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EFFECTS OF INULIN-TYPE FRUCTANS CONSUMPTION ON MINERAL INTESTINAL ABSORPTION AND BALANCE IN RATS FED CONTROL AND IRON-DEFICIENT DIETS*

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■ABSTRACT: In this study, the effects of inulin-type fructans (ITF) consumption on mineral absorption (Ca, Mg, Cu, Fe and Zn) and balance (Ca and Mg) were evaluated in rats fed control and iron-deficient diets. Each of 24 male Wistar rats (4-wk old) was assigned to 1 of 4 groups derived from 2×2 factorial design with 2 levels of added Fe (0 and 35mg/kg) and ITF (0 and 100g/kg) for 33 days. The food intake was determined daily and body weight recorded every two days. Food and demineralized water were offered *ad libitum*. Feces and urine were quantitatively collected for 5 days from day 23rd of the test period, pooled, and stored at -20ºC for mineral analysis. ITF consumption increased Ca absorption ($P<0.05$) only in non-deficient rats, whereas Mg absorption was positively affected irrespective of Fe status of the animals. Unexpectedly, Cu absorption was impaired by ITF. Furthermore, Fe deficiency negatively affected the absorption of Mg and Zn ($P < 0.001$). In conclusion, ITF consumption positively affected the absorption of Ca and Mg. However, Fe and Zn absorption were unchanged and Cu was negatively affected by ITF supplementation. The effects of ITF consumption on Cu homeostasis is unknown and should be considered in future research.

■KEYWORDS: Inulin-type fructans; calcium; magnesium; intestinal absorption; rats.

INTRODUCTION

The characterization of food matrices and the knowledge about their influence on mineral absorption and bioavailability have been the aim of several studies over the past years. In particular, the bioavailability of Fe present in food depends on its chemical form, the presence of dietary factors which influence its absorption and also the Fe status of the individual. In this context, the effect of dietary fibers on mineral bioavailability has been studied as a function of positive evidences resulting from the consumption of fermentable fibers, e.g. inulin-type fructans (ITF; fructooligosaccharides [FOS] and inulin) in the large intestine. [11, 14, 20, 23]

Inulin-type fructans selectively stimulate the metabolic activity of certain bacterial species, thus leading to a favorable environment to the mineral absorption. [20,23] Bacterial fermentation of such fibers produces short-chain fatty acids, [3] which results in decreased luminal pH and increased mineral solubility. [7] The fermentation is also accompanied by increased blood flow and vasodilatation of the large intestine arteries.[26] Increased number and volume of cells, as well as quantity, extension and bifurcation of intestinal crypts have also been observed, which might contribute to an increased absorptive surface.[11,14,19]

The interaction between Fe and other minerals has been well established in studies with animals and humans, and it may occur in a direct or indirect way. The direct interactions are usually competitive phenomena which take place during intestinal absorption or tissue utilization, whilst the indirect interactions take place when the mineral is involved in the metabolism of another mineral, in a way that the deficiency of one mineral causes an impaired function of the other.[15] Despite the fact that these interactions are based on chemical similarity of the minerals, the mechanism involved in these processes still shows controversial results. Hence, from the knowledge that the assessment and intervention on Fe status cannot be considered isolatedly and that several factors, both physiological and nutritional, could interfere with the mineral absorption, transport and storage, in this study we evaluated the influence of ITF (a diet component which has

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been systematically related to the promotion of mineral absorption[23]) on mineral absorption (Ca, Mg, Cu, Fe and Zn) and balance (Ca and Mg) in rats fed control and Fe-deficient diets.

MATERIAL AND METHODS

Diets and Animals

Experimental diets were supplemented with Raftilose P95 (Orafti-Active Food International, Tienen, Belgium) donated by Clariant S/A (São Paulo, Brazil). According to the analysis certificate, this product contains 94.96% FOS consisting of molecules (GF_n) with an average degree of polymerization (DP) of 4. ITF was obtained by a partial enzymatic hydrolysis of inulin, which was extracted from chicory (*Chicorium intybus*) roots. The experimental diets were modified from AIN-93G[18] (Table 1). Ferric pyrophosphate ($Fe_4(P_2O_7)_3$), an Fe source of low bioavailability, was obtained from Fermavi Eletroquímica Ltda (São Paulo, Brazil). ITF levels in the Raftilose P95 were subtracted from those of sucrose and starch. Fe as $Fe_4(P_2O_7)_3$ was replaced with sucrose in the AIN-93G mineral mix of the Fe-deficient diets. The following factors were used for energy calculations: 4, for carbohydrates and proteins, 9 for lipids and 1 for ITF.[21]

A total of 24 male Wistar rats (4 weeks old), initially weighing 54-56g, were obtained from the colony of the Faculty of Pharmaceutical Sciences, University of São Paulo. The experimental protocol was approved by the Commission on Ethics in Animal Experiments of the Faculty of Pharmaceutical Sciences of the University of São Paulo (CEEA 88/2005 FCF-USP) according to the guidelines of the Brazilian College on Animal Experimentation. All rats were housed in individual stainless-steel wire-mesh cages under a controlled temperature (22°C ± 2°C) with a 12-h light-dark cycle (lights off from 8 PM to 8 AM).

Experimental Design

Each of the animals was assigned to 1 of 4 groups derived from a 2×2 factorial design with 2 levels of added Fe (0 and 35mg/kg) and ITF (0 and 100g/kg) for 33 days. The animals underwent five days of acclimatization before the start of the experimental period. The food intake was determined daily and body weight recorded every two days. Food and demineralized water were offered *ad libitum*. The feed efficiency was determined as the weight gain per gram of food intake at the end of the experiment.

At the end of the experimental period, the rats were anesthetized with a 1:1:0.4:1.6 (v/v/v/v) mixture of ketamine (10mg/kg; Vetaset, Fort Dodge, Iowa, USA), xylazine (25mg/kg; Virbaxil 2%, Virbac, São Paulo, Brazil), acepromazine (2 mg/mL; Acepran 0.2%, Univet S/A Indústria Veterinária, São Paulo, Brazil) and demineralized H_2O. After the anesthesia had caused unconsciousness, blood was withdrawn from the abdominal aorta for determination of Hb concentrations. Hb and Fe intake values were used for estimating the Fe content of Hb (Hb Fe pool), calculated assuming a total blood volume of 6.7% of the rat body weight, and a Fe content in Hb of 0.335% (wt/wt), according the equation: [16]

Table 1 – Formulation of experimental diets.

Ingredients (%)	CON	ITF
Casein[1]	23.20	23.20
Fiber	5.00	5.00
Soybean oil[2]	7.00	7.00
L-cystine	0.30	0.30
Choline bitartarate	0.25	0.25
Vitamin mix[3]	1.00	1.00
Mineral mix[4]	3.50	3.50
Sucrose	10.00	-
Corn starch	49.93	49.00
Raftilose P95[5]	-	10.75

CON, control group; ITF, inulin-type fructans group.

[1] 78.2% protein;

[2] Cargill Agrícola S/A;

[3] AIN-93-VX vitamin mixture; [9]

[4] Modified from a mineral mixture (AIN-93G-MX). [9] CON and ITF diets contained an additional 35mg Fe/kg diet. Iron as $Fe_4(P_2O_7)_3$ was replaced with sucrose in the mineral mix of the Fe[-]diets.

[5] Orafti Active Food International (Clariant, São Paulo, Brazil), 94.96% ITF.

Hb Fe pool (mg) = [body weight (g) × Hb (g/L) × 6.7 × 0.335]/10,000

Feces and urine were quantitatively collected for 5 consecutive days from day 23 of the test period, pooled, and stored at -20ºC for further mineral analysis.

Chemical composition of experimental diets

The following components were determined: moisture, ash and total lipids; [2] and protein by the micro-Kjeldahl method[1] (conversion factor of 6.25). Dietary Ca, Mg, Fe, Cu and Zn concentrations were determined by atomic absorption spectrophotometry (AAS; AAnalyst 100, Perkin Elmer, USA) employing a hollow cathode lamp at 422.7, 202.6, 248.3, 324.8 and 213.9nm, respectively, and slits of 0.7, 1.3, 0.2, 1.3 and 1.3nm, respectively, after wet digestion (HNO_3:H_2O_2, 5:1; v/v) and addition of 0.1% (wt/v) lanthanum as La_2O_3 (for Ca and Mg analyses), as previously described. [13] The working standard solutions were prepared by diluting $CaCl_2$, $MgCl_2$, $FeCl_3$, $CuCl_2$ and $ZnCl_2$ (Titrisol, Merck, Darmstadt, Germany). The results obtained by the determination of mineral concentration in the different diets are listed in Table 2.

Apparent mineral absorption and balance

Dry feces (105°C, 15 h) were milled, and the powdered samples, as well as the urine samples, were utilized for mineral analyses, as described previously for the mineral analyses of the diets. Apparent absorption and balance were calculated by the following equations:

1) Apparent absorption (%) = 100 × (ingestion – fecal excretion)/ingestion
2) Mineral balance (%) = 100 × (ingestion – [fecal excretion + urinary excretion]) /ingestion

Statistical Analysis

An analysis of variance (ANOVA) was performed with a 2×2 factorial design (two levels of ITF and Fe in the diets), with the aid of the software ESTATISTICA (version 7). When the analyses revealed effects with $P<0.05$, a Tukey's test (Graphpad Instat, Graphpad software, version 2.00) was carried out for comparing the means between the groups, adopting a confidence interval of 95%.

RESULTS

Body Weight Gain, Food Intake and Efficiency

Considering that the initial weight of the groups was similar, ITF consumption after 33 days of experiment affected the body weight only in the animals fed the Fe-deficient diets (P=0.004; Table 3). Fe-deficient anemic rats supplemented with ITF had a lower total food intake (P=0.012) compared to the controls. However, there were no statistically significant differences in the food efficiency among the groups.

Fecal output, apparent intestinal absorption and balance of minerals

Table 3 also shows the results for dry weight and moisture of feces collected between days 23 and 27 of the test period. No statistically significant changes in the fecal output (dry basis) were found among the groups. However, ITF consumption resulted in a considerable increase ($P<0.05$) in the percentage of moisture in the feces in comparison to the control animals.

A higher content of Ca was observed in the feces and urine of the control group (results not shown), which was reflected in a higher intestinal absorption ($P<0.05$; Figure 1 A) and balance ($P<0.05$; Figure 2) in ITF-fed rats compared with control non-deficient rats. Moreover, apparent Mg absorption was increased in ITF non-deficient rats ($P<0.05$; Figure 1 A) whilst Mg balance remained unchanged (Figure 2). Unexpectedly, ITF impaired Cu absorption in both non-deficient and Fe-deficient animals. Intestinal absorption of Fe and Zn were not affected by ITF supplementation in the present experimental conditions (Figure 1 B). Furthermore, Fe deficiency also impaired Zn absorption, irrespective of the presence of ITF in the diet.

Iron status parameters

The consumption of Fe-deficient diets led to a moderate but significant decrease in Fe status parameters (Hb concentration, Hb Fe pool; $P<0.001$) comparatively to control diets (130g/L and 7.6mg for the control group *vs.* 90g/L and 4.5mg for the Fe-deficient group, respectively) whereas such parameters were not affected by ITF consumption.

Table 2 – Mineral composition (mg/kg) of the experimental diets.

Components	CON	ITF	Fe[-]	Fe[-]FTI
Calcium	5506.3 ± 49.5	5432.9 ± 110.0	5140.9 ± 34.0	5357.2 ± 77.8
Magnesium	413.98 ± 12.65	424.11 ± 10.42	442.77 ± 14.67	454.44 ± 10.5
Iron	68.0 ± 3.0	64.6 ± 2.2	12.6 ± 0.5	12.3 ± 0.4
Copper	12.2 ± 0.2	7.8 ± 0.2	12.0 ± 0.3	7.3 ± 0.2
Zinc	48.1 ± 2.3	45.3 ± 1.6	38.2 ± 3.2	39.8 ± 0.8

Results expressed as mean ± SD (n = 3).

Table 3 – Body weight gain, food intake and fecal output of Fe-deficient rats fed a control diet or a 10% ITF diet for 33 d. [1]

Variable	Dietary treatment				*P* value		
	CON	ITF	Fe[−]	Fe[−]ITF	ITF	Fe[−]	ITF × Fe[−]
Initial body wt (g)	54.5 ± 3.4[a]	54.8 ± 3.2[a]	55.8 ± 2.9[a]	56.0 ± 2.8[a]	NS	NS	NS
Final body wt (g)	239.5 ± 24.1[a]	209.8 ± 25.0[ab]	218.3 ± 27.2[ab]	191.7 ± 28.4[b]	NS	NS	< 0.01
Food intake (g/33 d)	481.9 ± 39.3[a]	432.5 ± 73.2[ab]	454.0 ± 41.4[ab]	386.0 ± 47.7[b]	< 0.05	NS	NS
Food efficiency	0.38 ± 0.02[a]	0.36 ± 0.01[a]	0.36 ± 0.03[a]	0.35 ± 0.06[a]	NS	NS	NS
Fecal output (dry g/5d)	6.9 ± 1.3[a]	6.0 ± 1.3[a]	6.2 ± 1.0[a]	6.7 ± 1.8[a]	NS	NS	NS
Fecal water content (%)	17.7 ± 4.3[a]	25.5 ± 3.6[b]	20.5 ± 2.0[a]	27.1 ± 5.3[b]	< 0.05	NS	NS

[1] Results expressed as mean ± SD (n = 6);
Mean values followed by the same superscript letter are not significantly different (P<0.05).

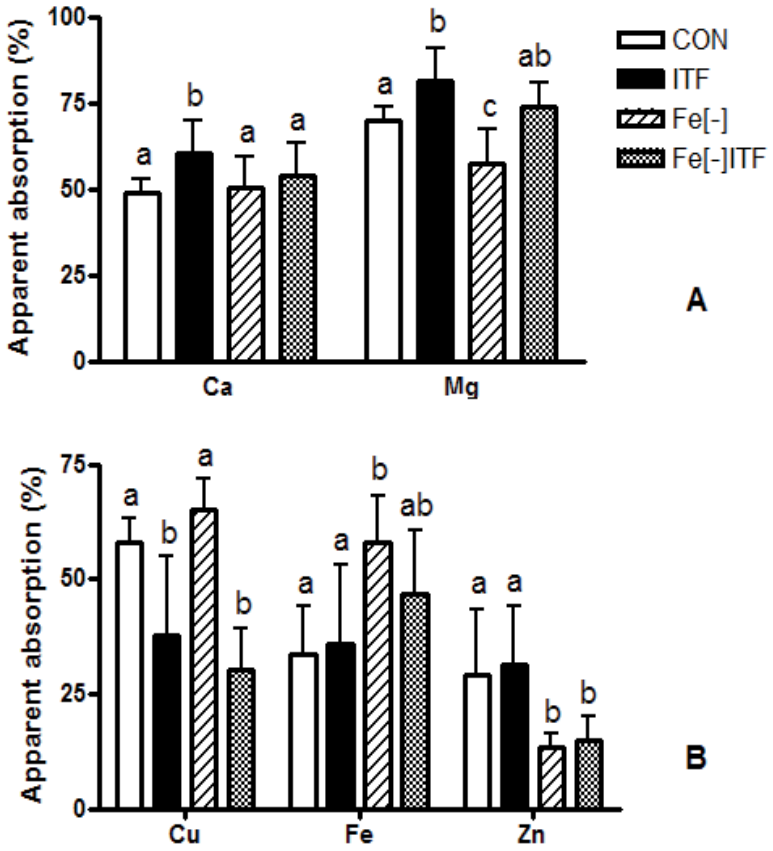

FIGURE 1 – Apparent absorption of macrominerals (calcium and magnesium) (A) and microminerals (copper, iron and zinc) (B) of Fe-deficient rats fed a control diet or a 10% ITF diet for 33 d. The results are expressed as mean ± SD (n = 6). The differences between the means of the groups were assessed through ANOVA followed by a Tukey's post-hoc test. [a,b,c,d] Different superscript letters indicate significantly different values (P<0.05).

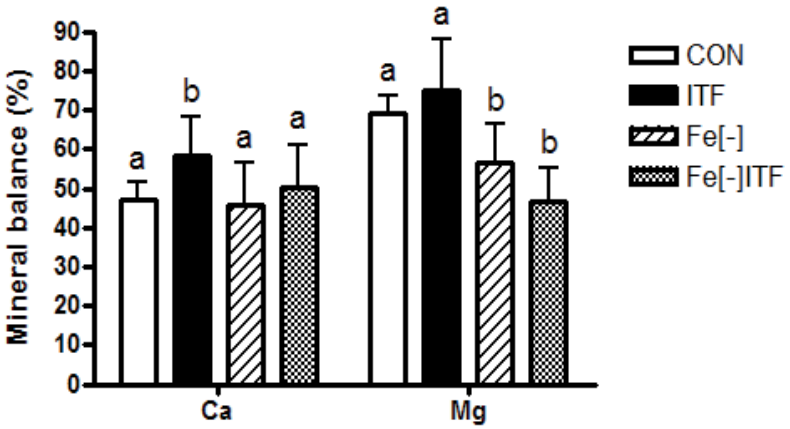

FIGURE 2 – Calcium and magnesium balance of Fe-deficient rats fed a control diet or a 10% ITF diet for 33 d. The results are expressed as mean ± SD (n = 6). The differences between the means of the groups were assessed through ANOVA followed by a Tukey's post-hoc test. [a,b] Different superscript letters indicate significantly different values (P<0.05).

DISCUSSION

Intestinal Fe absorption may be compromised by mineral interactions such as that between Ca and Fe, which is well known to limit Fe absorption in rats and humans.[8,24] However, little is known about Fe interaction with other minerals and food components (such as ITF) which influence mineral absorption in situations of Fe deficiency. In this study, the influence of ITF on macromineral (Ca and Mg) and micromineral (Cu, Fe and Zn) absorption, as well as mineral (Ca and Mg) balance was studied in control and in moderately Fe-deficient rats. After 33 d, 10% ITF in the diet increased Ca and Mg absorption in non-deficient rats and recovered Mg absorption which had been lowered by Fe deficiency. However, ITF had no effects on micromineral (Fe and Zn) absorption and on Fe status parameters in the present experimental conditions. Furthermore, Cu absorption was unexpectedly impaired by ITF irrespective of the Fe status of the animals.

In Fe-deficient rats, ITF consumption led to a lower final body weight compared to the controls. Furthermore, these animals consumed less food than those in the control group, which was observed in previous studies with healthy animals consuming diets with different ITF levels, [9,10,13] and was initially attributed to fluid accumulation in the intestine. ITF consumption causes an increase in intestinal content weight and more specifically in the water content, as these carbohydrates remain in solution in the chyme and contribute to the increase in the osmotic pressure, resulting in increased water flow to the intestinal lumen. [9]

Unlike other types of dietary fiber, non-digestible oligosaccharides (including ITF) stimulate intestinal absorption of some minerals in the large intestine. In the present study, our findings reinforce the positive influence of ITF on Ca and Mg absorption in healthy non-deficient animals. Many studies have systematically demonstrated that Ca and Mg absorption are improved by ITF supplementation in rat models, although the results may be dependent on the length of the feeding period and on the ITF DP. Moreover, such effects may also be affected by changes in the food matrix, such as dietary Ca content or the lipid composition of the diets. [5,7,9,10,19,27]

In this study, Fe deficiency resulted in decreased intestinal Mg absorption whereas ITF supplementation re-established Mg absorption to levels compared with those of the control non-deficient group. The interaction between Mg and Fe has been reported; however, the mechanisms of interaction have not been established. Recently, we have observed that Fe-deficient rats had reduced bone Mg levels when compared to controls[12] and we supposed that increased tibia Mg mobilization might have happened to allow the erythrocyte survival in Fe-deficient rats. Furthermore, feeding diets with ITF did not correct bone Mg levels which had been lowered by Fe deficiency. Additional research is needed to clarify the effects of Fe deficiency on Mg homeostasis. Furthermore, apparent Zn absorption decreased in Fe-deficient rats irrespective of ITF in the diets. Contrarily to our results, Zn absorption was showed to be unchanged after 30 to 40 days of nutritional Fe deficiency in rats. [22] Pérèz et al., [17] however, observed that an acute Fe deficiency in rats exacerbated the inhibitory effect of Fe on Zn absorption. Accordingly, a down-regulation in ZIP8 (SLC39A8; a Zn transporter) expression was observed in the small intestine epithelium of Fe-deficient rats when compared to controls. [4]

Inulin-type fructans had no effects on Fe and Zn absorption as well as on Fe status in the present experimental conditions. In addition, contrarily to what could be expected, Cu absorption was negatively affected by ITF consumption. Studies on the effect of ITF on Cu absorption are relatively scarce in the literature. At present, it seems that the ITF DP plays a paramount role in the observed effects, as the studies using high-DP ITF or a mixture of low- and high-DP ITFs show more positive effects. [23] On the other hand, feeding low-DP ITF led to a negative[25] or no effects[6] in Cu absorption. For example, Wolf et al.[25] reported that a 50 g low-DP ITF (FOS)/kg as part of a purified (AIN-93G) diet decreased Cu absorption in healthy growing rats. The authors suggested that the decrease in Cu absorption could be due to an increase in the intestinal pool of bile-excreted Cu or an increase in the bile influx of Cu into the intestine what might ultimately increase the fecal excretion of the mineral.

In summary, ITF improved Ca and Mg absorption in non-deficient rats and recovered Mg absorption which had been impaired by Fe deficiency. These results reinforce the effects of these food components as macromineral absorption enhancers. However, Fe and Zn absorption were unchanged and Cu was negatively affected by ITF supplementation. The effects of ITF consumption on Cu homeostasis is unknown and should be considered in future research.

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■RESUMO: Neste estudo, os efeitos do consumo de frutanos do tipo inulina (FTI) foram avaliados na absorção (Ca, Mg, Cu, Fe e Zn) e balanço (Ca e Mg) de minerais em ratos alimentados com rações controle e deficientes em Fe. Ratos Wistar machos (n = 24; 4 semanas de idade) foram distribuídos em 4 grupos seguindo um delineamento

fatorial 2×2, com 2 níveis de Fe adicionado (0 e 35mg/kg) e FTI (0 e 100g/kg), por 33 dias. O consumo de ração foi determinado diariamente e o peso corporal a cada 2 dias. Ração e água desmineralizada foram oferecidas *ad libitum*. Fezes e urina foram quantitativamente coletadas por 5 dias a partir do 23º dia, agrupadas e armazenadas a -20ºC, para análise de minerais. O consumo de FTI aumentou a absorção de Ca (P< 0,05) somente nos ratos não-deficientes, considerando que a absorção de Mg foi positivamente afetada, independente do *status* de Fe dos animais. De maneira não esperada, a absorção de Cu foi prejudicada pelo consumo de FTI. Além disso, a deficiência de Fe afetou de maneira negativa a absorção de Mg e Zn (P<0,001). Como conclusão, o consumo dos FTI afetou de maneira positiva a absorção intestinal de Ca e Mg. Entretanto, a absorção de Fe e Zn não foi afetada e a absorção de Cu foi prejudicada pela suplementação de FTI. Os efeitos do consumo de FTI na homeostase de Cu são desconhecidos e devem ser considerados em trabalhos futuros.

■PALAVRAS-CHAVE: Frutanos do tipo inulina; cálcio; magnésio; absorção intestinal; ratos.

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