Effects of two fermentable carbohydrates (inulin and resistant starch) and their combination on calcium and magnesium balance in rats

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Resistant starch and inulin are complex carbohydrates that are fermented by the microflora and known to increase colonic absorption of minerals in animals. The fermentation of these substrates in the large bowel to short-chain fatty acids is the main reason for this increase in mineral absorption. The purpose of the present study was to examine the potential synergistic effect of a combination of these two fermentable carbohydrates. For this purpose, thirty-two adult male Wistar rats weighing 200 g were used in the present study. The rats were distributed into four groups, and fed for 21 d a fibre-free basal purified diet or diet containing 100 g inulin, or 150 g resistant starch (raw potato starch)/kg diet or a blend of 50 g inulin and 75 g resistant starch/kg diet. After an adaptation period of 14 d, the rats were then transferred to metabolic cages and dietary intake, faeces and urine were monitored for 5 d. The animals were then anaesthetized and caecal Ca and Mg absorption were measured. Finally, the rats were killed and blood, caecum and tissues were sampled. Ca and Mg levels were assessed in diets, faeces, urine, caecum and plasma by atomic absorption spectrometry. Our results confirmed that inulin and resistant starch ingestion led to considerable caecal fermentation in the three experimental groups compared with the control group diet. Moreover, both carbohydrates significantly increased the intestinal absorption and balance of Ca and Mg, without altering the plasma level of these two minerals. Interestingly, the combination of the studied carbohydrates increased significantly the caecal soluble Ca and Mg concentrations, the apparent intestinal absorption and balance of Ca, and non-significantly the plasma Mg level. In conclusion, a combination of different carbohydrates showed synergistic effects on intestinal Ca absorption and balance in rats. Further studies with other types of carbohydrate combinations should be carried out to extend these findings.

Fermentable carbohydrates: Inulin: Resistant starch: Short-chain fatty acids: Calcium and magnesium: Intestinal absorption: Rat

Many reports have indicated that Ca can be significantly absorbed and Mg is mainly absorbed from the large intestine, namely the caecum and the colon (Ebel & Gunther, 1980; Allen, 1982; Hardwick et al. 1990; Brink & Beynen, 1992; Kayne & Lee, 1993). Fermentable carbohydrates reach the large intestine to be used by the local microflora. They may stimulate bifidobacteria and lactobacilli growth in the intestine, which has been proposed to be connected with health-promoting functions (Gibson et al. 1995; Kruse et al. 1999). Many other beneficial health effects for fermentable carbohydrates have been already reported, concerning diabetes and lipid metabolism and cancer prevention (de Deckere et al. 1993; Younes et al. 1995; Jackson et al. 1999). Several investigations have demonstrated that rats fed fermentable carbohydrates absorbed more Ca and Mg than control rats, despite an increase in total faecal mass (Ohta et al. 1994, 1995, 1996; Delzenne et al. 1995).

Carbohydrate fermentation can influence the intestinal absorption of Ca and Mg in many ways. The short-chain fatty acid (SCFA), fermentation products, are responsible for decreasing caecal content pH, which in its turn increases mineral solubility to improve mineral absorption. The SCFA can also directly influence mineral absorption by

Abbreviations: RS, resistant starch; SCFA, short-chain fatty acids.
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complexing with the minerals leading to an increase in their absorption. The caecal enlargement can also increase the surface area exchange to improve mineral absorption (Demigne et al. 1989; Rémyés et al. 1993; Younes et al. 1993). These different aspects are closely linked to the nature of studied fermentable carbohydrates and to mineral concentrations (Ohta et al. 1994, 1995, 1996; Younes et al. 1996). The enhancing effect of inulin on Ca and Mg absorption has been investigated several times in animals (Levrat et al. 1991a; Delzenne et al. 1995) and human subjects (Coudray et al. 1997a; Coudray & Fairweather-Tait, 1998; Van den Heuvel et al. 1998). Resistant starches (RS) have been examined in animals and also showed enhancing effects on Ca and Mg absorption (Rayssiguier & Rémyés, 1977; Moraes et al. 1996; Younes et al. 1996; Lopez et al. 1998). Foods are a complex mixture containing different relative molecular masses and concentrations of fermentable carbohydrates. The complexity and the variety of these later components may allow maintenance of high fermentation activity throughout the large intestine, which can increase their beneficial effects on mineral absorption. Only a few studies have tried to determine the combined effects of different fermentable carbohydrates on SCFA production and hence mineral absorption (Topping et al. 1985; Campbell et al. 1997).

The aim of the present study was to investigate the potential synergistic effect of a blend of two different fermentable carbohydrates on fermentation variables and on the caecal as well as the apparent intestinal absorption of Ca and Mg and on their plasma level in rats.

**Materials and methods**

**Animals and diets**

Thirty-two male Wistar rats (derived from the colony of laboratory animals of the National Institute of Agronomic Research, Clermont-Ferrand/Theix, France) were fed a commercial pellet diet (U.A.R., Villemonoisson s/Orge, France) until body weights reached about 200 g (8 weeks). Groups of eight rats were fed for 21 d a basal (fibre-free) purified diet, or diets containing 100 g inulin/kg, 150 g RS/kg or a blend of 50 g inulin and 75 g RS/kg (Table 1). RS was a crude potato starch supplied by Louis François, St-Maur, France. It is worth noting that up to 750 g/kg starch was RS (Andrieux et al. 1989). Inulin (or chicory inulin) was supplied by ORAFTI, Tienen, Belgium. The degree of polymerization of this inulin ranges from 3 to 50, with an average of 9. Inulin contains 1000 g indigestible carbohydrate/kg while crude potato starch contains only 750 g fermentable carbohydrates/kg. That is why we have chosen an inulin:crude potato starch ratio of 1:1.5 to obtain similar levels of fermentations, and to compare then, the effect of their combination. Diet Ca and Mg levels were about 7500 and 900 mg/kg respectively. The rats were provided with fresh food and distilled water daily; these were available ad libitum. During the period of adaptation (14 d), the rats were housed two per cage (wire-bottomed to limit coprophagy) and maintained in a temperature-controlled room (22°C) with the dark period from 20.00 hours to 08.00 hours. Following the adaptation phase, rats were individually housed for additional 7 d in metabolic cages fitted with urine–faeces separators to collect faeces and urine (experimental phase). Food consumption and body weight were recorded twice per week during the adaptation phase, then daily during the 7 d experimental phase. Urine (acidified by HCl, final 5 mmol/l) and faeces were collected during the last 5 d of the experiment for determination of mineral balance. The animals were maintained and handled according to the recommendations of the Institutional Ethic Committee of the University of Clermont-Ferrand, France.

**Sampling procedures**

The rats were killed just after the dark period (between 08.00 and 10.00 hours), because caecal fermentation was still very active. After anaesthesia (40 mg sodium pentobarbital/kg), the rats were maintained on a warming plate at 37°C. The procedure of blood sampling for the measurement of arteriovenous difference across the caecum has been described previously (Demigne & Rémyés, 1985). For blood flow measurement, bromosulphthalain in saline

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**Table 1.** Composition of experimental diets (g/kg)

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Fibre-free</th>
<th>Inulin</th>
<th>Resistant starch</th>
<th>Inulin+resistant starch</th>
</tr>
</thead>
<tbody>
<tr>
<td>Casein</td>
<td>160</td>
<td>160</td>
<td>160</td>
<td>160</td>
</tr>
<tr>
<td>Wheat starch*</td>
<td>720</td>
<td>620</td>
<td>570</td>
<td>595</td>
</tr>
<tr>
<td>Inulin</td>
<td>0</td>
<td>100</td>
<td>0</td>
<td>50</td>
</tr>
<tr>
<td>Crude potato starch</td>
<td>0</td>
<td>0</td>
<td>150</td>
<td>75</td>
</tr>
<tr>
<td>Corn oil</td>
<td>50</td>
<td>50</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>AIN salt mix†§</td>
<td>60</td>
<td>60</td>
<td>60</td>
<td>60</td>
</tr>
<tr>
<td>AIN vitamin mix‡§</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
</tbody>
</table>

* Wheat starch was extensively purified and contains 0 g of resistant starch or NSP/kg.
† AIN salt mixture (g/kg): calcium phosphate dibasic 500, sodium chloride 74, potassium citrate monohydrate 220, potassium sulfate 52, magnesium oxide 24, manganese carbonate (430–480 g Mn/kg) 3.5, ferric sulfate (160–170 g Fe/kg) 6, zinc carbonate (700 g Zn/kg) 1.6, copper carbonate (530–550 g Cu/kg) 0.3, potassium iodate 0.1, sodium selenite 0.1, chromium potassium sulfate 0.55, sucrose, finely powdered 118.
‡ AIN vitamin mixture (mg/kg): thiamine hydrochloride 600, riboflavin 600, pyridoxine hydrochloride 700, nicotinic acid 3000, d-calcium pantothenate 1600, folic acid 200, biotin 20, cyanocobalamin (vitamin B12) 1, retinyl palmitate (vitamin A) pre-mix 1600, dl-α-tocopheryl acetate 20000, cholecalciferol (vitamin D3) 250, menaquinone (vitamin K2) 50, sucrose, finely powdered 972.9 g.
§ Mineral and vitamin mix were purchased from UAR (Villemonoisson, Epinay-sur-Orge, France).
range comparisons by Fisher’s least-significant difference procedures. Values of $P < 0.05$ were considered significant.

**Results**

**Food intake, body weight and caecal fermentation variables**

Neither the daily food intake nor the body-weight gain were significantly different between the experimental groups. As expected, the presence of fermentable carbohydrates in the diets resulted in an enlargement of the caecum and lowered caecal pH compared with the control group (Table 2). The results of the rats fed the combination of the carbohydrates were statistically similar to those of the rats fed the carbohydrates separately. With regard to the SCFA, fermentable carbohydrate ingestion increased significantly ($P < 0.05$) the molar concentration of SCFA in the caecum, particularly in the RS and inulin + RS groups (Table 3). The effect of fermentable carbohydrates on the production of SCFA became more marked when the results were expressed as the amount of SCFA per caecum, RS group exhibiting the greatest produced amount of SCFA. The profile of SCFA was changed in the caecum in the presence of fermentable carbohydrates. Thus, the molar proportions of propionate and butyrate were raised at the expense of acetate in rats fed inulin or RS respectively. In rats fed the combination of fermentable carbohydrates, both propionate and butyrate molar proportions were increased (Table 3).

**Caecal absorption of calcium and magnesium**

Table 4 shows that caecal fermentation of the tested carbohydrates increased significantly the solubility of Ca and Mg (4-fold for Ca and 2-fold for Mg) in the caecum. This has as consequence of increasing the bioavailability of these two minerals. Moreover, rats fed fermentable carbohydrates had a higher caecal blood flow than the control group. Consequently, the caecal Ca and Mg absorption increased significantly in the rats fed fermentable carbohydrates. The rats fed the blend of both fermentable carbohydrates had a higher soluble caecal concentration of both Ca and Mg accompanied by a higher caecal Mg absorption compared with the rats fed with either of the studied fermentable carbohydrates separately (Table 4). However, the difference in the caecal Mg absorption was not statistically significant.

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**Table 2. Effects of dietary conditions on daily food intake, daily weight gain and variables of caecal development**

<table>
<thead>
<tr>
<th>Diets</th>
<th>Daily food intake (g)</th>
<th>Daily weight gain (g)</th>
<th>Caecum weight (g)</th>
<th>Wall caecum weight (g)</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SE</td>
<td>Mean</td>
<td>SE</td>
<td>Mean</td>
</tr>
<tr>
<td>Fibre-free</td>
<td>22.2a</td>
<td>1.2</td>
<td>6.06a</td>
<td>0.44</td>
<td>2.54b</td>
</tr>
<tr>
<td>Inulin</td>
<td>21.0a</td>
<td>0.9</td>
<td>5.58a</td>
<td>0.36</td>
<td>5.39b</td>
</tr>
<tr>
<td>Resistant starch</td>
<td>22.5a</td>
<td>0.9</td>
<td>6.03a</td>
<td>0.57</td>
<td>6.03b</td>
</tr>
<tr>
<td>Inulin+resistant starch</td>
<td>22.2a</td>
<td>1.0</td>
<td>5.50a</td>
<td>0.32</td>
<td>5.12b</td>
</tr>
</tbody>
</table>

*a,bMean values within a column with unlike superscript letters were significantly different (P < 0.05).  
* For details of diets and procedures, see Table 1 and p. 480.

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**Analytical procedures**

SCFA were measured by GLC on aliquots of supernatant fractions of caecal contents as previously described (Demigné et al. 1980). Ca and Mg were determined on the plasma, caecal supernatant fractions (soluble), urine, and, after mineralization (0.8 M–HCl, 12 h at 800°C), on the untreated caecal samples (total) and faecal materials. After an adequate dilution, mineral concentrations were measured by atomic absorption spectrophotometry (Perkin-Elmer 400; Perkin-Elmer, Norwalk, CT, USA) at wavelengths of 422 nm (Ca) and 285 nm (Mg).

**Calculation and data analysis**

The entire caecal content was calculated as: caecal concentration ($\mu$mol/ml) × caecal water (ml); and caecal absorption as (caecal vein)−(artery) difference ($\mu$mol/ml plasma) × caecal plasma flow (ml/min). For the determination of digestive balance, food and faeces samples of each rat collected during 5 d were homogenized before mineral analysis. Intake of Ca via the drinking water was considered to be negligible (<0.5 mg/l) compared with that from the diet and was not considered.

Values are given as values with their standard errors and, where appropriate, significance of differences between mean values was determined by ANOVA and multiple mean values was determined by ANOVA and multiple
As shown in Table 5, the daily intake of Ca was similar, whereas daily faecal excretion of Ca was significantly different between the four studied groups. This is because intestinal absorption of Ca (mg/d) was significantly increased in the groups fed the fermentable carbohydrates compared with the control group. Moreover, this increase was significantly higher in the group fed the blend of both fermentable carbohydrates than that observed in rats fed one of the studied fermentable carbohydrates, separately (Table 5). Urinary Ca excretion was also increased in the
Table 6. Effects of dietary fibre conditions on the plasma content of calcium and magnesium*
(Mean values with their standard errors for eight rats per group)

<table>
<thead>
<tr>
<th>Diets</th>
<th>Plasma Ca (mm)</th>
<th>Plasma Mg (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SE</td>
</tr>
<tr>
<td>Fibre-free</td>
<td>2.77a</td>
<td>0.05</td>
</tr>
<tr>
<td>Inulin</td>
<td>2.68a</td>
<td>0.04</td>
</tr>
<tr>
<td>Resistant starch</td>
<td>2.83b</td>
<td>0.07</td>
</tr>
<tr>
<td>Inulin+resistant starch</td>
<td>2.92b</td>
<td>0.08</td>
</tr>
</tbody>
</table>

*Mean values within a column with unlike superscript letters were significantly different (P < 0.05).
* For details of diets and procedures, see Table 1 and p. 480.

Discussion

It has often been reported that vegetable products, rich in indigestible dietary carbohydrates, may affect the bioavailability of minerals. Impairment of Ca and Mg absorption by dietary fibre is traditionally ascribed to the phytic acid content or to the uronic acid content of fibre fractions (McCance & Widdowson, 1942; Reinhold et al. 1976). However, when fermentable carbohydrates like RS and inulin were ingested, there was no decrease, but an increase in Ca and Mg absorption. The beneficial effects of fermentable carbohydrates on mineral absorption in rats were reported for the first time in our laboratory in 1977 (Rayssiguier & Rémesy, 1977). This beneficial effect is now well documented with different fermentable carbohydrates in many nutritional conditions in animals (Demigné et al. 1980; Ohta et al. 1994, 1995, 1996; Delzenne et al. 1995; Campbell et al. 1997; Yanahira et al. 1997) and in some studies with human subjects (Van den Heuvel et al. 1999; Coudray et al. 1997a, Tahiri et al. 2001). However, such effects are dependent on the type of fermentable carbohydrates (dose, structure and relative molecular mass) and on the duration of the experiment (Lajvardi et al. 1993; Campbell et al. 1997). The aim of the present study was to investigate the potential synergistic effect of a blend of two different fermentable carbohydrates on the fermentation variables and on the caecal and the apparent intestinal absorption of Ca and Mg in rats. Our results showed clearly that a combination of different carbohydrates had synergistic effects on intestinal Ca and Mg absorption and on plasma Mg level in rats.

The caecum of the rat is the main site of degradation of fermentable carbohydrates. As expected, our present results, obtained in a relatively short-term experiment lasting 3 weeks, indicate that both inulin and RS are extensively fermented in the rat caecum leading to significant changes in caecum content and wall weights, SCFA production and caecal lumen pH. The ingestion of fermentable carbohydrates induced caecal development and caecal weight rise was at least 2-fold greater than in the control group, resulting in a greater exchange surface area in the caecum. This development of the caecal wall is due to a combination effect of both hypertrophy and hyperplasia (Rémesy et al. 1993; Levrat et al. 1991a). Caecal SCFA concentration was the most important in the group fed the blend of inulin and RS, which may reflect higher activity of fermentation in these rats than in those received one of the studied fermentable carbohydrates singly. Similar results have been reported by Topping et al. 1985. They observed that concentrations of SCFA in caecal fluid did not differ between rats fed gum arabic or cellulose, but were raised in rats fed the mixture. SCFA play an important role in the enhancement of caecal mineral absorption (Scharrer & Lutz, 1990, 1992; Trinidad et al. 1999). The acidic caecal pH resulting from ingestion of the fermentable carbohydrate diets is caused by the greater level of total SCFA production (Younes et al. 1996; Le Blay et al. 1999). This reduction in caecal pH leads to greater solubilization of Mg and Ca so that the biologically available concentration of these minerals is increased (Lutz et al. 1991; Rémesy et al. 1993). Indeed, it was shown that the reduction in the ileal pH was inversely related to apparent absorption of Mg (Heijnen et al. 1993). Another possible hypothesis is that SCFA contribute directly to the enhancement of Mg and Ca absorption via a cation exchange mechanism (Trinidad et al. 1996, 1999). SCFA in a protonated form are absorbed across the apical membrane and undergo dissociation within the intracellular environment. The increased intracellular H⁺ are then secreted from the cell into the lumen in exchange for Mg²⁺ or one Ca²⁺. SCFA may also directly influence intestinal mineral absorption by forming a lower charge complex with Mg or Ca which can across the cell membrane (Lutz et al. 1991; Trinidad et al. 1996, 1999). Finally, SCFA
may also responsible for the rise in caecal blood flow and may thus increase the overall mineral input, as reported in the colon (Kvietys & Granger, 1981).

Ca and Mg are absorbed from both the small and the large intestine, which includes the caecum. According to our results, the fermentable carbohydrates ingestion resulting in the lowering of lumen pH led to an increase in the soluble mineral fractions in the caecum (4-fold for Ca and 2-fold for Mg). Again, the combination of both studied fermentable carbohydrates was more effective in increasing soluble Ca and Mg concentrations in the caecum than each fermentable carbohydrates fed singly. This is very important, because the solubility of a mineral is a major determinant for its absorption. Moreover, the caecal blood flow was significantly increased in the rats receiving the fermentable carbohydrates compared with the control group. This phenomenon has been already observed (Demigne et al. 1989). Consequently, the process of caecal absorption of Ca and Mg was enhanced in parallel with the dietary intake of fermentable carbohydrates, and large amounts of Ca and Mg were absorbed via the caecum in the rats fed the fermentable carbohydrate diets (3- to 4-fold) compared with the control diet. This is in agreement with the increase in SCFA production and the increase in the higher solubility of Ca and Mg observed in the rats fed the fermentable carbohydrates in the present experiment. The rats fed the blend of fermentable carbohydrates presented the higher Ca and Mg soluble caecal concentration with a high Mg caecal absorption rate, which reflects a potential synergistic effect between the two fermentable carbohydrates.

The results of the present study showed that apparent intestinal absorption of Ca and Mg increased significantly \( (P < 0.05) \) in rats fed the fermentable carbohydrates separately. Interestingly, the increase in apparent intestinal absorption of Ca and Mg was more important in the rats fed with the blend of two studied fermentable carbohydrates, which reached a significant threshold when the Ca absorption was expressed in both mg/d and in percentage terms. This is in agreement with the results of caecal mineral absorption. In the present study, feeding rats with fermentable carbohydrates had a comparable effect on the intestinal absorption of Ca and Mg. Thus, increasing divalent cation absorption in the large intestine did not seem to be accompanied by a lower absorption of these cations in the small intestine under the conditions of the present study.

The improvement of apparent intestinal Ca absorption by the fermentable carbohydrates singly and in combination was not accompanied by significant modifications in plasma Ca levels. Such results are in accordance with previous observations (Levrat et al. 1991a; Delzenne et al. 1995; Lopez et al. 1998, 2000). Moreover, the increase in apparent intestinal Mg absorption by the fermentable carbohydrates, fed singly, was not accompanied by significant modifications in plasma Mg levels, but fermentable carbohydrates in combination led to significantly \( (P < 0.05) \) higher plasma Mg levels than in the control or the inulin groups. Indeed, Ca blood homeostasis is efficiently controlled, whereas that of Mg is less tightly controlled. In keeping with this, many reports in the literature indicate that a high intake of dietary Mg or an improvement in intestinal Mg absorption are generally accompanied by an increase in plasma Mg levels (Rayssiguier & Rémésy, 1977; Navas & Cordova, 1996; Coudray et al. 1997b).

In conclusion, the feeding of inulin or RS significantly \( (P < 0.05) \) increased the apparent intestinal absorption of Ca and Mg in rats without significant change in their plasma levels. Interestingly, the combination of these different fermentable carbohydrates showed significant synergistic effects on intestinal Ca absorption and on plasma Mg levels in rats. These results are relevant because foods are very complex and may contain many types of fermentable carbohydrates in each meal. The non-purified vegetable products have the double interest of being both rich in minerals and an important source of fermentable carbohydrates which can enhance the intestinal absorption of these minerals. Further studies with other types of fermentable carbohydrates deserve to be carried out to extend these findings.

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