroone.[20] Labeled mevalonolactone was separated from unreacted HMG CoA by column chromatography using AG1-X8 resin (200 to 400 mesh, formate form, BioRad, Paris, France). The specific activity of the enzyme is calculated after correcting for counting efficiency and recovery of mevalonolactone from the column.

Presentation of Results

The cecal pool was calculated as the cecal concentration (millimolar) × cecal content volume (milliliters). The portal balance was calculated as ([portal vein] − [artery]) × portal blood flow, the hepatic balance as ([hepatic vein] − [afferent]) × hepatic blood flow, and the afferent concentration was calculated from the portal vein and artery, considering their respective blood flow.

Values are presented as the mean ± SEM, and where appropriate, the significance of differences between mean values was determined by ANOVA and multiple-range comparisons by Fisher's protected least significant difference procedures (StatView 512+, Brain Power, Calabasas, CA). Where it was necessary to achieve homogeneity of variance, the data were subjected to logarithmic transformation. Values of P less than .05 were considered significant.

RESULTS

Effect of Dietary β-CD on Weight Gain and Cecal Fermentations

Table 1 shows that the presence of 2.5% β-CD in the diet had no effect on the daily weight gain, whereas there was a

Table 1. Effect of Dietary β-CD on Growth of Rats and on the Cecal Parameters

		Cecum		
Diet	Daily Weight Gain (g)	Total Weight (g)	Wall Weight (g)	pH
Control	5.7 ± 0.3	3.2 ± 0.2	0.7 ± 0.1	7.21 ± 0.08
2.5% β-CD	5.5 ± 0.2	4.4 ± 0.3*	0.9 ± 0.1	6.70 ± 0.08*
5% β-CD	4.9 ± 0.2*†	6.3 ± 0.7*†	1.2 ± 0.2*	5.78 ± 0.06*†

*P < .05 *v* control.

†P < .05, 5% β-CD *v* 2.5% β-CD.

Table 2. Effect of Dietary β-CD on Cecal SCFA

Diet	Acetic Acid (mmol/L)	Propionic Acid (mmol/L)	Butyric Acid (mmol/L)	Total SCFA (mmol/L)	Cecal SCFA Pool (μmol)	SCFA Molar Ratio (Ac/Pr/Bu)
Control	47.9 ± 3.8	20.4 ± 1.2	11.9 ± 1.2	80.2 ± 4.8	183 ± 10	60/25/15
2.5% β-CD	47.5 ± 4.4	41.9 ± 2.8*	11.6 ± 3.1	101.0 ± 7.2*	358 ± 28*	47/41/12
5% β-CD	79.5 ± 5.2*†	66.2 ± 4.8*†	12.7 ± 1.6	158.4 ± 8.8*†	793 ± 41*†	49/41/10

Abbreviations: Ac, acetic acid; Pr, propionic acid; Bu, butyric acid.

*$P < .05$ *v* control.

†$P < .05$, 5% β-CD *v* 2.5% β-CD.

slight but significant decrease in rats fed the 5% β-CD diet. However, there was no significant difference in the daily food intake between the diet groups. Rats fed β-CD diets showed a significant enlargement of the cecum (+38% and +97% with the 2.5% and 5% levels, respectively), which principally corresponded to an increase of the cecal content weight. The cecal wall was significantly heavier only in rats adapted to the 5% β-CD diet. The cecal enlargement was accompanied by an acidification of the cecal content, which decreased to 6.72 (2.5% β-CD) or 5.80 (5% β-CD; Table 2). In parallel, there was a striking alteration in the concentration and molar ratio of cecal SCFA. Compared with the controls, the 2.5% β-CD group had a 26% higher SCFA concentration, which was entirely due to a higher concentration of propionic acid. In the 5% β-CD group, the total SCFA concentration was particularly high (~160 mmol/L), reflecting the presence of a high concentration of acetic acid and of propionic acid (up to 66.2 mmol/L). With the two β-CD diets, the molar ratio of propionic acid was particularly high (41%), and it must be noted that both the propionic acid concentration and the SCFA pool in the cecum were virtually proportional to the β-CD level in the diet.

Digestive and Hepatic Balance of SCFA

Figure 1 shows that SCFA absorption was proportional to the cecal SCFA pool, except for butyric acid, which appears in relatively small quantities in portal blood because of its metabolization by the large intestine mucosa. The hepatic balance of propionic and butyric acid reflected a complete uptake of these acids by the liver, and the quantities of propionic acid metabolized in the liver were proportional to the β-CD level in the diet. Acetic acid was present in concentrations higher than or similar to those of propionic acid in the portal vein, but there was no net uptake of acetic acid by the liver (a small but significant net production was noted in the control and 2.5% β-CD diet groups).

Effect of β-CD on Cecal and Fecal Steroids

Rats fed the β-CD diets showed a significant increase in the cecal concentration of bile acids (Table 3), which in conjunction with the cecal enlargement resulted in a dramatic elevation of the cecal bile acid pool (+123% and +265% with the 2.5% and the 5% β-CD levels, respectively). Concurrently, there was a reduced percentage of the soluble form of bile acids, from 50% in control rats to approximately 25% in rats fed the β-CD diets. The cecal pool of soluble bile acids was significantly greater than in controls only in rats adapted to the 5% β-CD diet. The total concentration of Ca in the cecum was very high in control rats (~450 mmol/g), and it was decreased in parallel to the cecal enlargement (to 198 mmol/g in rats fed the 5% β-CD diet). On the other hand, the cecal concentration of soluble Ca was elevated in parallel to the dietary β-CD level: soluble Ca represented 9.3% of total Ca in rats fed the 5%

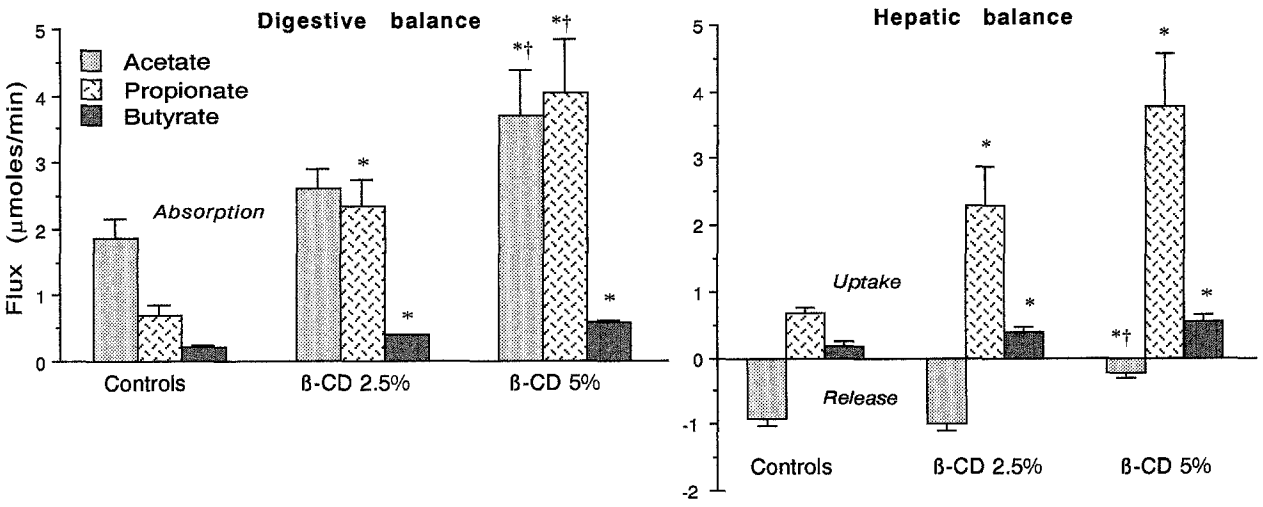

Fig 1. Effect of the dietary level of β-CD on digestive and hepatic balances of SCFA. *$P < .05$: significantly different from controls. †$P < .05$: significant difference between rats fed the 2.5% or 5% β-CD diets.

Table 3. Effect of Dietary β-CD on Cecal Ca and Bile Acids and on the Fecal Excretion of Neutral and Acidic Steroids

	Cecum					Fecal Excretion (μmol/d)	
Diet	Total Ca (mmol/g)	Soluble Ca (mmol/L)	Total Bile Acids (mmol/g)	Bile Acids Cecal Pool (μmol)	Soluble Bile Acids (%)	Neutral Steroids	Bile Acids
Control	453 ± 17	6.4 ± 0.5	3.4 ± 0.2	7.4 ± 0.3	51	14.2 ± 1.5	8.8 ± 0.8
2.5% β-CD	277 ± 14*	12.2 ± 1.2*	4.6 ± 0.4*	16.5 ± 2.2*	27	16.1 ± 1.2	14.8 ± 1.6
5% β-CD	198 ± 15*†	18.5 ± 1.6*†	5.1 ± 0.6*	26.9 ± 3.1*†	23	21.2 ± 2.1*†	30.2 ± 4.1

*$P < .05$ *v* control.
†$P < .05$, 5% β-CD *v* 2.5% β-CD.

β-CD diet, as compared with 1.4% in control rats. The fecal excretion of sterols and bile acids was considerably elevated by the β-CD diets. It must be noted that the β-CD effect seems more potent on bile acids than on sterols excretion: compared with controls, bile acid excretion was 1.7- or 3.4-fold greater in rats fed the 2.5% or 5% β-CD diets, respectively.

Effect of β-CD on Lipid Metabolism

As shown in Table 4, β-CD in the diet elicited a cholesterol-lowering effect (−20%) together with a more potent triglyceride-lowering effect (−43%) with the 5% β-CD diet. This effect on plasma lipids was slightly higher with the 5% than with the 2.5% β-CD diet. In parallel, the β-CD diets strongly induced the activity of microsomal HMG CoA reductase (up to 6.4-fold with the 5% β-CD diet); in contrast, liver cholesterol was not noticeably decreased by the β-CD diets.

Plasma lipoproteins were fractionated by gradient ultracentrifugation and further analyzed for lipids and protein content. Cholesterol was not uniformly decreased (Fig 2). It was significantly decreased in the very–low-density lipoprotein (VLDL), low-density lipoprotein (LDL), and (to a lesser extent) high-density lipoprotein 1 (HDL_1) fractions, whereas it was unchanged in the HDL_2 fraction, which represents the major vehicle of plasma cholesterol in the rat. Data for protein and phospholipid concentrations in lipoprotein fractions indicate changes that were practically parallel to those for cholesterol, except for VLDL, the protein concentration of which was less responsive to the diet conditions than the concentrations of the various lipid categories. It is noteworthy that the 2.5% β-CD level was practically as effective as the 5% level on the plasma lipoprotein profile. As shown in Fig 3, it appears that the triglyceride concentration was also markedly decreased in rats fed the β-CD diets (~ −45%) in the triglyceride-rich lipoprotein fractions, essentially VLDL, in the present conditions of low-fat diets.

DISCUSSION

Early investigations in this domain came to the conclusion that due to its ring structure, β-CD was slowly hydrolyzed in the large intestine and a portion would be excreted intact in the feces.[21] However, recent data in man and experimental animals (and the present data) support the view that β-CD is extensively hydrolyzed[9,11,12] when consumed in moderate quantities. The fact that if present in a percentage as low as 2.5% in the diet, β-CD fermentation results in a doubling of the propionic acid concentration is an interesting feature. Such a pattern of fermentation has been observed with readily fermented substrates such as amylomaize starch[22] or inulin.[23] However, even with inulin a high production of propionic acid took place at a relatively low (6.5 to 6.0) or even definitely acidic (<6.0) cecal pH, in agreement with the classic concept that the SCFA molar ratio in digestive fermentations is chiefly governed by pH conditions.[24] The present results suggest that carbohydrates themselves, especially oligosaccharides (β-CD, fructans[9,23]), are particularly effective in promoting propionic acid production by the microflora even when the luminal pH is higher than 6.5.

SCFA are rapidly absorbed and transferred, via the portal vein, to the liver. Propionic acid is quantitatively taken up by the liver, where it yields CoA derivatives and intermediates of the Krebs cycle that may affect a variety of metabolic pathways.[25] In addition, propionic acid has an inhibitory effect on the hepatic uptake of acetic acid, as previously shown on isolated hepatocytes.[26] The actual importance of this effect on acetyl CoA metabolism (lipogenesis, cholesterogenesis) is still to be ascertained, but the fact that there is a net release of acetate in rats fed the 2.5% β-CD diet suggests that this metabolism could be altered.

Cyclodextrin has the ability to accommodate in its cavity a variety of suitably sized organic molecules such as bile acids and sterols or fatty acids. By its entrapment of bile acids in the ileum, β-CD increased their cecal pool, and this effect was practically proportional to the dietary level of

Table 4. Effect of Dietary β-CD on Plasma Lipids, and on Liver Cholesterol and HMG CoA Reductase Activity

	Plasma				
Diet	Cholesterol (mmol/L)	Triglycerides (mmol/L)	Phospholipids (mmol/L)	Liver Cholesterol (mg/g)	Microsomal HMG CoA Reductase [pmol/(min · mg protein)]
Control	1.52 ± 0.08	1.24 ± 0.02	1.38 ± 0.03	1.70 ± 0.06	18 ± 3
2.5% β-CD	1.30 ± 0.05*	0.84 ± 0.02*	1.22 ± 0.02*	1.86 ± 0.09	46 ± 6*
5% β-CD	1.21 ± 0.05*	0.70 ± 0.06*†	1.15 ± 0.02*†	1.99 ± 0.10*	116 ± 17*†

*$P < .05$ *v* control.
†$P < .05$, 5% β-CD *v* 2.5% β-CD.

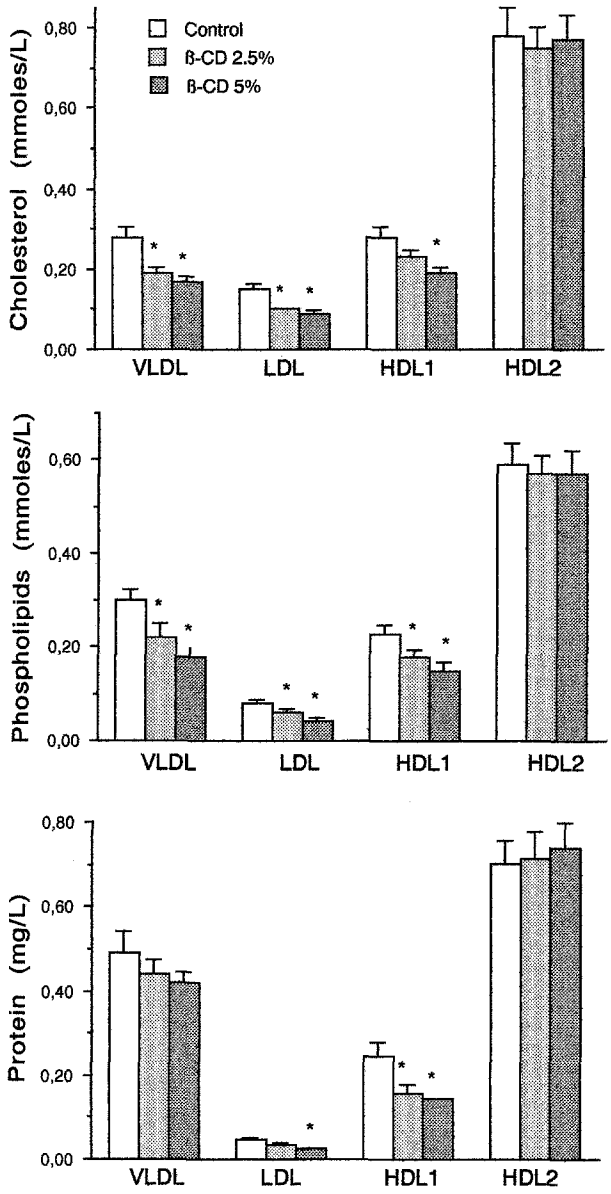

Fig 2. Effect of the dietary level of β-CD on the cholesterol, phospholipid, and protein repartition in the different plasma lipoprotein classes. The triglyceride-rich lipoproteins (essentially VLDL) corresponded to the fractions with a density lower than 1.006. LDL, HDL_1, and HDL_2 corresponded to the fractions within a density range of 1.006 to 1.040, 1.040 to 1.080, and 1.080 to 1.16, respectively. *P < .05: significantly different from controls.

β-CD. It has been reported that the presence of sequestrants (cholestyramine) not only causes an increase in bile acids output from the ileum, but also inhibits their passive absorption in the colon.[27] In the present study, the solubility of bile acids in the large intestine was low despite the breakdown of β-CD, and this may represent a factor limiting bile acid salvage in the large intestine. SCFA produced by the microbial fermentation of β-CD contributed to solubilize a portion of the cecal Ca pool,[7] which, in turn, plays a role in the insolubilization of bile acids (besides a role of pH itself[28]). This point is critical with fermented carbohydrates such as β-CD, which are liable to release in the medium the guest molecules encapsulated in the upper gastrointestinal tract. There are apparently other processes that limit the availability of soluble bile acids in the large intestine, such as binding to bacteria or insoluble calcium salts. The binding of bile acids on insoluble materials is favored by acidic pH conditions.[7] This may explain how fibers devoid of binding capacity for bile acids in the small intestine nevertheless enhance the fecal excretion of bile acids.[7,9] When dietary manipulations are designed to impair the absorption of bile acids in the small intestine, it must be kept in mind that these molecules (especially the dehydroxylated metabolites) are potentially cytotoxic for the large intestine mucosa.[29,30] Fortunately, the acidification of the cecal content should depress the formation of secondary bile acids by the microflora[4]; furthermore, Riottot et al[31] have shown that dietary β-CD prevents the intestinal absorption of lithocholic acid and promotes the disposal of this cytotoxic bile acid from the colon. It must be noted that the increase of steroid excretion is largely due to the changes in bile acids rather than in sterol excretion.[31] This suggests that in the conditions prevailing in the small intestine, bile acids are more effectively entrapped by β-CD than sterols are.

It appears that in the rat, a species that uses HDL as the primary lipoprotein involved in cholesterol transport, the decrease of plasma cholesterol was chiefly the result of a reduction in LDL and VLDL cholesterol. In addition, there was a significant decrease in HDL_1 cholesterol, which is generally parallel to a decrease in apolipoprotein E content.[16,32] The decrease of HDL_1 cholesterol could be explained by a reduced formation or an enhanced removal. The low level of triglyceride-rich lipoprotein (TGRLP; hence a depressed supply of the surface components for HDL_1 formation) is consistent with the first view. Nevertheless, a faster removal of HDL_1, which plays a major role in the reverse transport of cholesterol, is conceivable since plasma cholesterol was decreased in rats fed β-CD. It is noteworthy that β-CD has the potential to decrease LDL cholesterol with little effect on HDL cholesterol, which could be of interest in humans.

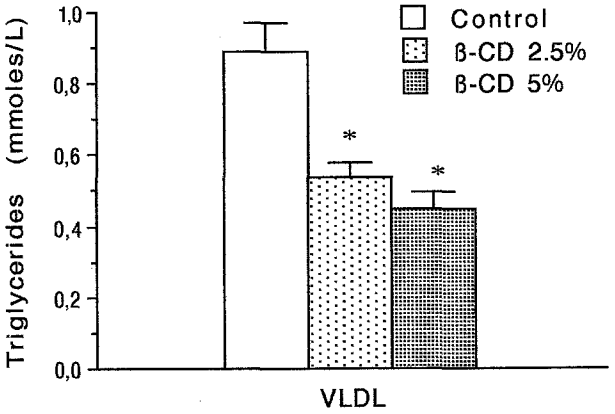

Fig 3. Effect of the dietary level of β-CD on the triglyceride concentration in triglyceride-rich lipoproteins (essentially VLDL, d < 1.006). *P < .05: significantly different from controls.

When the demand for cholesterol increases in the liver, a similar signal mechanism could be involved in the expression of both HMG CoA reductase and the apolipoprotein B/E receptor.[33,34] The cholesterol-lowering effect of complex carbohydrates or steroid sequestrants is frequently accompanied by a striking induction of the microsomal HMG CoA reductase,[35] provided that the dietary cholesterol level is low.[9,16,36] Conceivably, this effect might have been more pronounced with microsomes isolated in the middle of the dark period.[37,38] It is noteworthy that the cholesterol-lowering effects of the two β-CD levels did not markedly differ, whereas HMG CoA reductase activity was much higher with the 5% level than with the 2.5% level. This could reflect the rate of liver cholesterogenesis if it was to be proportional to the magnitude of the fecal loss of steroids; furthermore, both bile acid synthesis and cholesterogenesis are tightly connected in microsomes.

β-CD has consistently displayed a more potent effect on triglycerides than on cholesterol in plasma,[9,30] especially in the TGRLP fraction. Whether binding of bile acids by β-CD causes a malabsorption of neutral lipid is questionable with the low-fat diets used in the present studies. It has been shown that feeding fermented carbohydrates causes a decrease in the activity of the major enzymes of the lipogenesis pathway, together with a depressed rate of fatty acid synthesis (using ^3H_2O) in the liver.[39,40] However, this effect was obtained with a relatively high percentage of nonavailable carbohydrates in the diet, and hence a lower glucose absorption and insulinemia. The triglyceride-lowering effect of β-CD seems difficult to connect to a direct effect of propionic acid, since a previous study has shown that the addition of 2.5% Ca propionate in the diet failed to change significantly the plasma concentrations of triglycerides or cholesterol.[9] Thus, the question arises as to whether losses in cholesterol affect triglyceride synthesis, for example, by directing acetyl CoA toward the very active cholesterogenesis. This hypothesis is supported by the fact that the hepatic uptake of portal acetic acid was very low, but it must be kept in mind that there are other sources of acetyl CoA (blood lactate, glycolysis). Triglyceride release by the liver may also be limited by a shortage in cholesterol for VLDL synthesis (but no change in liver cholesterol was observed in the present study). Whatever the mechanisms, it is interesting to consider the use of β-CD to decrease the TGRLP, particularly in situations of hypertriglyceridemia in human subjects.

In conclusion, β-CD has interesting lipid-lowering effects, especially on plasma triglycerides. The production of SCFA from β-CD fermentation certainly plays a minor role to explain its effects on lipid metabolism. However, by acidifying the large intestine content and solubilizing Ca, cecal SCFA contribute to inhibit the passive reabsorption of bile acids. The hypotriglyceridemic effect has been shown here with a low-fat diet, but it remains to be verified whether this effect is still observed with high-fat or hypertriglyceridemic diets, for example, those rich in fructose.

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