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

ELISVÂNIA FREITAS DOS SANTOS[1]; KATHIA HITOMI TSUBOI[1]; MARINA RACHEL ARAÚJO[1]; NELSON ADAMI ANDREOLLO, TBC-SP[2]; CELIO KENJI MIYASAKA[3]

A B S T R A C T

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 absorptionin 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[1].

Very extensive gastric resections are known to cause bone disorders (osteoporosis/osteomalacia)[2,3], possibly related to impaired capacity to incorporate dietary calcium[1,4]. 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[4].

Several reports suggest that indigestible carbohydrates such as fructo-oligosaccharides[5,6], inulin[7], hydrolyzed guar gum[8] with polydextrose[4] and galacto-oligosaccharides stimulate and increase calcium absorption in rats and mineral absorption in the intestine[9-11]. 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[12].

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 thiopental (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.
1. Laboratory of Enzymology and Experimental Carcinogenesis, Campinas State University (UNICAMP) – Campinas, São Paulo, Brazil; 2. Professor, Department of Surgery, Faculty of Medical Sciences, Campinas State University – UNICAMP, Campinas, São Paulo, Brazil; 3. Assistant Professor, Center for Biological and Health Sciences, Cruzeiro do Sul University, Sao Paulo – SP, Brazil.

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[13]. 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 galacto-oligosaccharide.

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[14]. 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[10].

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% HNO_3 and H_2O_2 in Hostaflon closed tubes under pressure heated in the microwave (DGT-100 Plus® ripe, 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)[10].

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.

Ingredients, g/Kg	Control Diet	GOS Diet[1]
Corn Starch [2]	466	466
Maltodextrin [2]	155	155
Casein [3]	140	140
Soy Oil[4]	40	40
Cellulose [5]	50	50
Saccharose [6]	100	50
GOS [1]	0	50
Mineral Mixture [7]	35	35
Vitamin Mixture [7]	10	10
L-Cystine [8]	1.8	1.8
Choline Bitartrate [9]	2.5	2.5
Tert-butyl hydroquinone[10]	0.008	0.008

[1] Galacto-oligosaccharide (GOS 50 g/kg diet) (50-55% oligosaccharide), manufactured in Japan, Yakult Industry and Commerce, São Paulo, São Paulo, Brazil)
[2] Maltodextrin and corn starch (Corn Products Brazil - Ingredients Industry Ltda, Mogi Guaçu, São Paulo, Brazil).
[3] Plury Química Ltda, Diadema, São Paulo, manufactured by Naarden Agro Products-Holland.
[4] Liza Brand, Cargill Brazil, Uberlandia, Minas Gerais, Brazil.
[5] Microcel Brand, Blanver Farmoquímica Ltd., Cotia, São Paulo, Brazil.
[6] Union Refinery, Assis, São Paulo, Brazil.
[7] Prepared according to the AIN-93M formulation by M. Cassab Comércio e Indústria Ltda, São Paulo, São Paulo, Brazil.
[8] Synth Brand C1027.01.AE, Diadema, São Paulo, Brazil.
[9,10] Sigma 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[6]. This reduction in food intake may be associated with the smaller size of the stomach and was observed in another study[15].

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[1,15].

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

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

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

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

Treatment/Diets	Initial Weight (g)	Final Weight (g)	Food Ingestion (g/day)
Sham			
Control (n = 10)	264.50 +/- 3.43 **a**	421.60 +/- 7.66 **a**	25.84 +/- 2.48 **a**
GOS (n = 10)	259.30 +/- 6.51 **a**	411.40 +/- 6.23 **a**	27.00 +/- 3.01 **a**
Gastrectomy			
Control (n = 8)	221.88 +/- 4.74 **b**	375.00 +/- 9.94 **b**	21.02 +/- 1.10 **b**
GOS (n = 9)	242.00 +/- 3.63 **b**	387.00 +/- 8.70 **b**	23.45 +/- 1.10 **b**

deficiency of calcium absorption. They also suggest that the mucous lining of the stomach may have a calciotropic agent, the gastrocalcin, which stimulates the absorption of calcium from bone[16]. 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[17].

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[4,6]. The galacto-oligosaccharide supplementation increased the apparent calcium absorption in rats fed with a normal diet without calcium[10]. 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[9] and also that both the large and small intestines are possibly responsible for the increased absorption of calcium[4].

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[4]. After gastric resection the calcium bone content is reduced[4,6,18-21] and becomes more pronounced as time passes[20].

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

We thank The National Council of Scientific and Technological Development (CNPq), the Biomineral Chemical Analysis Laboratory Ltd. and Johnson & Johnson® for the surgical sutures.

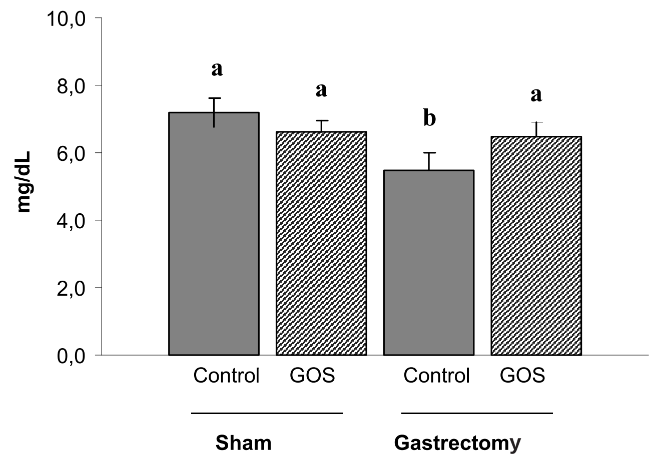

Figure 1 - *Values of serum calcium (mg / dL) in the four subgroups of animals.*

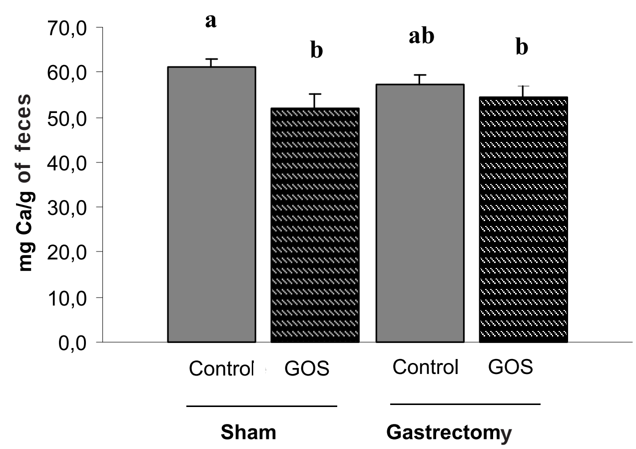

Figure 2 - *Concentration of fecal calcium (mg/g of feces) in the animals of the four subgroups.*

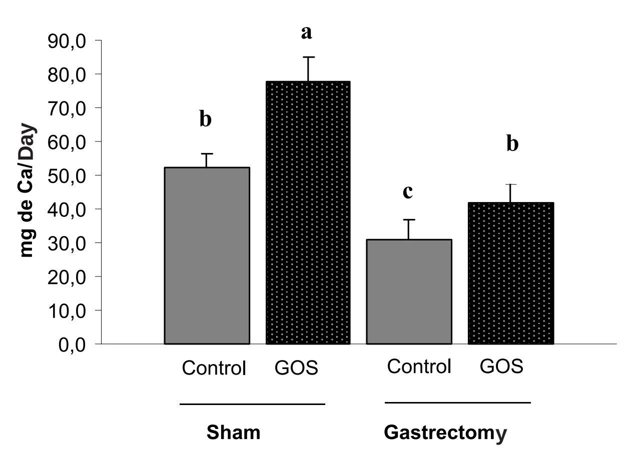

Figure 3 - *Apparent calcium absorption (mg/day) in animals of the four subgroups.*

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 galacto-oligossacarídeo ou uma dieta contendo (galacto-oligossacarídeo - 50g/kg de dieta) durante oito semanas. Os animais foram divididos em quatro subgrupos: sham-operados e não gastrectomizados com galacto-oligossacarídeo, sham-operados e gastrectomizados sem galacto-oligossacarídeo. No dia final do estudo, o sangue total foi coletado para determinação da concentração de cálcio sérico.* ***Resultados:*** *Na dieta no grupo com galacto-oligossacarídeo 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 galacto-oligossacarídeo do que no grupo com dieta controle.* ***Conclusão:*** *A dieta com galacto-oligossacarídeos previne a osteopenia em ratos parcialmente gastrectomizados.*

Descritores: *Prebióticos. Oligossacarídeos. Gastrectomia. Cálcio. Ratos.*

REFERENCES

1. Axelson J, Persson P, Gagnemo-Persson R, Håkanson R. Importance of the stomach in maintaining calcium homeostasis in the rat. Gut 1991; 32(11):1298-302.
2. Koga S, Nishimura O, Iwai N, Kishi K, Takeuchi T, Hinohara T, et al. Clinical evaluation of long-term survival after total gastrectomy. Am J Surg 1979; 138(5):635-9.
3. Nilas L, Christiansen C, Christiansen J. Regulation of Vitamin D and calcium metabolism after gastrectomy. Gut 1985; 26(3):252-7.
4. Hara H, Suzuki T, Aoyama Y. Ingestion of the soluble dietary fibre, polydextrose, increases calcium absorption and bone mineralization in normal and total-gastrectomized rats. Br J Nutr 2000; 84(5):655-61.
5. Ohta A, Ohtsuki M, Baba S, Adachi T, Sakata T, Sakaguchi E. Calcium and magnesium absorption from the colon and rectum are increased in rats fed fructooligosaccharides. J Nutr 1995; 125(9):2417-24.
6. Ohta A, Ohtsuki M, Hosono A, Adachi T, Hara H, Sakata T. Dietary fructooligosaccharides prevent osteopenia after gastrectomy in rats. J Nut 1998; 128(1):106-10.
7. Rémésy C, Levrat MA, Gamet L, Demigné C. Cecal fermentations in rats fed oligosaccharides (inulin) are modulated by dietary calcium level. Am J Physiol 1993; 264(5 Pt 1):G855-62.
8. Hara H, Nagata M, Ohta A, Kasai T. Increases in calcium absorption with ingestion of soluble dietary fiber, guar-gum hydrolysate, depende on the caecum in partially nephrectomized and normal rats. Br J Nutr 1996; 76(5):773-84.
9. Chonan O, Watanuki M. Effect of galactooligosaccharides on calcium absorption in rats. J Nutr Sci Vitaminol 1995; 41(1):95-104.
10. Chonan O, Watanuki M. The effect of 6'-Galactooligosaccharides on bone mineralization of rats adapted to different levels of dietary calcium. Int J Vitam Nutr Res 1996; 66(3):244-9.
11. Chonan O, Takahashi R, Watanuki M. Role of activity of gastrointestinal microflora in absorption of calcium and magnesium in rats fed beta1-4 linked galactooligosaccharides. Biosci Biotechnol Biochem 2001; 65(8):1872-5.
12. Hirama Y, Morohashi T, Sano T, Maki K, Ohta A, Sakai N, Yamada S, Sasa R. Fructooligosaccharides prevent disorders of the femoral neck following gastrectomy in growing rats. J Bone Miner Metab 2003; 21(5):294-8.
13. Reeves PG, Nielsen FH, Fahey GC Jr. AIN-93 purified diets for laboratory rodents: final report of the American Institute of Nutrition ad hoc writing committee on the reformulation of the AIN-76A rodent diet. J Nut 1993; 123(11):1939-51.
14. Shiga K, Nishimukai M, Tomita F, Hara H. Ingestion of difructose anhydride III, a non-digestible disaccharide, prevents gastrectomy-induced iron malabsorption and anemia in rats. Nutrition 2006; 22(7-8):786-93.
15. Sakai K, Aramaki K, Takasaki M, Inaba H, Tokunaga T, Ohta A. Effect of dietary short-chain fructooligosaccharides on the cecal microflora in gastrectomized rats. Biosci Biotechnol Biochem 2001; 65(2):264-9.
16. Sakai K, Ohta A, Shiga K, Takasaki M, Tokunaga T, Hara H. The cecum and dietary short-chain fructooligosaccharides are involved in preventing postgastrectomy anemia in rats. J Nut 2000; 130(6):1608-12.
17. Zittel TT, Zeeb B, Maier GW, Kaiser GW, Zwirner M, Liebich H, et al. High prevalence of bone disorders after gastrectomy. Am J Surg 1997; 174(4):431-8.
18. Santos EF, Tsuboi KH, Palu BF, Araujo MR, Andreollo NA, Miyasaka CK. Partial gastrectomy associated to anterior truncal vagotomy: alterations in metabolism of the calcium. Experimental study in rats. ABCD Arq Bras Cir Dig 2009; 22(2):105-9.
19. Santos EF, Tsuboi KH, Araújo MR, Ouwehand AC, Andreollo NA, Miyasaka CK. Dietary polydextrose increases calcium absorption in normal rats. ABCD Arq Bras Cir Dig 2009; 22(4):201-5.
20. De Prisco C, Levine SN. Metabolic bone disease after gastric bypass surgery for obesity. Am J Med Sci 2005; 329(2):57-61.
21. Schölmerich J. Postgastrectomy syndromes—diagnosis and treatment. Best Pract Res Clin Gastroenterol 2004; 18(5):917-33.

Received 29/04/2010
Accepted for publication 01/07/2010
Conflict of interest: none
Source of funding: none

How to cite this article:
Santos EF, Tsuboi KH, Araújo MR, Andreollo NA, Miyasaka CK. Dietary galactooligosaccharides increase calcium absorption in normal and gastrectomized rats. Rev Col Bras Cir. [periódico na Internet] 2011; 38(3). Disponível em URL: http://www.scielo.br/rcbc

Correspondence to:
Elisvânia Freitas dos Santos.
E-mail: elisvania@gmail.com