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## Control of iron-deficiency anaemia in Brazilian preschool children using iron-fortified orange juice

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### Abstract

Different fortified foods have been used for the control of iron-deficiency anaemia in children. In Brazil, a low cost, abundant and culturally accepted food but not yet used for fortification, is orange juice. To evaluate the usefulness of fortified orange juice, 50 preschool children enrolled in a day-care centre in the town of Pontal, Southeast Brazil, received two flasks of 200 ml orange juice fortified with 20 mg ferrous sulfate heptahydrate, providing 2 mg elemental/100 ml, from Monday to Friday for 4 months (a total of 84 days). Capillary haemoglobin and z scores of the anthropometric indicators weight-for-age, weight-for-height and height-for-age were determined at the beginning of the study and after 4 months. Mean haemoglobin increased from  $10.48 \pm 1.66$  to  $11.60 \pm 1.09$  mg/dl ( $p = 0.00003$ ) and the prevalence of anaemia (Hb < 11 mg/dl) decreased from 60 to 20%. No significant alterations in the mean z scores of the anthropometric indicators were observed. The acceptance of fortified juice was excellent and no undesired effect was observed. We conclude that the consumption of iron-fortified orange juice is an adequate strategy to complement iron intake in preschool children and, therefore, to treat and prevent iron-deficiency anaemia. © 2003 Elsevier Science Inc. All rights reserved.

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## 1. Introduction

Iron-deficiency anaemia is currently the most frequent nutritional disorder worldwide, with different countries being affected to a different extent. In developing countries, iron deficiency is estimated to affect more than 3.5 billion people [1]. Some countries, such as the United States, were able to reduce the prevalence of anaemia, or to keep it at a low level through different preventive measures such as food fortification [2]. In Brazil, regional studies have shown a substantially increasing prevalence even among populations with better acquisitive power [3].

The adverse effects of iron deficiency on child development justify the search for urgently needed preventive and control measures [4]. The present trend is to adopt differential strategies according to the age range affected and cultural characteristics of the population [5]. In developed countries, different foods have been fortified, including milk, cereals, sugar and water [6–8]. In Brazil, a possible and adequate but still unused food for fortification is orange juice, based on the fact that Brazil is a large world producer of this fruit and that orange juice is inexpensive, culturally accepted and appreciated by the population. In addition, the vitamin C present in orange juice facilitates not only the absorption of iron added to the juice, but also the absorption of iron present in other consumed foods [9–12].

The aim of the present study was to evaluate the efficacy of orange juice fortified with ferrous sulfate in the control of iron-deficiency anaemia in a child population showing a high prevalence of this disease.

## 2. Materials and methods

The study was conducted at the day-care centre Centro de Convivência Infantil in the town of Pontal, São Paulo, Southeast Brazil. Fifty of the 200 children enrolled in the institution were selected by drawing lots, ranging in age from 12 to 72 months. At time zero, each child had its weight and height measured by standard, internationally accepted techniques [13]. On the same occasion, personal data were obtained and blood was collected for the determination of capillary haemoglobin using a previously calibrated HemoCue1 [14] apparatus, which was adjusted after every 10 measurements. The z scores of the weight-for-age, age-for-height and height-for-age indicators were calculated according to the NCHS standards [15] using the EpiInfo2000 software [16].

The children received 400 ml of iron-fortified orange juice in two daily portions, one between breakfast and lunch and the other between lunch and dinner from Monday to Friday for 4 months on 84 consecutive days. The preparation used was a commercial product available on the Brazilian market, with 9000 flasks of 200 ml divided into four lots being donated by the manufacturer. During the manufacturing process, 10 mg of ferrous sulfate heptahydrate ( $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ ) is added to each 100 ml of reconstituted concentrated orange juice and the mixture is homogenised in shaking tanks in order to obtain a solution that contains 4 mg iron per 200-ml flask. Concentrations of less than 3 mg/100 ml have been found to be adequate to maintain the organoleptic properties of orange juice [17]. Despite

possible biological variations in the vitamin C content per flask, vitamin C was added to the juice provided for the study by the manufacturer when necessary in order to obtain a concentration of 70 mg per 200-ml flask.

The iron concentration in the four juice lots was determined by atomic absorption spectrophotometry. One flask was randomly removed from each lot and shaken for a few seconds and a 5.0-ml aliquot was obtained after opening the flask. The sample was then diluted by adding 20 ml 2% HCl in order to fit the sensitivity and reliability range of the apparatus (absorbance interval of 0.1 to 0.4) and to prevent iron hydrolysis [18]. According to international recommendations, the solution was filtered through quantitative filter paper for slow filtration [19] in order to separate fibres present in the juice, which may interfere with the capillary suction of the apparatus by obstructing it. Absorbance was read with a Shimadzu AA-680/G V-5 atomic absorption spectrophotometer.

The patients were submitted to daily clinical follow-up throughout the 4 months of the study in order to detect problems resulting from the use of the fortified juice or from diseases that may interfere with the results. Attendance at the day-care centre and the amount of effectively ingested juice were recorded daily for each child.

After 4 months (time one), the children were again submitted to nutritional anthropometry and capillary haemoglobin determination. The z scores at times zero and one were compared by the Student *t*-test, with alpha set at 5%. The prevalence of anaemia (capillary haemoglobin < 11 mg/dl) at the two time points was also determined. All parents signed an informed consent form for their children to participate and the study was approved by the Ethics Committee of the University of Ribeirão Preto.

### 3. Results

The total amount of juice offered was 33,600 ml per child. Mean consumption over a period of 84 days was  $24,029 \pm 6,641$  ml, corresponding to  $286 \pm 79$  ml a day. Thus, iron and vitamin C intake were, on average,  $5.7 \pm 1.6$  and  $100 \pm 28$  mg/day, respectively. A chickenpox epidemic occurred during the study period and nine children had to leave the day-care centre, although only for 2 weeks. One child presented diarrhea on the day after the beginning of the use of fortified juice, but the picture disappeared within 4 days even with continuous ingestion of the juice. No other problems related to the digestive tract were observed during the study period. The percentage of children being absent from the day-care centre during the study period among those participating in the study was 22.5%, but there were 26 children with more than 20% absence, 12 of them with more than 30% absence and four with more than 50%.

The iron concentration in the juice lots offered to the children was 3.57 (lot 2), 4.24 (lot 4), 3.71 (lot 6), and 3.94 mg/200 ml (lot 9), with a mean of 3.87 mg/200 ml for the four lots. As can be seen, the concentrations obtained for each sample and the mean concentration were close to the concentration reported on the product label (4 mg/200 ml).

Table 1 shows the z scores of the anthropometric indicators and the haemoglobin values before and after the use of iron-fortified orange juice for 4 months. No variation in the z

Table 1

Mean ( $\pm$  SD) scores of the indicators weight-for-age, weight-for-height and height-for-age and capillary haemoglobin at times zero and one

	Time zero	Time one	<i>p</i>
Weight-for-age z score	$-0.06 \pm 0.96$	$-0.03 \pm 0.95$	0.71
Weight-for-height z score	$0.16 \pm 0.78$	$0.16 \pm 0.99$	0.98
Height-for-age z score	$-0.23 \pm 0.96$	$-0.16 \pm 0.94$	0.073
Haemoglobin (mg/dl)	$10.48 \pm 1.66$	$11.60 \pm 1.09$	0.00003

scores was observed for any of the three anthropometric indicators, while capillary haemoglobin increased significantly between time zero and time one.

Fig. 1 shows the prevalence of anaemia at times zero and one. A significant reduction in prevalence can be observed. Twenty-seven of the 30 initially anaemic children (60%) showed increased haemoglobin values, with 22 of them no longer being anaemic, two showing reduction (from 10.7 to 10.3 and from 10.6 to 10.1 mg/dl) and one child maintaining the same value (10.8 mg/dl). Of the 20 initially non-anaemic children, nine (40%) showed increased haemoglobin values, two maintained the same value and in nine children this value was reduced, with two children becoming anaemic (from 11 to 9.7 and from 12.1 to 9.3 mg/dl). Of the 11 children showing reduced haemoglobin values, six presented more than 30% absence from the day-care centre during the study period and one child had chickenpox.

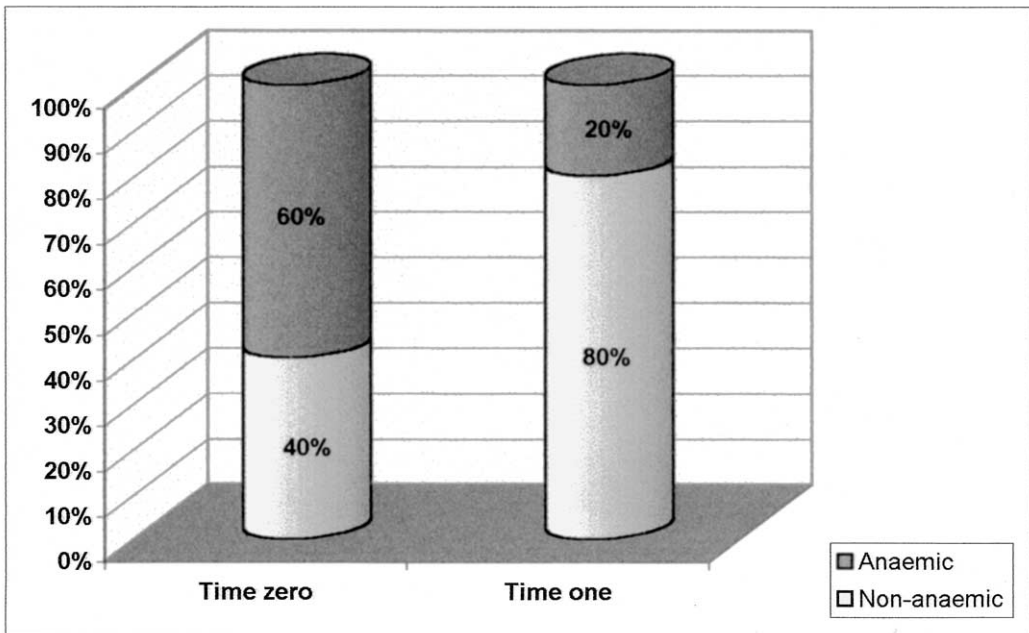


Fig. 1. Prevalence of non-anaemic (haemoglobin  $\geq$  11 mg/dl) and anaemic children (haemoglobin  $<$  11 mg/dl) at times zero and one.

#### 4. Discussion

Since the control of iron deficiency by a single strategy is currently considered to be a highly difficult task, many countries have been seeking local alternatives appropriate for their reality, such as the fortification of dressings, rice, flour, snacks, and biscuits, among others [5]. Brazil is one of the main world producers of oranges, representing about 35% of the world production, a value corresponding to about 78 billion fruits per year [20]. Therefore, it is highly appropriate to imagine that orange juice, although still unused, is an adequate vehicle for iron fortification to control iron-deficiency anaemia in Brazilian preschool children.

A previous study from our group showed a 68.7% prevalence of anaemia among preschool children at the institution studied here [21], and therefore the search for a strategy to combat iron-deficiency anaemia other than the commonly used one, i.e. oral iron supplementation, is imperative. In fact, a large number of children frequenting the institution had used at some time ferrous sulfate drops, but this fact did not have any impact on the prevalence of anaemia, which, despite being high, was closely similar to that observed in other parts of Brazil and in most third world countries. Food intake, also evaluated in the above study, provided about 9.8 mg of iron per day, but the low bioavailability and the presence of absorption inhibitors rendered this intake insufficient for the maintenance of an adequate iron nutritional status [21]. Therefore, the innovative strategy of using iron-fortified orange juice was chosen and an industrialised product was offered in order to test its capacity of providing the iron that was lacking in the diet of the children. The decision to use a commercial product was made based on the urgent need, in the opinion of the authors, to act together with the community, forgoing a long and laborious process of food technology to obtain a not only nutritionally adequate but organoleptically satisfactory fortified juice. The only concern of the team was to determine by atomic absorption spectrophotometry whether the iron concentration in the juice reported by the manufacturer corresponded to that observed in the product. The concentration was indeed found to be within adequate limits.

It is a recognized fact that vitamin C improves the absorption of iron present in ingested food [9,10,11,12]. On this basis, for a better demonstration of the results obtained in the present study, it would have been important to include a control group consisting of children who would receive only juice with no iron added. However, we opted for the exclusion of such group due to two factors: first, as previously reported [21], the diet of the children at the day care centers studied already supplied vitamin C corresponding to 70.8% of RDA. Second, due to ethical questions since we knew that, with the iron supplementation contained in fortified juice, the hemoglobin values of the children would certainly tend to increase. Thus, we thought it would be inappropriate to submit a group of children with a high prevalence of anemia [21] to two blood collections without the benefit of receiving the iron supplementation during the 4 months of the study.

A possible criticism to the proposed strategy may derive from some studies suggesting an association between the use of large amounts of fruit juice and a reduction in growth rate and the occurrence of obesity [22,23]. Although many authors did not observe this correlation [24–26], in the present study we evaluated possible changes in the z scores of anthropometric indicators, with no changes being observed during the 4 months of fortified orange juice use.

Another matter of concern may be a possible iron excess; however, the mean amount consumed per day (5.7 mg) was similar to the amount found in a portion of 100 g of beef, characterising the present study as a fortification strategy that only aims at approximating daily iron intake to internationally recommended standards [27], without risks of intoxication and adverse effects for the children involved.

The use of iron-fortified orange juice was found to be adequate to reduce the prevalence of anaemia, without side effects or acceptance problems of the product on the part of the children or their relatives. The prevalence of anaemia decreased by 20% within only 4 months of intervention and most children showed an increase in haemoglobin values. However, some children maintained or even reduced haemoglobin levels, with two of them becoming anaemic. External factors probably contributed to this finding, mainly the large number of absences from the day-care centre as a result of the July school holidays. Although the day-care centre does not interrupt its activities during this month, many mothers take out their children during this period, a fact that would lead to lower than predicted amounts of ingested juice during the 4 months. The presence of diseases can be also considered an interfering factor, especially typical winter viral infections, which were indeed highly prevalent, and the occurrence of a chickenpox epidemic during the study period.

Despite the short duration of the study, we believe that the continuous use of fortified juice, incorporated into the menu of the day-care centre, can lead to the recovery of all children with iron deficiency and, more important, prevent other children from becoming deficient. Therefore, this practice represents an important strategy for combating a severe public health problem, i.e. childhood anaemia.

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