Effect of dietary fibre mixture on growth and intestinal iron absorption in rats recovering from iron-deficiency anaemia

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There is concern regarding the possible negative effects of ingestion of dietary fibre on growth and intestinal Fe absorption in infants. The aim of the present study was to compare the effect of a fibre mixture on the growth and the intestinal absorption of Fe in rats with Fe-deficiency anaemia with that of a diet without fibres. Faecal weight and caecal pH were also evaluated. According to the Hb depletion–repletion model, twenty-two male weaned Wistar rats were fed the AIN93-G diet without Fe until Fe-deficiency anaemia was induced with Hb < 70 g/l. The anaemic rats were divided into two groups: (1) fibre mixture group – fed 100 g of fibre mixture/kg of diet (soya polysaccharide, inulin, resistant starch, Arabic gum, fructo-oligossaccharide and cellulose) (*n* 11); (2) control group – fed without fibres (*n* 11). All diets had 157 mg of ferric citrate (30 mg of elemental Fe) added to lead to recovery from anaemia. Fe intestinal absorption was measured by Hb repletion efficiency (HRE) and apparent Fe intestinal absorption. The HRE was 44·8 (SD 9·5) % in the fibre mixture group and 43·0 (SD 9·5) % in the control group (*P*=0·664). The apparent Fe absorption was 46·2 (SD 16·5) and 47·2 (SD 10·2) % (*P*=0·861) in the fibre mixture and control groups, respectively. The faecal weight median was 6·17 g in the fibre mixture group and 2·11 g in the control group (*P*<0·001). The caecal pH was in the same order: 6·11 (SD 0·59) and 7·07 (SD 0·34) (*P*<0·001). Both the groups consumed similar quantities of diet, and growth was similar in both the groups. The fibre mixture had no influence either on growth or on Fe intestinal absorption in rats recovering from anaemia. This mixture favoured an increase in faecal weight and a decrease in caecal pH.

Dietary fibre: Growth: Iron absorption: Iron-deficiency anaemia: Rats

Dietary fibres, in accordance with their type, can be fermented to different degrees in the different segments of the colon[1]. The fibre fermentation results in the formation of SCFA that reduce the intestinal pH, bringing about greater solubility of minerals and an increase in peristalsis[2]. From a clinical point of view, it is believed that dietary fibres can reduce the risk of chronic diseases such as diabetes, CVD, obesity, cancer and intestinal constipation[3].

Intestinal constipation occurs with elevated prevalence in unweaned infants[4]. The start of the symptoms is associated with the introduction of complementary foods, including lacteal formulae[5]. Studies indicate that children with chronic constipation consume less dietary fibres than healthy children[6]. In this manner, low dietary fibre consumption is considered a risk factor for chronic constipation, and, to this end, a diet rich in dietary fibres is considered not only a preventative measure against the development of constipation, but also a part of the treatment[7,8].

The recommendations for the consumption of dietary fibres (American Health Foundation and Dietary References Intakes) do not take into consideration the first year of life due to the possible negative effects that the fibres can have about on growth and the absorption of minerals[9,10], including Fe.

Fe deficiency shows an elevated prevalence in unweaned infants, and can bring about Fe-deficiency anaemia and other repercussions, among which is the deficit in neuropsychomotor development[11–13].

The majority of the studies that evaluated the effects of fibres on the bioavailability of Fe in the intestine are based on a single test meal[14], apparent absorption[15,16], or *in vitro* methods[17,18] with results that are not always in agreement. On the other hand, bioavailability and the intestinal absorption of Fe, as well as food intake and weight gain, can be evaluated in a long-term basis by the way of regeneration of Hb in rats, with Fe anaemia in the growth phase with prior induced Fe-deficiency anaemia[19–21].

Based on the theoretical principle that diet contains different types of fibres that are fermented to different levels in the colon, with a potential increase in the beneficial effect of dietary fibres, the food manufacturers formulated a supplement with six different types of fibres with proportions that approximate to that proclaimed in an ideal diet[22]. The beneficial effects of this fibre mixture in the prevention of constipation were observed in patients who consumed an enteral diet[23,24]. On the other hand, whether or not this fibre mixture can have an influence on Fe absorption, food intake and growth was not evaluated.

Thus, the present study was carried out with the objective of evaluating whether or not a fibre mixture with 32·0 % of soya polysaccharides, 12·5 % inulin, 9·0 % resistant starch, 24·0 % Arabic gum, 10·5 % fructo-oligosaccharides and 12·0 % cellulose interferes with the intestinal absorption of Fe, food intake and growth of anaemic rats during their growth phase. The biological effects of this mixture on the caecal pH and weight and faecal humidity were also evaluated.

Experimental methods

Animals and experimental design

A total of twenty-two male Wistar rats weaned at 21 d, with 55·35 (SD 4·25) g mean body weight, from the laboratory animal house (Experimental Model Development Center) at the Federal University of Sao Paulo (Brazil) were used for the present experiment.

During the whole study period, the rats received *ad libitum* deionised water (MilliQ Plus system, Millipore Corporation, Sao Paulo, SP, Brazil) and rations. They were kept in individual metabolic cages (Nalgene Metabolic Cages 650-0100, Rochester, NY, USA), under a 12 h light cycle, at a temperature of 23 ± 1°C.

The experimental study was divided into two phases to perform the rat model depletion–repletion methods. In the first phase, Fe-deficiency anaemia was induced. In the second phase, the effects of the fibre mixture on Fe bioavailability were evaluated. The study protocol was approved by the Research Ethics Committee of the Federal University of Sao Paulo, and was conducted in compliance with the International Guiding Principles for Biomedical Research Involving Animals (Council for International Organisations of Medical Sciences) – Geneva, 1985.

Induction of Fe-deficiency anaemia

To induce Fe-deficiency anaemia, the rats were fed the AIN93-G diet without Fe addition, as recommended by the American Institute of Nutrition[25], for 2 or 3 weeks until they became anaemic, with Hb lower than 70 g/l; this was done to use the rat depletion–repletion model methods[26]. Following the induction of Fe-deficiency anaemia, the rats were randomised into two similar groups according to weight, length, Hb level, packed cell volume and number of weeks necessary to obtain Hb lower than 70 g/l. Therefore, fourteen rats were randomised after 2 weeks and eight rats after 3 weeks, a total of twenty-two rats with eleven in each group.

Experimental diets

The experimental diets (AIN93-G, modified) were offered to the anaemic rats for 3 weeks in each group: (1) fibre mixture group: 50 g of cellulose/kg of AIN93-G was replaced with 100 g of a dietary fibre mixture composed of 32·0 % soya polysaccharide, 12·5 % inulin, 9·0 % resistant starch, 24·0 % Arabic gum, 10·5 % fructo-oligosaccharides and 12·0 % cellulose (Stimulance multi-fibre; Milupa, Fulda, Germany, and imported and distributed by Support, Brazil); (2) control group: the 50 g of cellulose/kg of AIN93-G was replaced with 100 g of maize starch/kg of feed, as suggested in the literature[27]. In both the

groups, similar quantities of elemental Fe were added to the feeds (30 mg/kg of diet) in the form of hydrated ferric citrate (Merck®, Frankfurt, Hesse, Germany). To increase the sensitivity of the model for the evaluation of the recovery Hb mass, Fe was offered at a quantity that was less than the recommendation for AIN93-G (35 mg/kg of ration), which allowed for the identification of even slightly poor Fe absorption, as reported previously[19–21].

To produce the feed given to the fibre mixture group, the quantity of dietary fibre mixture was subtracted from the total of maize starch contained in the AIN93-G diet. The diets were prepared in the Research Laboratory of the Pediatrics Department, Federal University of Sao Paulo, Brazil. The diet compositions are given in Table 1.

Evaluation of growth and Hb recovery

After the start of the experimental diets, the weight and length of each rat were measured weekly. The rats were weighed on an electronic digital balance (CG LIBROR L-600; CG Instrumentos Científicos Limited, São Paulo, SP, Brazil) with a maximum capacity of 600 g and a sensitivity of 0·1 g. The length expressed in cm was measured using the technique described by Hugues & Tanner[28], and it was obtained by summing the measures of the body and tail. A blood sample was collected from the tail to determine Hb and packed cell volume levels. Hb was determined by the cyanometha-haemoglobin method and microhaematocrit by the Wintrobe method (Perkin Elmer, model Victor3 1420 − 012 Wallac, Turku, Finland)[29]. Hb repletion efficiency (HRE) was determined according to the following formula[26,30]:

$$HRE = ((\text{final total body Hb Fe (mg)} - \text{initial total body Hb Fe (mg)})/\text{total Fe intake (mg)}) \times 100.$$

Table 1. Composition of the AIN93-G diet, prepared in the laboratory, modified by the substitution of cellulose with either the fibre mixture or maize starch (control diet) and a reduction in the level of iron (30 mg/kg diet)

Ingredients (g/kg)	Fibre mixture group (*n* 11)	Control group (*n* 11)
Maize starch	447·89	579·49
Cellulose	0·00	0·00
Fibre mixture	131·60	0·00
Casein	195·52	200·00
Sugar	100·00	100·00
Soya oil	69·58	70·00
L-Cystine	3·00	3·00
Choline bitartrate	2·50	2·50
t-Butyl hydroquinone	0·014	0·014
Vitamin mixture (Roche®)*	10·00	10·00
Mineral mixture (Roche®)†	35·00	35·00
Ferric citrate (19 %)	0·1572	0·1572

Macronutrient and micronutrient composition of the fibre mixture/100 g: carbohydrate 11·8 g; proteins 3·4 g; total fats 0·32 g; Ca 300 ng; Fe 0 mg; Na 60 mg; K 410 mg; P 80 mg; dietary fibre 76 g (46 g of soluble fibre and 30 g of insoluble fibre).
* Composition in mg: nicotinic acid 30: pantothenate 15: pyridoxine 6: thiamin 5; riboflavin 6; folic acid 2. Composition in μg: vitamin K 750; D-biotin 200; vitamin B₁₂ 25. Composition in IU: vitamin A 4000; vitamin D₃ 1000; vitamin E 75.
† Composition in mg (essential minerals): Ca 5000; P 1561; K 3600; S 300; Na 1019; Cl 1571; Mg 507; Zn 30; Mn 10; Cu 6; iodine 0·2; Mo 0·15; Se 0·15; Si 5; Cr 1; fluoride 1; Ni 0·5; B 0·5; Li 0·1; V 0·1; with no iron.

Total body Hb Fe content was calculated using the following formula:

Hb Fe (mg) = (body weight (g) × 0·075 ml blood/g body weight) × (Hb (g/ml)) × (3·35 mg Fe/Hb (g)).

The amount of experimental diets consumed was measured daily. This allowed for the calculation of the weekly and total feed efficiency, which indicates how much of the ration is responsible for the weight gain of the rats. The feed efficiency was calculated using the following formula[31]:

Feed efficiency = (weight gain (g)/consumption of diet (g)).

Apparent absorption of iron, fresh weight and faecal humidity

During the last week of the experiment, 0·1 g of carmine-pink dye (Merck®, Frankfurt, Hesse, Germany) was added to the diets of each rat and the faeces excreted were collected for three consecutive days, beginning from the point at which the faeces became red. About 72 h after the addition of carmine-pink, another dye (aniline blue, soluble in water, INLAB, Sao Paulo, SP, Brazil) was added to the diets. Faeces collection was terminated when blue-coloured faeces were first excreted.

The faeces collected during the 3 d were stored in a freezer (−20°C). After the third day of faeces collection, the wet weight of the faeces was measured with an electronic analytical balance (Mettler Toledo model AB204, Greisensee, Zürich, Switzerland), with a sensitivity of 0·1 mg. The faeces were then dried in an oven at 105°C. After 22 h, the faeces were weighed at 30 min intervals, until a constant weight was obtained. The faecal humidity was calculated according to the formula[20,32]:

Fresh faecal weight − dry faecal weight/fresh faecal weight × 100.

On the same analytical electronic balance, we measured, in a sequence, 500 mg of dry faeces from each rat, which had been divided into two samples of 250 mg (duplicate) and subjected to acid digestion with both nitric acid (65 %) and perchloric acid (70 %). The Fe content (μg/g dry faeces) was then analysed in an atomic absorption spectrophotometer (model Perkin-Elmer – 5100 PC, Norwald, CT, USA)[33]. During the same period that the faeces were collected, the quantity correspondent to the Fe ingested (μg) and excreted was also estimated.

To calculate the percentage of apparent absorption of Fe, the following formula was applied[20]:

Quantity of Fe ingested (μg)

− quantity of Fe excreted (μg)/quantity of Fe ingested (μg)

× 100.

The total quantity of Fe excreted was calculated according to the relation between Fe content in dry faeces and total dry faecal weight using the following formula: Fe excreted, μg = ((Fe (μg) × total dry faecal weight (g))/1 g of dry faeces (g)).

Evaluation of hepatic iron and caecal pH

After 3 weeks of the dietary treatment, the rats were killed and their livers and caecums were extracted.

The fresh livers were weighed in an analytical balance (Metler Toledo model AB204) with a sensitivity of 0·1 mg[19–21]. The hepatic Fe was also determined after liquid digestion of the dried tissue with nitric and perchloric acids, by means of spectrophotometry, similar to the method used for the faeces as described by Marks et al.[33].

The pH of the material extracted from the caecum was checked using a pH meter (Micronal pH meter, B374, Sao Paulo, SP, Brazil) between 6.00 and 10.00 hours, when intestinal fermentation is most active[34].

Statistical analysis

The results are expressed as means and standard deviations, medians and percentages, or the distributions of the variables. For the comparison of groups, Student's t test was applied when the variables were normally distributed, and the Mann–Whitney test was applied when the variables were not normally distributed.

All statistical and graphic tests were performed with the program Jandel–Sigma Stat 3.1 (Jandel Corporation, San Rafael, CA, USA)[35], and the null hypothesis was rejected at the 0·05 (5 %) level.

Results

Baseline data

The mean values for weight at the beginning of the dietary treatment after the induction of Fe-deficiency anaemia were 136·53 (SD 24·87) g for the rats in the fibre mixture group and 140·56 (SD 20·67) g for the rats in the control group ($P=0·683$). The mean values for body length, body + tail, were 30·54 (SD 2·28) and 30·41 (SD 2·04) cm ($P=0·892$), respectively. The mean values for Hb were 5·76 (SD 0·67) and 58·0 (SD 0·67) g/l ($P=0·890$), respectively. The mean packed cell volume values were 20·27 (SD 2·10) % and 20·54 (SD 2·70) % ($P=0·794$), respectively. Consequently, the two groups studied were similar in their weight, length, Hb and packed cell volume values at the beginning of the fibre mixture or control diet intake. The values obtained for Hb and packed cell volume demonstrated that the diet was sufficient to induce anaemia in the rats[30,31].

Effect of fibre mixture on faecal weight and humidity and caecal pH

The medians (25th and 75th percentiles in parentheses) for the wet weight of the faeces collected over 3 d between days 17 and 19 of the experimental diets were 6·17 (4·87−9·13) g in the fibre mixture group and 2·11 (1·52−2·39) g in the control group, which were significantly different ($P<0·001$). The faeces of the fibre mixtur group were more humid (39·41 (SD 20·24) %) than those of the control group (29·58 (SD 13·83) %), although the difference was not statistically significant ($P=0·199$). The caecal pH was 6·11 (SD 0·59) for the fibre mixture group and 7·07 (SD 0·34) for the control group, which were significantly different ($P<0·001$).

Food intake and growth

During the experiment, the rats in both the groups had similar weekly food intake, weights and lengths. The final body weight in the fibre mixture group was 265·16 (SD 19·35) g, and in the control group, it was 273·35 (SD 20·34) g ($P=0.345$). The final body lengths were 39·02 (SD 1·57) and 39·15 (SD 0·70) cm ($P=0.795$), respectively. Thus, the relationship between weight gain and the weekly consumption of diet, representing the feed efficiency, was also similar between the groups. Table 2 shows the weekly food intake parameters for each group: total food intake (g) and feed efficiency. There was no statistically significant difference between the groups.

Hb recovery: apparent absorption of Fe and Hb repletion efficiency

Table 2 shows the increase in the weekly values for Hb (g/l) in the two groups after the start of the dietary treatment. The results demonstrate that the recovery from previously induced anaemia was similar in both the groups.

The mean values for packed cell volume during the first week in the fibre mixture and control groups were 27·91 (SD 2·39) and 27·54 (SD 3·11) % ($P=0.761$), respectively. During the second week, the values were 32·82 (SD 3·03) and 34·09 (SD 3·21) % ($P=0.350$), respectively, and during the third week, they were 36·73 (SD 3·47) and 35·45 (SD 2·73) % ($P=0.350$), respectively.

The mean value for the apparent absorption of Fe, which corresponds to the difference between the total Fe ingested and the total Fe excreted in the faeces, was similar in both the groups: 46·18 (SD 16·48) % in the fibre mixture group and 47·22 (SD 10·23) % in the control group, which were not significantly different ($P=0.861$). The final mean value for the HRE did not differ between the two groups: 44·8 (SD 9·51) % in the fibre mixture group and 43·0 (SD 9·46) %

in the control group ($P=0.664$). Weekly data analysis (not shown) indicated that during 3 weeks of the present study, the fibre mixture effect on HRE was similar to that observed in the control group (Fig. 1).

Hepatic iron

The fresh weight of the liver was equal to 12·81 (SD 1·06) g in the fibre mixture group and 13·23 (SD 1·77) g in the control group ($P=0.514$). The levels of hepatic Fe in the dry tissue of the fibre mixture and control groups were 188·73 (SD 42·31) and 172·18 (SD 58·02) μg/g ($P=0.454$), respectively, which were not significantly different.

Discussion

The model used in the present study was adequate to show that the fibre mixture did not reduce Fe absorption, food intake and weight gain of the rats. A biological effect expected for this fibre mixture was observed, that is, a reduction of the caecal pH and an increase in the faecal volume were observed. In spite of the faecal humidity having been halved in the group that received the fibre mixture, statistical significance was not reached. Some of the effects of such a mixture on the gastrointestinal tract have been previously evaluated in human subjects[23,24]; nevertheless, the absorption of minerals was not evaluated.

The intestinal absorption of Fe was similar in the two groups, when evaluated both by the weekly HRE and by the balance of Fe carried out during the third week of the study. Both indicated bioavailability of approximately 45 % of Fe. It is worth emphasising that this is the only study in which the effect of this fibre mixture on mineral absorption was evaluated, demonstrating that this fibre mixture does not compromise the intestinal absorption of Fe.

Also, the analysed fibre mixture did not bring about a restriction of food intake and growth, as has been observed

Table 2. Data obtained from the weekly assessment of food intake (g), feed efficiency (g/g) and Hb recuperation (g/l) of the rats of the fibre mixture and control groups

(Mean values and standard deviations, medians and percentiles (25th–75th))

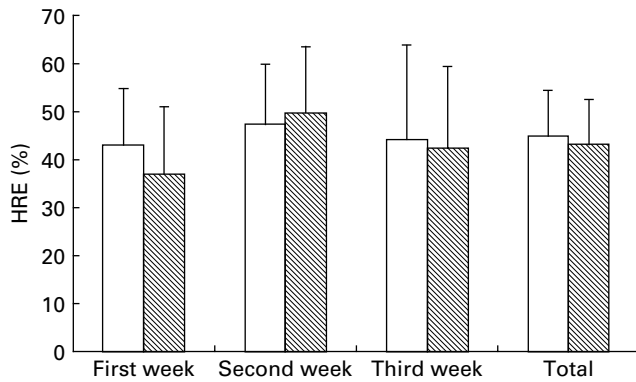|	Fibre mixture group (*n* 11)		Control group (*n* 11)		
	Mean/median	SD/percentiles	Mean/median	SD/percentiles	*P*
Food intake (g)*					
First week	116·64	13·68	115·09	12·09	0·781
Second week	124·26	10·78	129·64	11·10	0·262
Third week	134·46	12·63	141·22	11·10	0·198
Total	375·37	31·99	385·95	30·47	0·436
Hb recuperation (g/l)*					
Basal	57·6	6·6	58·0	7·0	0·890
First week	75·3	8·6	70·4	8·9	0·205
Second week	91·2	9·3	88·9	11·1	0·522
Third week	105·3	14·0	101·6	11·1	0·504
Feed efficiency (g/g)†					
First week	0·43	0·38–0·45	0·41	0·37–0·43	0·278
Second week	0·36	0·31–0·39	0·38	0·31–0·39	0·646
Third week	0·31	0·20–0·33	0·29	0·24–0·34	0·974
Total	1·11	0·94–1·16	1·10	0·93–1·16	1·000

* Student's *t* test.
† Mann–Whitney test.

Fig. 1. Effects of fibre mixture on Hb repletion efficiency (HRE) in the fibre mixture and control groups. Data were analysed using Student's *t*-test. Values are means with their standard errors (*n* 11 per group). There was no significant difference between the two groups during each week and total study period (*P* > 0·05). □, Fibre mixture group; ▨, control group.

in previous studies with cellulose, wheat bran and guar gum[19,20]. Feed efficiency was also considered in the present study, and it was identical for both the groups, which displayed no significant differences in the amount of diet consumed, weight gain, body length or fresh liver weight.

Thus, the non-existence of a negative effect of this fibre mixture on growth and Fe absorption must be confirmed in future studies on human infants by considering the importance of the introduction of dietary fibres in complementary foods to guarantee not only the maintenance of the normal intestinal rhythm, but also the development of a dietary habit in which the dietary fibres are in adequate quantity and quality.

Traditionally, insoluble fibre is considered to prejudice the absorption of minerals. However, the addition of soya polysaccharide (32·0 %) and cellulose (12·0 %) to the fibre mixture did not interfere with the absorption of Fe, thus verifying the results of other studies[19,20,34,36]. The insoluble fibres, cellulose being one among them, which is fermented only slightly in the distal portions of the colon, have shown a great capacity to retain water, contributing to the faecal bulk and thus increasing the faecal volume[20]. The soya polysaccharide, despite being considered an insoluble fibre, is strongly fermented in a portion of the ascending colon, and also contributes substantially to the increase in the faecal weight and faecal humidity[32]. The fibres, when fermented, contribute to the increase in the faecal biomass, which is consistent with the increase in the faecal volume[37].

The faecal analysis showed that the faecal weight was greater in the fibre mixture group than in the control group. The significant difference in the median weight of the fresh faeces collected over 3 d in both the groups may have been partially influenced by the addition of the insoluble fibres (soya polysaccharide and cellulose) to the mixture[32]. The faecal humidity values demonstrate that the fibre mixture generated a medium percentage of humidity, higher than that in the control group, although the difference was not statistically significant.

The increase in faecal weight and faecal humidity may be related to the increase in the rate of intestinal transit, as in the theory proposed by Burkitt[38]. Greater faecal volume is related to an increase in intestinal motility, whereas an increase in faecal humidity provides greater softness to

the faeces. The addition of the fibre mixture to an enteral diet promoted a reduction in the intestinal transit time, measured with a radio-pack marker; however, no effect of the fibre mixture on faecal weight was observed in human subjects in a study conducted by Skil *et al.*[23]. In terms of the fermentation of the fibre mixture used in the present study, Schneider *et al.*[24] observed that when the same mixture was fed to human subjects in an enteral diet, it promoted an increase in total SCFA production, especially butyrate, and an increase in the total bacterial score. In the present study, the reduction in the caecal pH and the increase in the caecal volume of the group that consumed the mixture suggest that the fibres underwent fermentation in the proximal portion of the colon, and that it had been maintained over the colon due to the different degrees of fermentation of the fibre mixture.

In summary, the dietary fibre mixture supplement, containing six different types of fibres with different solubility and fermentability, did not interfere with the growth or Fe absorption of rats with Fe-deficiency anaemia in their growth phase, although it caused an increase in the faecal weight and humidity, and a reduction in the caecal pH. Consequently, the consumption of this dietary fibre mixture had no negative nutritional effects in the rats evaluated. These preliminary findings in animal experiments must be confirmed in further studies involving human infants using appropriate methods for evaluation of intestinal Fe absorption.

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