Dietary galactooligosaccharides increase calcium absorption in normal and gastrectomized rats

**Influência da dieta com galactooligossacarídeos sobre a absorção de cálcio em ratos normais e gastrectomizados**

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**ABSTRACT**

**Objective:** To determine whether the galactooligosaccharide stimulates calcium absorption in partially gastrectomized rats. 

**Methods:** Animals were submitted to laparotomy (sham-operated control) and partial gastrectomy (Billroth II) in groups of 20. They were fed a control diet (AIN-93M) without galacto-oligosaccharide or a diet containing (galacto-oligosaccharide – 50g/kg diet) for eight weeks. The animals were divided into four groups: sham-operated and non-gastrectomized with galactooligosaccharide, sham-operated and gastrectomized without galactooligosaccharide. On the final day of the study, whole blood was collected for determination of serum calcium concentration. 

**Results:** In the group with galactooligosaccharides calcium excretion in feces was significantly lower than the group without prebiotics. The apparent calcium absorption in gastrectomized and normal rats was higher in groups fed with galactooligosaccharide than in the control diet group. 

**Conclusion:** The ingestion of galactooligosaccharides prevents osteopenia in partially gastrectomized rats.

Key words: Prebiotics. Oligosaccharides. Gastrectomy. Calcium. Rats

**INTRODUCTION**

The stomach is important for calcium absorption, since pepsin and hydrochloric acid act together to produce soluble calcium from food insoluble phosphate complexes. Very extensive gastric resections are known to cause bone disorders (osteoporosis/osteomalacia), possibly related to impaired capacity to incorporate dietary calcium. Hypochlorhydria due to gastric resection may impair the absorption of insoluble calcium because the acid is the most important factor to the solubilization of insoluble calcium salts.

Several reports suggest that indigestible carbohydrates such as fructo-oligosaccharides, inulin, hydrolyzed guar gum with polydextrose and galacto-oligosaccharides stimulate and increase calcium absorption in rats and mineral absorption in the intestine. This effect comprehends the production of short chain fatty acids (SCFA) resulting from fermentation in the large intestine, which stimulate the proliferation of intestinal epithelial cells and reduce luminal pH.

Thus, this study aimed to determine whether the ingestion of galacto-oligosaccharide stimulates calcium absorption in partially gastrectomized rats.

**METHODS**

**Animals and surgical procedure**

Forty male Wistar rats of 250.0 ± 5 g body weight were kept in collective cages in a room with controlled temperature (22 ± 1 °C), humidity (60-70%), a 12-hour light / dark cycles (lights on at 07 : 00), with diet and deionized water ad libitum. The animals were randomly assigned to two groups of 20 animals each. Twenty underwent anterior truncal vagotomy with partial gastrectomy (Billroth II). The sham-operated (20 animals) underwent the same surgical stress, where the abdominal cavity was kept open for 45 minutes, the time length of gastrectomy. The rats were anesthetized with thiopenital (25 mg/kg, intravenously). The experimental protocol was approved by the Ethics Committee for Animal Research conducted in the Post-Graduate Department of Food and Nutrition (DEPAN), Faculty of Food Engineering (FEA) and the Department of Surgery, Faculty of Medical Sciences (FCM), Campinas State University – UNICAMP, Campinas - São Paulo, Brazil.
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Experimentation (EAEC), Campinas State University – UNICAMP (number of record. 839-1, 06/08/2005).

Experimental groups and diets
After 15 days of the procedure, the rats were randomly divided into four experimental subgroups (gastrectomy versus sham-operated, control diet versus polydextrose) and fed specific diets for eight weeks. Three animals died during the experiment without apparent cause.

The control and experimental diet were prepared according to AIN 93M formulation. The galacto-oligosaccharide (50% to 55% of oligosaccharides, manufactured in Japan, Yakult® Industry and Commerce, São Paulo, SP, Brazil) was added to 50 g/kg diet for substitution of the saccharose of the control diet.

Table 1 shows the composition of two experimental diets (control and galacto-oligosaccharide). Half of the gastrectomized and sham-operated rats were fed the control diet, and the other half with dietary galactooligosaccharide.

The animals had free access to deionized water throughout the observation period. For prevention of megaloblastic anemia, the rats were given supplements of vitamin B12 (intramuscular Cyanocobalamin 0.5 mg/kg; 5000 Cianotrat® - Delta Therapy Institute Ltd., Indaiatuba, São Paulo, Brazil) every two weeks, starting one week after the operation. The sham group received sodium chloride 0.9%.

Then the animals were placed in individual metabolic cages for three days at 15, 35 and 55 days of the experiment, to collection of feces.

Weight gain and dietary intake of the animals were monitored three times a week for eight weeks.

Analytical methods
Calcium levels were determined by a commercially available colorimetric method (Laborlab, Guarulhos, SP, Brazil).

The obtained dried feces were weighed and grinded. Diets and powdered dried feces were calcinated at temperatures increased linearly up to 550° C for 6 h and then at 550° C for 18 h in the muffle (Fornitec Industry and Trade Ltd.®, São Paulo, Brazil). The samples were digested with 65% HNO₃ and H₂O₂ in Hostafion closed tubes under pressure heated in the microwave (DGT-100 Plus®, Jundiaí, São Paulo, Brazil). Calcium measurements in the feces and diet were carried out by optical emission-IRIS AP (Thermo Jarrell Ash®, Franklin – Massachusetts – USA) in the specialized Biomineral Chemical Analysis Laboratory, Campinas, São Paulo, Brazil and the calculations were: apparent calcium absorption (mg/day) = calcium intake (mg/day) - calcium excretion in the feces (mg/day). The femur was removed and freed from the muscles after the death of the animals and frozen. The bone thus obtained was burned in an oven at 600° C to obtain clear ash. Measurements of bone calcium were performed by optical emission spectrometer IRIS-AP also in the Biomineral Chemical Analysis Laboratory.

Table 1 - Composition of experimental diets.

<table>
<thead>
<tr>
<th>Ingredients, g/Kg</th>
<th>Control Diet</th>
<th>GOS Diet¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn Starch ²</td>
<td>466</td>
<td>466</td>
</tr>
<tr>
<td>Maltodextrin ²</td>
<td>155</td>
<td>155</td>
</tr>
<tr>
<td>Casein ³</td>
<td>140</td>
<td>140</td>
</tr>
<tr>
<td>Soy Oil</td>
<td>40</td>
<td>40</td>
</tr>
<tr>
<td>Cellulose ⁵</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Saccharose ⁶</td>
<td>100</td>
<td>50</td>
</tr>
<tr>
<td>GOS ¹</td>
<td>0</td>
<td>50</td>
</tr>
<tr>
<td>Mineral Mixture ⁷</td>
<td>35</td>
<td>35</td>
</tr>
<tr>
<td>Vitamin Mixture ⁷</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>L-Cystine ⁸</td>
<td>1.8</td>
<td>1.8</td>
</tr>
<tr>
<td>Choline Bitartrate ⁹</td>
<td>2.5</td>
<td>2.5</td>
</tr>
<tr>
<td>Tert-butyl hydroquinone ¹⁰</td>
<td>0.008</td>
<td>0.008</td>
</tr>
</tbody>
</table>

¹Galacto-oligosaccharide (GOS 50 g/kg diet) (50-55% oligosaccharide), manufactured in Japan, Yakult Industry and Commerce, São Paulo, São Paulo, Brazil.
²Maltodextrin and corn starch (Corn Products Brazil - Ingredients Industry Ltda, Mogi Guaçu, São Paulo, Brazil).
³Plury Química Ltda, Diadema, São Paulo, manufactured by Naarden Agro Products-Holland.
⁴Liza Brand, Cargill Brazil, Uberlandia, Minas Gerais, Brazil.
⁵Microcel Brand, Blanver Farmoquímica Ltd., Cotia, São Paulo, Brazil.
⁶Union Refinery, Assis, São Paulo, Brazil.
⁷Prepared according to the AIN-93M formulation by M. Cassab Comércio e Indústria Ltda, São Paulo, São Paulo, Brazil.
⁸Synth Brand C1027.01.AE, Diadema, São Paulo, Brazil.
⁹Sigma Chemical Co., St. Louis, Mo., USA.
Statistical analysis
The results were submitted to analysis of variance (ANOVA) using the Duncan test for comparison of means. Data were analyzed in two (treatment and diet) and three ways (treatment, diet and time). Differences were considered significant when p < 0.05. Data are expressed as mean and standard error of the mean (SEM – Statistica® 6.0 for Windows).

RESULTS

Weight gain and food intake
The initial body weight in sham-operated animals was significantly higher than in the gastrectomized rats (P < 0.05). Final weights in both groups of gastrectomized rats were significantly lower than those in sham-operated rats (P < 0.05). On the other hand, the total body weight gain did not differ significantly between gastrectomized and sham groups (P > 0.05). The food intake of gastrectomized rats was significantly lower when compared to sham-operated rats (P < 0.05) (Table 2).

Wet weight and dry stool
In sham-operated and gastrectomized rats the average wet and dry weight of feces was significantly higher in rats fed with galacto-oligosaccharide diet than in rats that received the control diet (P < 0.05) (Table 3).

Serum calcium concentration
Serum calcium in gastrectomized rats that received control diet was significantly lower when compared to the other three groups (P < 0.05) (Figure 1).

Fecal calcium and apparent calcium absorption
The concentration of calcium in the feces of sham-operated and gastrectomized rats that received diets with galacto-oligosaccharide was lower (P < 0.05) when compared to the control diet (Figure 2). The apparent calcium absorption in sham-operated rats that received diets with galacto-oligosaccharide was higher when compared to animals fed with the control diet (Figure 3). The apparent calcium absorption in gastrectomized rats that received the diet with galacto-oligosaccharide was also significantly higher when compared to those receiving the control diet, not differing, however, from the sham-operated group with control diet (P < 0.05).

Concentration of bone calcium
Calcium concentration in the femur of sham-operated rats with control diet was significantly lower when compared to the other three groups (P < 0.05).

DISCUSSION

This study confirms observations of lower food intake (Table 2) in gastrectomy rats. This reduction in food intake may be associated with the smaller size of the stomach and was observed in another study.

Serum calcium was significantly lower in animals with gastric resection; however, when they were fed with galacto-oligosaccharide there was significant increase in serum calcium concentration (Figure 1). Decreased serum calcium in gastrectomized rats suggests that gastrectomy reduces the absorption of calcium, results previously reported by other authors.

The authors who have studied the issue emphasize that this is a process similar to the one of humans, where gastrectomy induces progressive

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Table 2 - Initial body weight, final body weight and food consumption.

<table>
<thead>
<tr>
<th>Treatment/Diets</th>
<th>Initial Weight (g)</th>
<th>Final Weight (g)</th>
<th>Food Ingestion (g/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (n = 10)</td>
<td>259.30 +/- 6.51 a</td>
<td>411.40 +/- 6.23 a</td>
<td>27.00 +/- 3.01 a</td>
</tr>
<tr>
<td>GOS (n = 10)</td>
<td>264.50 +/- 3.43 a</td>
<td>421.60 +/- 7.66 a</td>
<td>25.84 +/- 2.48 a</td>
</tr>
<tr>
<td>Gastrectomy</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (n = 8)</td>
<td>242.00 +/- 3.63 b</td>
<td>375.00 +/- 9.94 b</td>
<td>21.02 +/- 1.10 b</td>
</tr>
<tr>
<td>GOS (n = 9)</td>
<td>242.00 +/- 3.63 b</td>
<td>387.00 +/- 8.70 b</td>
<td>23.45 +/- 1.10 b</td>
</tr>
</tbody>
</table>

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Table 3 - Wet and dry weight of feces (g).

<table>
<thead>
<tr>
<th>Groups/Treatments</th>
<th>Weight of Feces</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Moist Weight (g)</td>
<td>Dry Weight (g)</td>
<td></td>
</tr>
<tr>
<td>Sham</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (n=10)</td>
<td>4.62 ± 0.22 b</td>
<td>4.12 ± 0.17 b</td>
<td></td>
</tr>
<tr>
<td>GOS (n=10)</td>
<td>5.68 ± 0.44 a</td>
<td>4.86 ± 0.33 a</td>
<td></td>
</tr>
<tr>
<td>Gastrectomy</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (n=8)</td>
<td>5.42 ± 0.46 b</td>
<td>4.73 ± 0.35 b</td>
<td></td>
</tr>
<tr>
<td>GOS (n=9)</td>
<td>6.38 ± 0.31 a</td>
<td>5.63 ± 0.24 a</td>
<td></td>
</tr>
</tbody>
</table>

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deficiency of calcium absorption. They also suggest that the mucous lining of the stomach may have a calcitropic agent, the gastrocalcin, which stimulates the absorption of calcium from bone. In humans undergoing partial and total gastrectomy, there is a decrease in serum calcium, suggesting that this is due to reduced release of calcium from food and increased intestinal flow due to the removal of the parts of the duodenum and jejunum. The sum of these factors causes a decrease in serum calcium.

Thus, it is shown that partial gastrectomy surprisingly reduces the apparent calcium absorption (Figure 3). The galacto-oligosaccharide supplementation decreased calcium excretion in the feces (Figure 2) and fully restored calcium absorption in normal (sham-operated) and gastrectomized rats (Figure 3).

The results suggest that this experimental protocol actually causes depletion of the mineral calcium absorption. Other studies also confirmed that total gastrectomy also reduces the absorption of calcium in rats. The galacto-oligosaccharide supplementation increased the apparent calcium absorption in rats fed with a normal diet without calcium. Data from this study suggest that the stimulatory effect of galacto-oligosaccharides on calcium absorption may be partly associated with increased solubility of calcium and the liquid contents into the cecal lumen and also that both the large and small intestines are possibly responsible for the increased absorption of calcium.

The galacto-oligosaccharide diet increased the concentration of bone calcium in sham-operated rats, but did not alter the concentration of bone calcium in partially gastrectomized rats. The bone is the main calcium storage site of the body and the results of this study showed that eight weeks of the experiment may not be enough to cause a relevant reduction in bone calcium after partial gastric resection. In a previous study there was decreased bone mineralization, however with total gastrectomy. After gastric resection the calcium bone content is reduced and becomes more pronounced as time passes.

Finally, one can conclude that feeding with galacto-oligosaccharide increases calcium absorption, preventing osteopenia after gastrectomy in rats, which may be relevant to decrease the risk of osteoporosis. Partial gastrectomy did not affect the concentration of bone calcium in 56 days (eight weeks) of experimental period. By using a model of partial gastrectomy for studies of bone alterations, we suggest a period longer than eight weeks.

Acknowledgements

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RESUMO

Objetivo: Verificar se o galactooligossacarídeo estimula a absorção de cálcio em ratos Wistar parcialmente gastrectomizados.

Métodos: Os animais foram laparotomizados (controle sham-operado) e parcialmente gastrectomizados (Billroth II) em grupos de 20 e 20 cada, respectivamente. Eles foram alimentados com uma dieta controle (AIN-93M), sem galactooligossacarídeo ou uma dieta contendo (galactooligossacarídeo - 50g/kg de dieta) durante oito semanas. Os animais foram divididos em quatro subgrupos: sham-operados e não gastrectomizados com galactooligossacarídeo, sham-operados e gastrectomizados sem galactooligossacarídeo.

No dia final do estudo, o sangue total foi coletado para determinação da concentração de cálcio sérico. Resultados: Na dieta no grupo com galactooligossacarídeo a excreção do cálcio nas fezes foi significativamente menor do que no grupo sem prebióticos. A absorção aparente de cálcio em ratos gastrectomizados e normais foi maior nos grupos alimentados com galactooligossacarídeo do que no grupo com dieta controle. Conclusão: A dieta com galactooligossacarídeos previne a osteopenia em ratos parcialmente gastrectomizados.


REFERENCES


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