Rev Saúde Pública 2007;41(4)

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Effect of iron fortification of flour on anemia in preschool children in Pelotas, Brazil

ABSTRACT

OBJECTIVE: Iron fortification of flour has been sanctioned by the Brazilian government since 2004. The objective of the study was to assess the impact of flour fortification on hemoglobin level in children under six.

METHODS: A time-series study was carried out in Pelotas, Southern Brazil, consisting of three assessments at a 12-month interval. In May 2004, before flour fortification, hemoglobin measurements were obtained in a probabilistic sample of 453 children. Twelve and 24 months later, samples of 923 and 863 children were studied, respectively.

RESULTS: The three groups studied were comparable in terms of demographic and socioeconomic characteristics. At baseline, mean hemoglobin was 11.3±2.8 g/dL. In the post-fortification period, means were 11.2±2.8 (at 12 months) and 11.3±2.5 g/dL (at 24 months), with no statistically significant difference among the three time periods studied ($p=0.16$).

CONCLUSIONS: Fortification had no effect on hemoglobin levels of the children studied. This finding could be partially due to inadequate flour intake and/or low bioavailability of dietary iron.

KEYWORDS: Food, fortified. Flour, analysis. Iron, dietary. Anemia. Hemoglobins, biosynthesis. Child, Preschool. Intervention studies. Time series studies.

INTRODUCTION

Anemia is an indicator of nutritional deficiency.* In developing countries, low intake of high-bioavailability iron is a major factor in the development of anemia and population-based interventions are required to remedy this condition. Iron fortification of widely consumed food products (mass or universal fortification) has been the strategy to better people's nutritional status in developing countries.[20]

Population-based studies in several Brazilian regions showed high anemia prevalences above 30%.[1,2,9,11,12,15-17] Hence, since July 2004, the Brazilian Ministry of Health has established that all wheat and corn flours produced should be iron fortified. A special administrative act** specified the dietary iron compounds to be used: dehydrated iron sulphate, iron fumarate, reduced

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Received: 11/21/2006
Approved: 2/28/2007

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*World Health Organization. Focusing on anemia. Geneva; 2004. Available at: http://www.paho.org/English/AD/FCH/NU/WHO04_Anemia.pdf [Access on 2/8/2006]

**Ministério da Saúde. Agência Nacional de Vigilância Sanitária. Resolução /RDC nº 344, de 13 de dezembro de 2002. Aprova o Regulamento Técnico para a Fortificação das Farinhas de Trigo e das Farinhas de Milho com Ferro e Ácido Fólico, constante do anexo desta Resolução. Revoga a resolução – RDC n0 15, de 21 de fevereiro de 2000 [lei na Internet]. Diário Oficial da União, Brasília, DF, 18 dez 2002. Available at: http://www.anvisa.gov.br/e-legis/

iron, and electrolytic iron; 325-mesh Tyler, iron and sodium ethylenediaminetetracetic acid (EDTA), and chelated iron bysglicin, as well as their amounts (4.2 mg iron/100 g flour).

Some studies showed the efficacy of fortification in increasing serum iron but the effectiveness of mass fortification programs has hardly ever been evaluated.[20] The purpose of the present study was to assess the effect of iron fortification of flour on hemoglobin levels in preschool children.

METHODS

A time-series study was conducted consisting of three assessments at a 12-month interval. Three cross-sectional surveys were carried out in the city of Pelotas, Southern Brazil. Hemoglobin levels were investigated in a group of children aged zero to five (baseline study) between May and July 2004, prior to compulsory iron fortification of flour.[2] Then, 12 and 24 months post-fortification implementation (2005 and 2006), hemoglobin levels were once more investigated in other groups of children of similar age and socioeconomic condition compared to the first group assessed. Not always the same group of children was study as, due to ethical issues, diagnosed cases of anemia were referred to treatment.

The sample size was estimated to detect a 0.5 g/dL difference in mean hemoglobin between pre- and post-intervention assessments. There were required 600 children aged zero to 71 months for each phase of the study, given a 95% confidence level (two-sided), 90% power and standard deviation of 1.7 g/dL hemoglobin.[11]

At baseline,[2] a sample by clusters was selected in two steps and primary sampling units were drawn from census tracts defined by the Instituto Brasileiro de Geografia and Estatística (IBGE – Brazilian Institute of Geography and Statistics) for 2000 Demographic Census.

Despite the fact that the original estimate of the sample size did not include the correction for the design effect,[19] the remaining samples studied were estimated after correcting the first sample size for the design effects identified at baseline (1.6 and 3.3 for children aged zero to 24 months and 25 to 71 months, respectively) as a way of assuring statistical power for comparisons. There were estimated 900 children required to the later steps of the study. Applying the same sampling criteria of the first phase, in order to identify 30 children per census tract, 40 tracts were required to be visited in the second and third phases. Seeing that tracts were arranged in an ascending order by income, there were selected tracts right before and after those studied in each earlier phase so that new tracts would have mean income similar to those previously studied.

At the three time points, the mothers of children were interviewed at home by previously trained nutritionists. The following data was collected on the children and their families: demographic (sex, age in months, skin color, birth weight, current weight and height); socioeconomic characteristics (current family income in reals, maternal and paternal schooling in full years, sanitation conditions and number of residents in the household); prior history of anemia (anemia diagnosed by a physician in the last year and drug therapy); morbidity in the last 15 days (diarrhea, cough, and fever); current dietary patterns (weekly intake of iron-fortified food); pattern of flour intake (low, intermediate, and high – constructed from tertiles of scores based on weekly intake of food made with flour such as breads, cookies, pastas, cakes and polentas: zero score for rarely/never, 2.5 for 2 to 3 days a week, 5 for 4 to 6 days a week and 7 for daily intake); breastfeeding; and intake of macro and micronutrients assessed using a 24-hour food recall (except on the day following Sundays and holidays) and analyzed through Virtual Nutri 1.0.* The amount of iron added to flour products was not included in the analysis.

Weight measures of children were obtained using digital electronic scales (Seca) with 150-kg capacity and 100-g precision (Unicef, Copenhagen). Children under two were weighed on their mothers' lap. Length measurements of children aged up to two years were obtained using anthropometers (Sanny), belt model, with 20 to 105-cm scale and 0.5-cm precision, with children lying down according to the standard procedure. Height of older children was obtained using an Alturaexata stadiometer with 35 to 213-cm scale and 0.1-cm precision, with children in the standing position.

Hemoglobin measurement in the peripheral blood was obtained through finger prick by nursing aids and readings were made in a portable hemoglobinometer (HemoCue AB, Sweden), which was calibrated daily as per the manufacturer's specifications. Hemoglobin concentration was expressed in g/dL and those children with hemoglobin below 11 g/dL were classified as anemic.[22]

Nutritional status was assessed using the National Center for Health Statistics[6] criteria for the comparison of anthropometric ratios obtained based on length/height and weight measures and age. New growth curves, published by the World Health Organization in 2006, were also used for assessing children aged from zero to 60 months.**

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* Philipp ST, Szarfarc SC, Latterza AR. Virtual Nutri [programa de computador]. Versão 1.0 for Windows. São Paulo: Departamento de Nutrição da Faculdade de Saúde Pública da USP; 1996.

** World Health Organization. Child growth standards. Geneva; 2006. Available at: http://www.who.int/childgrowth/en/ [Access on 10/17/2006]

Children were classified with growth deficit when their height/age Z score was below -2 standard deviations and with overweight when their weight/height ratio was greater than 2 standard deviations as proposed by the World Health Organization.[21]

Since food manufacturing companies may have started either earlier or later fortifying flour products, flour samples were collected at baseline to confirm that flour products consumed have not yet been fortified and at the post-fortification period to confirm that the same flour products have already been fortified. For that, in the first two time points (baseline and 12 months), wheat flour samples were collected in 10% of the households visited and systematically selected, and tested for iron concentration in the Laboratório de Química dos Alimentos (Laboratory of Food Chemistry, LQA) of Universidade Federal de Pelotas. At the same time, an inventory list of all labeled fortified flour products and pastas available in wholesale and retail stores in the city of Pelotas was made to check whether fortified products have been supplied. Samples of commercially available flour products were tested in the LQA and in the Instituto Adolfo Lutz in São Paulo as well for result validation.

Data were double entered using EpiInfo 6.04 and then its consistency was ascertained. All data analyses considered the sample design and were adjusted for standard errors of potential intra-cluster correlation using the svy commands of Stata 9.0.

First baseline and post-fortification groups were compared in terms of the distribution of potential confounder variables followed by a comparison of hemoglobin means between these groups. Then stratified analyses were conducted to detect any effect change by factors that could affect the impact of fortification.

To ascertain repeatability of data collected, 10% of interviews in each census tract were repeated by the field work supervisor using an abbreviated questionnaire. Interviewers did not know which households would be revisited. Kappa coefficients for the variables studied

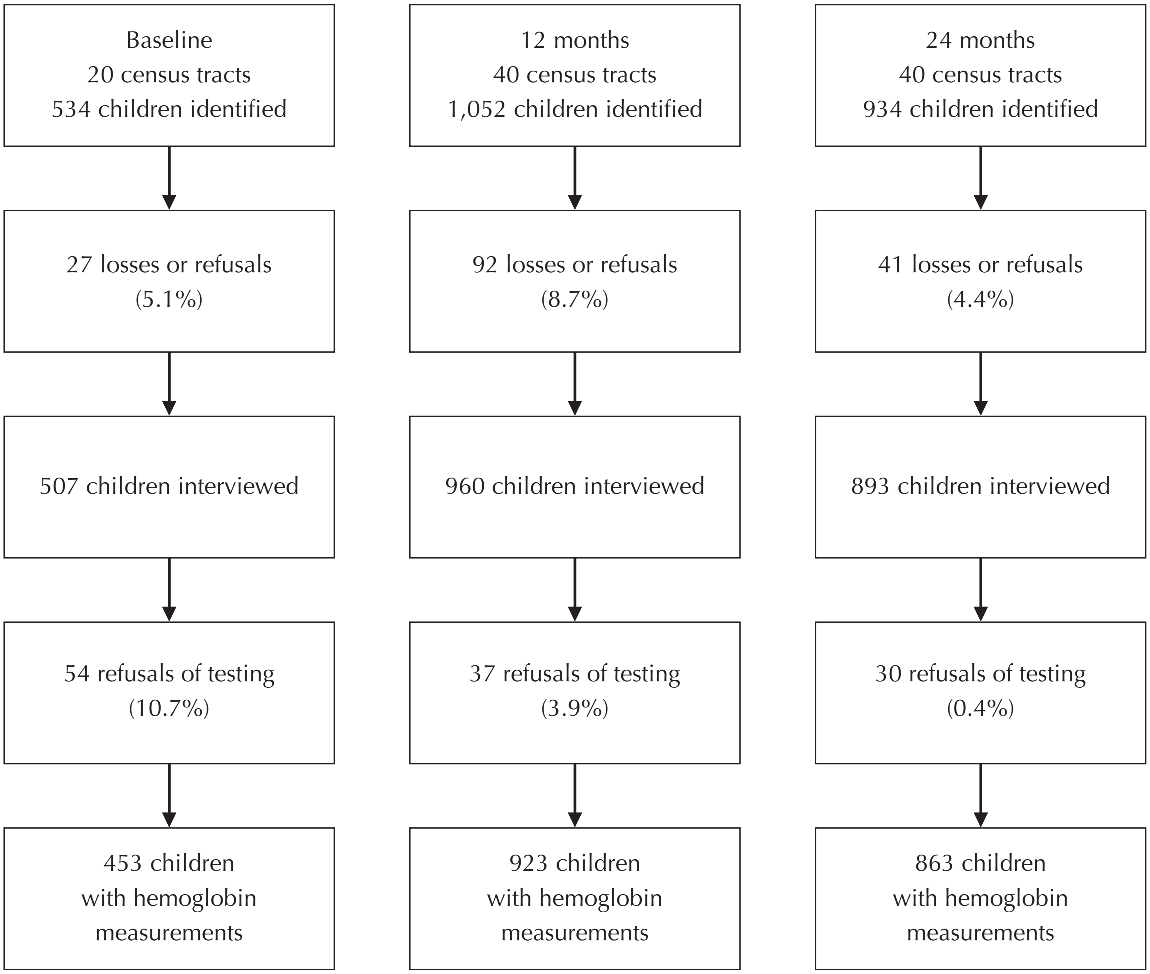

Figure. Descriptive flow chart of the number of children aged zero to five years identified in each step of the study and the number of losses and refusals: at baseline, and 12 and 24 months post-fortification. Pelotas, Southern Brazil, 2004-2006.

(skin color, maternal and paternal schooling) were all higher than 0.82 in the three steps of the study.

The study was approved by the Research Ethics Committee of the School of Medicine of Universidade Federal de Pelotas. The mother's or guardian's written consent was obtained before data and blood collection. The study did not imply in any risks to the children's health. Parents or guardians of those diagnosed with anemia were warned and their children were referred to health care services for treatment.

RESULTS

Figure describes the number of children identified in the three steps as well as refusals to participate in the interview and hemoglobin measurement. Losses ranged between 8% and 15% and information and hemoglobin measurement were obtained for 2,239 children. Refusals were directly associated to higher parental schooling and family income and females as well.

Tables 1 to 3 show the distribution demographic, socioeconomic, and nutritional characteristics, morbidity,

Table 1. Demographic and socioeconomic characteristics of the children studied. Pelotas, Southern Brazil, 2004–2006.

Characteristic	Baseline (N=507) %	Post-fortification 12 months (N=960) %	Post-fortification 24 months (N=893) %	p-value*
Gender				0.31
Male	52.7	50.2	54.0	
Female	47.5	49.8	46.0	
Skin color				0.08
White	74.5	68.8	77.6	
Non-white	25.5	31.2	22.4	
Age (months)				0.51
0–11	16.2	16.6	15.2	
12–23	12.6	16.5	15.9	
24–35	16.4	16.4	17.9	
36–47	17.0	15.0	16.4	
48–59	18.1	17.5	15.8	
60–71	19.7	18.0	18.8	
Maternal schooling				0.04
No schooling	6.3	2.4	1.2	
Up to 4 years	22.0	20.3	15.9	
5 – 8 years	38.4	43.1	40.4	
9 years or more	33.3	34.2	42.5	
Father's or partner's schooling				0.41
No schooling	3.5	3.0	2.3	
Up to 4 years	22.3	17.0	15.2	
5–8 years	45.1	51.0	47.4	
9 years or more	29.1	29.0	35.1	
Family income (minimum wages)				0.87
Less than 1	17.8	19.7	16.5	
1 – 2.99	51.4	53.9	54.9	
3 – 5.99	19.9	18.2	20.3	
6 or more	10.9	8.2	8.3	
Total residents in the household				0.70
2 – 4	46.7	49.1	52.0	
5 – 7	44.6	42.3	40.3	
8 – 14	8.7	8.6	7.7	

* Pearson's Chi-square

Table 2. Nutritional characteristics and morbidity of the children studied. Pelotas, Southern Brazil, 2004–2006.

Characteristic	Baseline (N=507) %	Post-fortification		p-value*
		12 months (N=960) %	24 months (N=893) %	
Low birth weight	10.8	8.3	8.8	0.40
Current nutritional status (NCHS)**				
Height/age < -2 SD	5.5	9.2	5.0	0.01
Weight/height > 2 SD	12.0	13.6	11.2	0.29
Current nutritional status (OMS)***				
Height/age < -2 SD	8.3	13.0	7.1	< 0.001
Weight/height > 2 SD	11.4	14.9	12.4	0.17
Prior diagnosis of anemia	40.0	42.5	36.2	0.07
Drug therapy for anemia in the last year	29.8	28.2	24.0	0.15
Diarrhea in the last 15 days	11.0	8.0	9.4	0.26
Cough in the last 15 days	57.0	47.4	51.1	0.07
Fever in the last 15 days	19.5	18.6	29.7	< 0.001

* Pearson's Chi-square
** National Center of Health Statistics, 1985
*** WHO, 2006 / children aged zero to 60 months only.

Table 3. Dietary patterns of the children studied. Pelotas, Southern Brazil, 2004–2006.

Characteristic	Baseline (N=507) %	Post-fortification		p-value*
		12 months (N=960) %	24 months (N=893) %	
Still breastfed	15.3	18.5	19.0	0.17
Weekly intake of food items:				
Red meat	80.1	83.7	87.8	0.03
Liver	22.4	24.4	20.2	0.37
Yolk	63.8	64.6	66.5	0.70
Bean	94.5	97.7	95.7	0.02
Bread	92.8	93.1	93.4	0.79
Pasta	89.5	86.0	90.1	0.04
Cookie	82.9	85.8	92.1	0.02
Pattern of flour intake:				0.12
Low	39.2	42.1	37.1	-
Intermediate	28.6	33.0	33.6	-
High	32.2	24.9	29.3	-

* Pearson's Chi-square

and dietary patterns in the groups of children included in each phase of the study.

The study groups did not show differences in the distribution by sex, skin color, age, family income, father's or partner's schooling and total residents in the household. As for maternal schooling, mothers of the children studied in the third step (at 24 months) were more educated. Height/age deficit was more often seen in children studied at 12 months post-fortification. The occurrence of "fever in the last 15 days" was more frequently observed in the group studied at 24 months post-fortification ($p<0.001$). In regard to dietary patterns, weekly intake of red meat and beans was different among the groups and more common among children studied post-intervention ($p=0.03$ and $p=0.02$ at 12 and 24 months, respectively). On the other hand, weekly intake of pasta and cookies was more frequent among children studied at 24 months post-fortification ($p=0.04$ and $p=0.02$ respectively). Mean iron intake was similar among children studied at the three time points: 8.42 ± 5.08 mg; 8.82 ± 7.59 mg and 8.84 ± 5.40 mg, respectively ($p=0.14$).

Table 4. Mean hemoglobin at the three steps of the study according to demographic and socioeconomic characteristics and dietary patterns. Pelotas, Southern Brazil, 2004–2006.

Characteristic	Mean hemoglobin (g/dL) ± SD Baseline	 12 months	 24 months	p-value*
Age in months				
0–11	10.9 ± 1.4	10.7 ± 2.5	10.3 ± 1.7	0.02
12 – 23	11.0 ± 1.6	10.4 ± 2.2	10.6 ± 2.2	0.07
24 – 35	11.0 ± 1.6	10.9 ± 2.1	11.2 ± 1.5	0.23
36 – 47	11.3 ± 1.8	11.4 ± 1.7	11.8 ± 1.1	0.02
48 – 59	11.8 ± 1.5	11.9 ± 1.6	11.8 ± 1.8	0.95
60 – 71	11.6 ± 2.0	11.7 ± 1.5	12.0 ± 1.5	0.14
Gender				
Male	11.4 ± 2.1	11.1 ± 2.4	11.4 ± 2.0	0.01
Female	11.2 ± 2.3	11.2 ± 2.3	11.2 ± 2.3	0.92
Family income (minimum wages)				
< 1	10.9 ± 1.4	11.0 ± 2.0	11.1 ± 2.5	0.78
1 – 2	11.3 ± 2.5	11.2 ± 2.5	11.2 ± 1.9	0.59
3 – 5	11.4 ± 1.0	11.5 ± 1.7	11.5 ± 1.2	0.87
6 or more	12.0 ± 0.8	11.1 ± 1.5	12.0 ± 1.5	<0.001
Maternal schooling (years)				
No schooling	10.9 ± 1.5	11.0 ± 1.5	11.1 ± 1.7	0.97
Up to 4	11.0 ± 2.7	11.4 ± 2.6	10.9 ± 2.2	0.06
5 – 8	11.3 ± 1.7	11.0 ± 2.3	11.2 ± 2.1	0.20
9 or more	11.7 ± 1.4	11.3 ± 2.3	11.6 ± 1.7	0.05
Weekly intake of red meat				
No	11.2 ± 1.8	10.9 ± 2.1	10.8 ± 2.1	0.30
Yes	11.4 ± 2.4	11.3 ± 2.9	11.5 ± 2.1	0.05
Weekly intake of beans				
No	11.3 ± 1.7	11.6 ± 1.8	11.0 ± 1.7	0.44
Yes	11.3 ± 2.8	11.2 ± 2.8	11.4 ± 2.5	0.03
Weekly intake of pasta				
No	11.1 ± 1.3	11.1 ± 2.6	11.0 ± 2.2	0.89
Yes	11.4 ± 2.8	11.2 ± 2.7	11.4 ± 2.2	0.04
All children	11.3 ± 2.8	11.2 ± 2.8	11.3 ± 2.5	0.16

*Anova

The evaluation of labels of flour products available in major wholesale and retail stores of the city showed that, of 34 brands available at baseline, three had nutritional information on iron fortification in the beginning of this period and 12 at the end of it. Corn flour and pasta products did not show any information on iron fortification on their labels. In the second and third steps of the study, all brands of flour and pasta products had this information on their labels.

The analysis of iron content in wheat flour samples collected in the households evidenced that, at baseline,[2] none had iron concentrations consistent with fortification, not even those products labeled fortified. However, the analysis of samples in the second phase of the study showed that about half of them had iron content equal to or higher than 4.2 mg/100 g flour.

The confirmatory analysis of iron content tested in samples of 23 different brands of flour products available at 12 months collection, carried out in the Instituto Adolfo Lutz, São Paulo, verified that five of them did not have iron content consistent with fortification.

Mean hemoglobin measurements were similar in the three steps of the study: 11.3 ± 2.8 g/dL, 11.2±2.8 g/dL, and 11.3 ± 2.5 g/dL at baseline, 12 months, and 24 months post-fortification, respectively (p=0.16).

Anemia prevalences were 30.2%, 41.5%, and 37.1% in the three steps of the study, respectively (p=0.02). Hemoglobin measurements were consistent with severe anemia (hemoglobin <7 g/dL)[22] in less than 1% of children studied.

There was no evidence of a clear impact of fortification on specific subgroups; some showed reduced mean hemoglobin while others had increased levels. Even though there were tested all the variables in the study, Table 4 shows only the analyses for those subgroups showing any changes. Children aged between zero and 11 months, males, belonging to families with higher income and born to mothers with higher schooling had a statistically significant reduction in mean hemoglobin levels in the second and third phases of the study, i.e., post-fortification. Similarly, those who consumed red meat and pasta at least one a week showed significantly reduced mean hemoglobin. On the other hand, children aged between 36 and 47 months, as well as those who had beans at least once a week, showed higher mean hemoglobin at 24 months post-fortification.

DISCUSSION

Positive experiences with iron-fortified flours have been described in European countries such as Sweden, Denmark, and Finland.[18]

Together with other interventions to reduce anemia, since 2001, African and Central Asiatic countries have implemented iron fortification of widely consumed food products such as wheat flour. At the same time, there have been developed studies for the assessment of the efficacy and effectiveness of such interventions.* A Sri Lanka study assessed the effectiveness of iron-fortified flour products before the implementation of fortification nationwide but no effect was evidenced on anemia among preschool children in areas where fortified food products were available.

Positive experiences have been reported in the Americas. In the US, food and milk infant formulas fortified with micronutrients can be accounted for reduced prevalence of anemia in children in the years between 1975 and 1984.[18] Venezuela's experience of 50% reduction of anemia prevalence among preschool children a year post-fortification of pre-cooked corn flour is the strongest evidence that high intake of fortified products directly benefits people.[8] In Chile, low prevalence of anemia among children under five has been attributed to iron fortification of wheat flour since 1950s, although there have not been new representative studies nationwide since 1975 to ascertain whether this effect remains.[10]

There are no studies in Brazil evaluating mass fortification of food products. The present study did not find any statistically significant difference in mean hemoglobin in children tested pre- and post-fortification of flour products. The analysis of subgroups did not show any evidences of a consistent impact on specific populations.

Given that it was an analysis of effectiveness where an intervention was tested under actual conditions, the study findings have to be interpreted based on certain considerations.[5] First it should be established whether the intervention has been actually provided to the population, in other words, whether flour products were actually fortified.

Instituto Adolfo Lutz analysis of iron content found in flour products available at 12 months post-fortification revealed that, of 23 brands tested, five did not have iron levels consistent with fortification.

Assuming that the intervention has been provided, the next step will be to ascertain whether these products have been effectively taken by the target population. As Mora[13] pointed out, low intake of fortified food can be accounted for lack of effect of fortification in children under six due to inadequate iron intake.

In the three steps of the present study, there was a similar pattern of flour intake in general. Although it does not assure that children in the study have been taking adequate amounts of food made with flour, which can increase iron supply to the body, it indicates that food intake cannot be considered a confounder in the analysis.

A Sri Lanka study showed that mean daily intake of 120 g of food made with wheat flour fortified with 6.6 mg iron per 100 g was not effective in increasing mean hemoglobin after 12- and 24-month follow-up.[14] In the present study, in addition to a 90 g mean intake of food made with flour in the population studied, iron added to flour products was lower (4.2 g/100 g). This can explain the lack of intervention effect but further studies are needed to accurately determine intake.

The lack of evidence of mass fortification interventions as effective strategies to reduce iron deficiency makes it difficult for policymakers to support the developing countries government's expenditure with such programs.[3] In addition to low intake of fortified food, poor documentation of iron levels using specific indicators, low bioavailability of iron used in fortified products, inadequate dietary practices resulting in low iron absorption; and failure to identify other causes of anemia[3] are some reasons for this lack of evidence.

In the present study, hemoglobin measurement was used to determine anemia cases. Isolated hemoglobin measurements show low sensitivity and specificity to diag-

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* World Health Organization. New Global alliance brings food fortification to world's poor. Global Alliance Improved Nutrition (GAIN) Press Release. Available at: www.who.int [Access on 2/8/2006]

nose iron deficiency anemia.[4] Other measures could be added such as transferrin saturation and serum ferritin. However, in field studies, these measures might imply in greater losses since mothers would have to take their children to laboratories for blood collection, which can affect the internal validity of the study. Or either, blood would have to be collected at home increasing costs. Moreover, the present study was an assessment of the effectiveness of a national program aiming at reducing the prevalence of anemia and thus it was reasonable to restrict the outcome to hemoglobin measures.

As for bioavailability, iron is the most difficult mineral to add to food products. High bioavailability compounds are those able to produce greater organoleptic changes. Another obstacle for iron use is posed by other dietary compounds that inhibit iron absorption. Phytic acid is found in cereal and vegetable grains and it is a major inhibitor of iron absorption.[7] In the present study, dietary data from the 24-hour food recall did not allow estimating the average consumption of phytates in the population studied.

Finally, anemia is unlikely to be caused by factors other than iron deficiency. In the study area, parasite infections and malaria are not considered causes of anemia. On the other hand, viral and bacterial infections, more often seen during winter time, are associated to iron depletion.[22] But as the three steps of the study were conducted at the same time of the year, the seasonality effect was controlled for. Although fever was more frequently reported by mothers in the third step of the study, when the analysis was stratified by this variable, mean hemoglobin was found to be similar at the three time points studied.

As a conclusion, iron fortification of flour products was not effective in increasing mean hemoglobin in children aged zero to 71 months after one and two years of intake. This can be partially explained by inadequate intake of flour, low bioavailability of iron added to food or even regular consumption of food rich in iron absorption inhibitors.

It is suggested the monitoring of iron compounds added to flour products as well as further studies on bioavailability.

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Supported by Fundo Nacional de Saúde, Ministério da Saúde (National Health Fund, Brazilian Ministry of Health - Process # 2799/2003).