Dietary Fructans Modulate Polyamine Concentration in the Cecum of Rats

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ABSTRACT

Nondigestible but fermentable dietary fructans such as oligofructose exert many effects on gut physiology through their fermentation end products such as short-chain fatty acids. Could other metabolites be produced in the gut and contribute to the physiologic effects of dietary fructans? The aim of the study was to evaluate the influence of oligofructose on putrescine, spermidine and spermine concentrations in the cecum, the portal vein and the liver of rats and to assess their involvement in cecal enlargement and the modulation of hepatic lipid metabolism. Putrescine, spermidine and spermine were quantified by HPLC in samples obtained from male Wistar rats fed a nonpurified standard diet (controls) or the same diet enriched with 10 g/100 g oligofructose (OFS) for 4 wk. OFS-fed rats had significantly greater cecal content and tissue weights. OFS almost doubled the concentration of putrescine in the cecal contents. The concentration of all three polyamines in the cecal tissue was significantly greater than in controls. The concentration of spermidine in portal plasma was lower in rats fed OFS, whereas the treatment did not affect the polyamine concentrations in the liver. The fermentation of dietary fructans contributed to an increase in the concentration of putrescine in the gut without modifying putrescine concentration in either the portal blood or liver. Moreover, the greater levels of polyamines in cecal tissue may be related to the cell proliferation resulting from OFS fermentation in the gut. J. Nutr. 130: 2456–2460, 2000.

KEY WORDS: polyamine • oligofructose • cecum • intestinal microflora • rats

The polyamines, spermidine, spermine and putrescine, are found in virtually all cells of higher eucaryotes. In intestinal cells, they are involved in several processes, including cell growth and differentiation (Bardocz et al. 1995, Löser et al. 1999), glucose transport (Johnson et al. 1995), intestinal motility (Fioramonti et al. 1994) and regulation of disaccharidase activities (Deloyer et al. 1996). Enteroocytes respond to luminal nutrients with large increases in polyamine synthesis, under the control of ornithine decarboxylase (ODC) activity, even though they are mature, nonproliferating cells (Johnson et al. 1995). In addition to the endogenous synthesis of polyamines inside the cell, exogenous sources of polyamines seem to be essential for small intestinal and colonic mucosal growth and development (Löser et al. 1999). Several sources of “exogenous polyamines” reaching the intestine are available. Polyamines may be absorbed per se as food components. Their absorption occurs mainly in the upper part of the gut, where they are involved in enterocyte maturation and metabolism (Bardocz et al. 1998). Polyamines found in the intestinal lumen may also be provided by pancreatic and biliary secretions, through desquamation or via their active secretion by intestinal cells (Benamouzig et al. 1997, Osborne and Seidel 1990). Some probiotics such as yeast can exert trophic effects on the small intestine by mediating the endoluminal release of spermine and spermidine (Buts et al. 1994). Moreover, as suggested by recent studies in humans and animals, polyamines may be synthesized from dietary fermentable substrates such as pectin or guar gum by bacteria in lower parts of the gut (ceccolon) (Noack et al. 1998, Satink et al. 1989). Inulin-type fructans are natural components of the diet that escape hydrolysis by mammalian digestive enzymes, but are largely fermented by colonic bacteria to produce a wide variety of compounds that may affect gut as well as systemic physiology, in both humans and rats (Roberfroid and Delzenne 1998, Van Loo et al. 1999). End products of carbohydrate fermentation are represented primarily by a limited number of carboxylic acids, especially short-chain fatty acids (SCFA) such as acetate, propionate and butyrate, which can have noticeable biological effects in tissues exposed to large concentrations including the colonic epithelium and, to a lesser extent, the liver. In the colon, the role of butyrate as a major energy fuel and control factor of cell proliferation has been established (Demigné et al. 1999, Koruda et al. 1990).

Fructans are completely fermented in the ceccolon by several bacterial strains; some of these bacteria such as bifidobacteria are able to acquire a proliferative advantage over other strains, as demonstrated in vitro in mixed bath culture and in vivo in humans (Gibson et al. 1995, Gibson and Roberfroid 1995). Several physiologic effects inside the gut may be related to the extensive fermentation of fructans by...
endogenous bacteria, i.e., a decrease in pH due to the production of short-chain carboxylic acids, an increase in calcium and magnesium absorption and a displacement of nitrogen excretion (Campbell et al. 1997, Delzenne et al. 1995, Rémyes et al. 1993). Moreover, feeding rats inulin-type fructans leads to cecal, but not colonic hyperplasia and to an increase in ODC activity in cecal wall cells (Rémyes et al. 1993).

In this study, we analyzed the influence of supplementing a diet with oligofructose (OFS), a fructan obtained by enzymatic hydrolysis of chicory inulin, on polyamine concentrations in the cecum, portal vein and liver of rats.

MATERIALS AND METHODS

All of the rats received care in compliance with NIH guidelines (NRC 1985). Male Wistar rats (n = 12; Iffa Credo, France), weighing 200 g at the beginning of the experiment were given either a standard diet (controls), commercially available (AO4, Villemoisson sur Orge, France), or the same diet enriched with 10% OFS (Raftilose P95, Orafti, Belgium) for 4 wk. The complete composition of the diets was described previously (Dautioul et al. 2000). The rats were anesthetized with pentobarbital (60 mg/kg). After laparotomy, blood from the portal vein was collected in sodium citrate solution and centrifuged at 1200 × g for 10 min at 4°C. The plasma and RBC were washed three times in 9 g/L NaCl to remove lymphocytes and monocytes and kept at −70°C until use. The liver was excised. The full cecum was weighed; the cecal contents were diluted with 2 mL of 9g/L NaCl, then centrifuged at 9000 g for 10 min. The supernatant was frozen at −70°C and kept for further polyamine analysis. The cecum and liver were weighed and then clamped in liquid nitrogen. Liver (fourfold dilution) and cecum (sixfold dilution) homogenates were prepared in sterile water using a Potter homogenizer.

The protein level was measured in cecal and liver homogenates by the method of Lowry et al. (1951).

Polyamine content in the samples was measured by HPLC (Bontemps et al. 1984). All samples had been stored under the same conditions before polyamine analysis, (−70°C; maximum 3 mo). Such storage did not modify polyamine content in pig RBC (data not shown). The tissue homogenate and cecal supernatant were treated using a procedure described previously (Kauas et al. 1997). The polyamines from the blood samples were extracted using the method of Moulinoux et al. (1991). After dansylchloride derivatization, polyamines were separated on a reverse-phase column (Lichrosart RP-18, Merck, Darmstadt, Germany).

Statistical analysis. Data are presented as means ± SEM; Student’s t test was applied to compare unpaired means. Statview512 + (BrainPower, Calabasas, CA) was used as software. The level of significance was set at P < 0.05.

TABLE 1

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>OFS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight, g</td>
<td>242 ± 7</td>
<td>220 ± 12*</td>
</tr>
<tr>
<td>Food intake, g/d</td>
<td>20.0 ± 0.8</td>
<td>20.0 ± 0.4</td>
</tr>
<tr>
<td>Cecum weight, g</td>
<td>5.08 ± 0.29</td>
<td>7.36 ± 0.28**</td>
</tr>
<tr>
<td>Total</td>
<td>2.96 ± 0.14</td>
<td>4.48 ± 0.35**</td>
</tr>
<tr>
<td>Content</td>
<td>1.86 ± 0.19</td>
<td>2.60 ± 0.18*</td>
</tr>
</tbody>
</table>

1 Results are means ± SEM, n = 6. The cecal content weight was calculated for each rat as follows: total weight − empty cecum weight (tissue). Mean dietary consumption corresponds to the mean of the daily consumption measured twice per week throughout the 4-wk treatment; *P < 0.05 and **P < 0.01 vs. control (unpaired t test).

RESULTS

The addition of OFS to the diet did not modify the daily food intake of rats but resulted in significantly lower body weight compared with controls (Table 1). OFS treatment significantly increased the cecal contents and cecal tissue weight by ~80%. Histologic examination of cecal tissue samples did not reveal any differences in the histologic pattern (cell proliferation, crypt depth, villous height) of cecal mucosa in OFS-fed and control rats.

Spermidine was the most abundant, whereas spermine represented only ~5% of the total polyamine concentration estimated in the cecal contents (Fig. 1). Treatment with OFS almost doubled the level of putrescine in the cecal contents but did not significantly modify the concentration of spermidine or spermine. In the cecal tissue, spermidine and spermine represented the major fractions (Fig. 2). Both of these polyamines as well as putrescine were significantly greater in the cecal tissue of OFS-fed rats.

Putrescine represented the most abundant polyamine in plasma, whereas spermidine was transported mainly in the RBC (Table 2). Polyamine concentrations in RBC were not affected by OFS treatment, but plasma spermidine concentra-
tion was significantly lower in OFS-fed rats. The concentration of spermine in the portal plasma was measured in all of the samples. In OFS-fed rats, it was 0.33 nmol/mL in one rat; in the other five rats, its concentration was below the limit of quantification (corresponding to 0.2 μmol/L) (see Table 2). Spermine concentration was measurable in all of the plasma samples from the control group. The hepatic concentration of the three polyamines was not modified by dietary OFS (Figure 3). In the liver, spermidine and spermine were the most abundant polyamines, whereas putrescine represented only 10% of the total polyamines measured.

DISCUSSION

Several studies report that nondigestible nutrients such as soluble dietary fibers, resistant carbohydrates (starch or oligosaccharides) and resistant protein strongly modify the gastrointestinal physiology, mainly through their fermentation in the large bowel (Campbell et al. 1997, Morita et al. 1999, Roberfroid and Delzenne 1998, Slavin 1999). This fermentation leads to the production of (SCFA), which have been proposed to be mediators of the systemic effect of such nondigestible/fermentable nutrients (Demigné et al. 1999). Could other metabolic by-products, namely, polyamines, play a role in the physiologic effects of nondigestible carbohydrates? In this study, OFS, an inulin-derived fructan, was added to the standard diet of rats for 4 wk. This treatment significantly decreased body weight after 4 wk, but food intake, measured weekly, was not significantly different between groups. However, the total energy intake was lower in OFS-fed than in control rats. This effect could be attributed to the lower energy value of the OFS-containing diet (13.23 kJ/g for OFS-containing diet vs. 14 kJ/g for control diet), using an estimated energy value for OFS of 6.3 kJ/g (Roberfroid et al. 1993). At the end of treatment, macroscopic analysis of the organs revealed cecal enlargement in OFS-fed rats, as previously described (Delzenne et al. 1995, Roberfroid and Delzenne 1998). The increase in the cecal contents reflects a huge proliferation of bacteria. Fructans may be metabolized by several types of bacteria, e.g., most species of bifidobacteria are able to use fructans as substrates (Gibson et al. 1995). An increase in the concentration of short-chain carboxylic acids, primarily acetate, but also propionate, butyrate and lactate was found in the cecum of rats fed inulin-type fructans (Campbell et al. 1997, Roberfroid et al. 1993, Van Loo et al. 1999). Among the polyamines tested, only the putrescine concentration was significantly greater in the cecal contents of OFS-fed rats. Noack et al. (1998) compared the influence of pectin, a fermentable dietary fiber, on cecal polyamine production in germfree and conventional rats. They showed that putrescine is the main endogenous polyamine secreted into the gut lumen from the gut mucosal cells (Noack et al. 1998). Our study did not provide any data relative to polyamine secretion by intestinal or cecal cells, and the involvement of such a mechanism would require further studies. Feeding rats fermentable dietary fiber increased the desquamation of intestinal and/or cecal cells, a phenomenon that could contribute to the release of putrescine in the gut lumen (Noack et al. 1998). Another source could result from a modified enterohepatic circulation of polyamines, leading to an increase in their pancreatic or biliary secretion (Osborne and Seidel 1990). Finally, the involvement of the intestinal flora in the modulation of polyamine concentration in the cecum must be questioned. Inulin-type fructans such as OFS are fermented mainly in mixed culture, and in human fecal samples in anaerobic batch cultures, by bifidobacteria, which predominate over the other strains; moreover, the addition of OFS (15g/d) in the diet significantly reduced the count of bacteroides, fusobacteria and clostridia populations in adult humans (Gibson et al. 1995). Do OFS reduce the number of bacterial strains able to metabolize polyamines, and/or do they promote the strains producing putrescine? The question remains open. The bacteria that induce putrescine production are gram-positive anaerobic cocci and fusobacteria, whereas members of the genus Bacteroides are considered spermidine producers (Noack et al. 1998). Testing the ability of bifidobacteria to produce polyamines could be an interesting way to determine their role in the modulation of polyamine cecal production by dietary fructans. The predominance of bacteroides in the cecal flora of conventional rats would explain why spermidine was the major polyamine found in the lumen of the cecum of rats from both groups in our study. The slight decrease (P = 0.07) in spermidine observed in the cecal contents of OFS-fed rats could be attributed to the decreased number of bacteroides.

In the cecal tissue, spermidine was the major polyamine, and its concentration was significantly higher in OFS-fed rats than in controls. The fact that all three polyamines were increased in the cecal wall of OFS-fed rats suggests a greater

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**TABLE 2**

Polyamine concentrations in the red blood cells and the plasma of the portal vein of rats fed control or oligofructose (OFS)-containing diets.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Putrescine</th>
<th>Spermine</th>
<th>Spermidine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Portal red blood cells, μmol/L RBC pellet</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>45.9 ± 4.11</td>
<td>9.1 ± 1.2</td>
<td>130 ± 14</td>
</tr>
<tr>
<td>OFS</td>
<td>38.1 ± 2.3</td>
<td>7.9 ± 0.7</td>
<td>121 ± 8</td>
</tr>
<tr>
<td>Portal plasma, μmol/L</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>8.2 ± 1.2</td>
<td>0.40 ± 0.03</td>
<td>1.48 ± 0.25</td>
</tr>
<tr>
<td>OFS</td>
<td>8.5 ± 0.3</td>
<td>0.33²</td>
<td>0.67 ± 0.05²</td>
</tr>
</tbody>
</table>

1 Results are means ± SEM, n = 6. * P < 0.05 vs. control (unpaired Student’s t test).
2 Spermine was detectable in the plasma of one rat only.

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**FIGURE 3** Polyamine concentrations in the liver of rats fed a control diet or the same diet containing 10 g oligofructose/100 g. Results are means ± SEM, n = 6.
endogenous synthesis, rather than greater uptakes of polyamines of bacterial origin. Increased activity of ODC, a key enzyme in polyamine synthesis, in the cecal tissue of fructan-fed rats has already been described (Rémesy et al. 1993) and could be linked to the cecal tissue hyperplasia observed in those rats; crypt cell proliferation in the cecum is stimulated by butyrate, as well as by propionate, albeit to a smaller extent (Demigné et al. 1999). In this study, we were unable to show histologically any increase in cell proliferation, even in the deepest zone of the crypts, where butyrate has been shown to promote DNA synthesis (Demigné et al. 1999).

In addition to its effect on the gastrointestinal tract, OFS exerts systemic effects on protein and lipid metabolism. Fructans reduce plasma urea in normal and nephrectomized rats, by enhancing urea N transfer into the large intestine with incorporation of this nitrogen into bacterial protein (Levrat et al. 1993, Younes et al. 1995). The addition of fructans to the diet of male Wistar rats for at least 3 wk has been shown to decrease fatty acid synthesis and esterification in the liver, thus reducing serum and hepatic triglyceride concentration (Delzenne and Kok 1999, Kok et al. 1996). SCFA (mainly propionate) have been proposed as the putative mediators of the "antilipogenic" effect of dietary fructans (Demigné et al. 1999).

In view of the results obtained in this study, we propose that the decrease in spermine and spermidine concentrations in the plasma of the portal vein of OFS-treated rats could also participate in lowering hepatic triglyceride. In fact, spermine was shown to promote fatty acid esterification by increasing phosphatidate phosphohydrolase translocation from the cytosol toward the microsomal fraction. It also stimulates mitochondrial and microsomal glycerol-3-phosphate acyltransferase activities (Bates and Sagerson 1981, Martin-Sanz et al. 1985, Pittner et al. 1986). The influence of polyamines on lipogenesis was shown only in adipose tissue and has never been studied in hepatic cells (Borland et al. 1994).


Martin-Sanz, P., Hopewell, R. & Brindle, D. N. (1985) Spermine promotes the translocation of phosphatidate phosphohydrolase from the cytosol to the microsomal fraction of rat liver and it enhances the effects of oleate in this tissue. J. Lipid Res. 26: 685–689.


