Original Research

Effect of Iron-Fortified Drinking Water of Daycare Facilities on the Hemoglobin Status of Young Children

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Keywords: anemia, anthropometry, daycare, children, foods, iron-fortification.

Background: Anemia is the most prevalent nutrition problem in young children. One possible strategy to prevent anemia is affordable fortification of drinking water.

Objective: The aim of this study was to evaluate the impact of iron-fortified drinking water of daycare facilities on the hemoglobin and anthropometric status of pre-school children.

Design: Hemoglobin (Hb) status, weight and height measurements were assessed in 160 pre-school children aged 6 to 59 m before and after 8 m consumption of iron- (12 mg/L) and vitamin C- (90 mg/L) fortified drinking water.

Results: Initially, 43.2% (69) of the children evaluated as being anemic decreased to 21% (37) at the end of study. At baseline, 42 (26.3%) children suffered from moderate anemia and 27 (16.9%) suffered severe anemia, but after iron fortification, total number of children suffering from moderate and severe anemia had decreased to 32 (20.7%) and 5 (3%), respectively. Weight-for-age (WAZ), height-for-age (HAZ) and weight-for-height (WHZ) Z-scores increased significantly from -0.84 ± 1.03 to 0.06 ± 1.10, -0.84 ± 1.11 to 0.54 ± 1.10 and -0.39 ± 0.94 to -0.18 ± 1.14, respectively ($p < 0.05$). Daycare personnel reported increased appetite and food consumption and decreased absenteeism during intervention.

Conclusion: Daily consumption of iron-fortified drinking water in daycare facilities is an effective, simple and inexpensive means of reducing and controlling for moderate and severe anemia in pre-school children.

INTRODUCTION

Anemia has been the center of numerous studies worldwide. Globally, it is estimated that 46% of school-aged children in developing countries are affected by anemia [1]. There have been calls from international conferences endorsing ambitious goals to reduce malnutrition by the year 2000. However, many of these goals have not been achieved. In 1992, the International Conference on Nutrition met with the objective among others, to endorse the 1990 World Summit for Children's goal of halving the 1990 prevalence of underweight in young children and substantially reducing anemia by the year 2000 [2]. Unfortunately little progress has been made toward the global elimination of iron deficiency. It has often been reported that iodine and vitamin A deficiency receive far greater attention and support [3].

Anemia has few overt symptoms, this being partly a reason for a lack of action. There is a shortcoming of knowledge of its serious and often permanent consequences to the cognitive developments of young children, and its negative impact on the health of all people [1]. To achieve results, current thinking must be directed at prevention measures that entail simple and low cost fortification models, easily adaptable to cultural environments targeting all children anemic or not.

Therapeutic supplementation is considered a direct measure, while nutrition education, iron fortification and public health measures are indirect measures and may be applied to prevent iron deficiency. Ideally, the fortification of widely consumed and centrally processed food staples with iron should be the priority by most developing countries, if iron-intake levels are to improve in children [4].

To confront anemia problems in developing countries requires commitment, principally innovative "user-friendly" approaches which are appropriate to their socioeconomic and cultural environments, and are simple and sustainable. One such approach is to use potable drinking water as a vehicle for

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Journal of the American College of Nutrition, Vol. 24, No. 2, 107–114 (2005)
Published by the American College of Nutrition

iron fortification to prevent iron deficiency anemia among low socioeconomic populations. The use of drinking water is a world wide established large-scale vehicle for fluoridation [5] and iodization [6] that resulted in positive gains for control and elimination of dental caries and goiter.

During the 1990s, the fortification of drinking water with iron and ascorbic acid to control anemia was tested in laboratory animals, and later in humans [7]. Results demonstrated that drinking water fortified with iron sulfate and ascorbic acid during eight months contributed to reducing anemia in daycare children [4,8]. A small study involving 31 pre-school children in a daycare facility resulted in significant decreases in anemia. To confirm the results of Dutra-de-Oliveira *et al.* (1996) [4], we tested the drinking-water approach for iron fortification in a large-scale intervention study investigating the feasibility and logistics of long-term iron fortification of drinking water in preschool children enrolled in municipal daycare centers in a socioeconomic deprived region in southeast Brazil.

SUBJECTS AND METHODS

Study Site and Subjects

The feasibility and long-term effects of iron fortification of drinking water to control and prevent iron-deficiency with and without anemia was conducted in 2001, from March to November. The study was done in the town of Diamantina (population 43,000), located at an altitude of 1,200 meters above sea level, in the Jequitinhonha Valley region of the State of Minas Gerais, Brazil. This area is characterized as having a low socio-economic population, with poor hygienic conditions, the principle occupation being diamond extraction. The estimated population of pre-school children attending daycare facilities was 400. At enrollment, 221 healthy pre-school children aged 6–59 months were randomly selected to participate in study during 8 months of intervention. The following municipal daycare facilities participated in the study: São Vicente de Paulo (SVP), Bela Vista (BV), Pequeno Príncipe (PP), Creche Cazuza (CC), Bom Jesus (BJ), Rio Grande (RG), Creche Nazaré (CN) and Maria Antônia (MA). Children attended daycare Monday thru Friday from 7 a.m. to 5 p.m. Daily attendance was recorded, and average number of absent-days per child during the intervention period were recorded. An invitation letter and consent forms were sent to parents whose children were enrolled in any one of the centers. All children who received parental consent had their baseline data measured. During this study, 61 (26.7%) children left the study. The reasons were as follows: family migration 33 (14.9%); 26 (11.8%) due to episodes of infectious diseases or absent from daycare more than 10 d; 2 (0.01%) were diagnosed with severe anemia (<7.0 g/dL), who were excluded, and referred to the community hospital for further evaluation and treatment. Therefore, 160 children participated during the 8 m intervention study. Socioeconomic status data (mother's education, monthly income and living situation) were collected via a questionnaire administered to mothers by trained nurses before the intervention began. The research protocol was approved by the Human Ethics Committee at the University of Brasília (UnB).

Anthropometric and Hemoglobin Status Measurements

Anthropometric measurements (weight and height) were obtained at baseline, 4 m, and 8 m by four examiners who followed standard procedures according to World Health Organization [9]. Infants were weighed naked (to nearest 100 g) on a digital infant scale (Seca Inc., Sydney, Australia). Recumbent length was measured to the nearest 0.1 cm on a portable infant measuring tape [10]. The hemoglobin (Hb) level of 160 children was determined on site by fingerprick blood samples at baseline, 4 m and 8 m of intervention using the HemoCue® (Hemo®Cue, Inc, Ängelholm, Sweden). Cut-off for anemia as a hemoglobin value (Hb) was established at mild (<11.0 g/dL); moderate (between 7.0–10.0 g/dL) and severe (<7.0 g/dL) [11]. Hemoglobin values were accordingly adjusted to <11.8 g/dL, <10.8 g/dL and <7.8 g/dL after compensating for altitude [12]. Return visits were made to obtain data of children who were absent.

Stool Analysis

Before and after the first four months of intervention, fecal exams were made in 109 of 160 randomly selected children to determine the worm burden using Stoll's dilution egg-count technique [13]. A 2 g sample of feces was added to 60 mL of 0.1 N NaOH and the flask was mixed during 1 minute. An aliquot of 0.20 mL was then put onto a clean slide upon which a cover slip was placed. Total number of eggs were counted microscopically and expressed as the number of eggs per gram of stool by multiplying by a factor of 200. Subjects testing positive for parasites were treated with 100 mg of mebendazole (Pluriverm®, São Paulo, Brazil) twice daily during 3 days as to remove helminthiasis as a confounder of both growth and hematological status. This was carried out by trained supervisors from each daycare. Mothers, whose children tested positive for *G. lamblia*, were encouraged to seek treatment at local health clinics.

Intervention

Six hundred milliliters of deionized water was placed in a 1L Erlenmeyer flask to prepare the concentrated iron and vitamin C solution. Thirty-six grams of iron sulfate—$FeSO_4$ $7H_2O$ (12 g elemental iron) and 54 g of ascorbic acid were added and mixed with the 600 mL deionized water. From the concentrated iron and ascorbic acid solution, 1 milliliter was added to each 1 liter of drinking water consumed at each daycare. Amber 20 mL vials with screw caps were used to store the concentrated solution during daily fortification of drinking water. New solution was prepared by a trained laboratory technician and delivered each Monday and stored in refrigerators at each daycare until use. Daycare did not operate on weekends. Total number of milliliters of iron-solution was calculated after estimating daily consumption of drinking water in each daycare. To facilitate dispensation, iron-concentrated solution was added to mineral-water plastic jugs (20L). Daycare workers were instructed on how to add the iron-vitamin C solution to drinking water. Each morning, plastic jugs were rinsed-out with water and newly filtered drinking water was prepared with iron-solution (any remaining iron-fortified drinking water from the day before was used for cooking rice, noodles, and beans or preparing corn meal for breakfast). Supervision occurred twice weekly on randomly chosen days to insure adherence and quality control. Children had *ad libitum* access to drinking water during the entire attendance (7 a.m. to 5 p.m.).

Quality Control of Water

Color and turbidity of iron-fortified drinking water with and without ascorbic acid were done according to Dutra-de-Oliveira *et al.* (1996) [4]. Briefly, 25 mL of iron-fortified water sample was analyzed immediately after preparation, and 3 vials containing same quantity solution were stored for a period of 1, 3 and 7 d at room temperature (27°C). Water color was quantitatively determined by a Hellige Water Tester (Hellige Inc., Garden City, NY). Results were expressed in mg Pt/L. Turbidity measures were quantitatively determined using same quantity and days as were used for color, but analyzed using a Micronal Turbidometer-B250 (Micronal FA, São Paulo, Brazil). Results were expressed in mg SiO_2/L.

Estimation of Nutrient Intake

The quantity of foods consumed and the Dietary Reference Intakes (DRI) of nutrients offered to pre-school children were estimated at the beginning of study by test-weighing samples of randomly collected food served in five of eight-daycare. Foods were weighed separately using a digital balance (Instrutherm BD-140, São Paulo, Brazil) to the nearest 1 g. Three plates were weighed and mean weight was subtracted from total weight of each food that was weighed three times. Liquids were measured to the nearest 10 mL using a standardized measuring cup (Quality Injetemp Ind., São Paulo, Brazil). Average daily water consumption (mL/d) was measured in 10 randomly selected children from each daycare (n = 80). Each of the 10 children from the 8 daycare was assigned a measuring cup with his or her name. Children were served the fortified drinking water from the plastic serving jug *ad libitum.* Quantity served was subtracted from quantity consumed and recorded during one day measurement. Data were recorded on a food evaluation form, analyzed with Virtual Nutri 1.0 software for Windows [14].

Statistical Analyses

The distribution of each variable was tested for normality before analyses, using the Kolmogorov-Smirnov goodness of fit test [15]. Where necessary, data were normalized using appropriate transformations. Data are presented as means ± standard deviations. Pearson's correlation test was used to examine the association between Hb, weight and height. Weight-for-age (WAZ), height-for-age (HAZ) and weight-for-height (WHZ) Z-scores were calculated by using National Center for Health Statistics reference data using the EPI-INFO software, version 6 (Centers for Disease Control, Atlanta, GA). Weight and height gains were calculated by adjusting for the actual length of the interval between measurements. The change of Hb from pre- and post-treatment was examined and compared using Student's *t* test and ANOVA with the Bonferroni's multiple comparison tests. Statistical analyses were performed with SPSS software program (version 10.0; SPSS Inc., Chicago). Difference between pre- and post-treatment parasitic infection was expressed in percent. The acceptable level of statistical significance for all tests was $p < 0.05$. Results are expressed as arithmetic means (± SD) and ranges.

RESULTS

Baseline data of socioeconomic and biological variables are presented in Table 1. There were no statistically significant differences in socio-economic indicators between family/mothers of the 69 anemic children. The outstanding features were that: 1.) Most of the children belonged to socio-economically deprived families as indicated by low education and income of mother. Approximately 78.5% (113) of mother's income was ≤R$200 reais (US$80) per month, which was also the same for the Brazilian national average for the same year reported [16]; 2.) Sixty percent of the family members slept more than two per bed; 3.) 7.6% (11) and 11.8% (17) responded as using either a latrine or open camp, and 4.) 13.2% (19) and 11.8% (17) of the mothers indicated that electricity was either shared from a neighboring household or did not have electricity.

Anthropometry

Results of anthropometric measurements at baseline were −0.84 ± 1.03, −0.84 ± 1.11 and −0.40 ± 0.94 for WAZ, HAZ and WHZ Z-scores, respectively (Table 2). During the trial there was a significant difference between at baseline and 8 m post-intervention ($p < 0.05$). There were significant positive mean Z-score increases in WAZ, HAZ and WHZ at 8 m to 0.06 ± 1.10, 0.54 ± 1.10 and −0.18 ± 1.14, respectively. This resulted in an overall increased difference in Z-score of 0.90 ± 0.50, 1.38 ± 0.64 and 0.21 ± 1.0 for WAZ, HAZ and WHZ, respectively.

Table 1. Baseline Characteristics of Pre-School Children[1,2]

Variables	Intervention
Biological characteristics, n	160
Hemoglobin (g/dL)	11.8 ± 1.3
Anaemic (n)	69
Non-anaemic (n)	91
Gender (male:female)	67:93
Age (y)	3.4 ± 1.1
Weight (kg)	13.6 ± 2.6
Height (cm)	94.0 ± 10.7
Socio-Economic Characteristics	
Mother's education (y)	4 ± 2
Sleeping arrangement (n)	
1 bed ≤2 members	57
1 bed >2 members	87
Safe water (n)	117
Sanitation (n)	
Toilet in home	116
Latrine	11
Use open camp	17
Electricity (n)	
Own meter	108
Share meter	19
No electricity	17
Mother's monthly income (n)	
≤200 reais[3]	113
>200 reais	31

[1] Values are means ± SD or numbers of children.
[2] Total number of mothers interviewed = 144.
[3] 1 real = 0.40 US $ at the time of data collection.

Hematology

Hematological parameters are shown in Tables 2, 3 and 4. At baseline, mean Hb was 11.8 ± 1.3 g/dL. At 8 months, mean Hb increased to 12.4 ± 0.93 g/dL ($p < 0.01$) (Table 2). The overall prevalence of mild, moderate severe anemia was measured as the percentage of individuals responding to iron-fortification during 8 m. Initially, 43.2% (n = 69) were evaluated as being anemic (<11.8 g/dL). At the end of study, prevalence decreased to 21%. Before intervention began, 26.3% (42) of the children suffered from moderate anemia and 16.9% (27) suffered from severe anemia. At 8 m, 20.7% (32) and 3% (5) continued to suffer from less moderate and severe anemia, respectively (Table 3). The number of non-anemic had risen from 91 (56.9%) at baseline, to 126 (78.8%) at the end of study. Hemoglobin levels during the study were also grouped according to age (Table 4). Anemia was twice as high in children 6 to 23 m old compared to those aged 24 to 59 m. There were statistically significant increases in Hb levels in 6 to 23.9 m ($p = 0.02$), 24 to 35.9 m ($p < 0.01$) and 36 to 47.9 m of age groups ($p < 0.01$). Hemoglobin levels in children older than 48 m increased (0.05 g/dL), but were not statistically significant ($p = 0.45$). Finally, there were no gender differences for hemoglobin (Hb). There was no statistically significant association between hemoglobin levels and parasite infection after the second exam during the last 4-months of the intervention (baseline $p = 0.82$, post-exam $p = 0.62$).

Stool Analysis

Stool analysis showed that 55% of selected children (109) were infected with one or more parasites. Before trial, 39.1% (68) were infected with *Ascaris lumbricoides*, 16.1% (28) infected with *Entamoeba histolytica*, 13.8% (24) infected with *Entamoeba coli* and 39.1% (68) tested positive for *Giardia lamblia.* After 4 months, stool reevaluation showed that re-infection was a common problem. Specifically, infection by *Ascaris* decreased to 24.1% (43), infection of *E. histolytica* increased to 20.1% (35), but was virtually unchanged for *E. coli* and *G. lamblia*, 12.1% (24) and 37.4% (65), respectively. Overall intestinal parasitic infection decreased 4.4% (55% to 50.6%) upon reexamination.

Quality Control of Water and Organoleptic Properties

The quality control of water fortified with ferrous sulfate, ferrous sulfate and ascorbic acid are presented in Table 5. When 12 mg iron/L as ferrous sulfate was added to water, there was a color increase noticeable on day 1. The color development was noticed only for iron, resulting in the highest reading (70 mg Pt/L). When same concentration of iron was mixed with 90 mg/L of ascorbic acid, there was no color development. Turbidity was also markedly present in water fortified with iron only, and insignificant when 90 mg/L of ascorbic acid was added with iron. Mean acidity (mean pH 6.7) decreased for iron

Table 2. Change in Variables before and 8 m Post-Intervention

Variables[1]	Before	After	Difference	*p*
Hemoglobin (g/dL)	11.8 ± 1.3	12.4 ± 0.93	0.60 ± 1.3	<0.01
Absenteeism[2]	3.5 ± 1.6	2.5 ± 1.1	1 ± 2.7	<0.01
Anthropometry measurements (z-score)				
W/A	−0.84 ± 1.03	0.06 ± 1.10	0.90 ± 0.50	<0.05
H/A	−0.84 ± 1.11	0.54 ± 1.10	1.38 ± 0.64	<0.05
W/H	−0.39 ± 0.94	−0.18 ± 1.14	0.21 ± 1.0	<0.05
Intestinal parasites[3] (%), n = 109	55	50.6	4.4	0.62

[1] Values are means ± SD in 160 children.
[2] Mean ± SD of child/days absent per month before intervention and after 8 m post-intervention.
[3] Children testing positive for parasites were treated with 100 mg mebendazol (Pluriverm®) before and retested 4 m during intervention.

Table 3. Prevalence and Magnitude of Anemia among Pre-School Children Aged 6 to 59 m Participating in 8 m of Iron-Fortified Drinking Water in Day-Care Centers

	Before		After	
	n	%	n	%
Normal	91	56.9	126	78.8
Moderate anemia	42	26.3	32	20.7
Severe anemia	27	16.9	5	0.03
Total	160	100	160	100

Table 4. Mean and SD of Hemoglobin (Hb) Levels at Baseline and 8 m Post-Intervention According to Age Group in Children Attending Day-Care

Age group (m)	n	Hemoglobin (g/dL)		
		Before	After	*p*
6 to 23.9	18	11.0 ± 1.4	11.7 ± 1.0	0.02
24 to 35.9	42	11.5 ± 1.5	12.5 ± 1.0	0.01
36 to 47.9	39	12.3 ± 1.2	12.8 ± 0.9	0.01
> 48	61	12.5 ± 1.2	12.6 ± 0.8	0.45
Total	160	11.8 ± 1.3	12.4 ± 0.9	0.01

after addition of ascorbic acid (mean pH 4.6). These results are in line with those from Dutra-de-Oliveira [8] using iron and ascorbic acid in water in daycare facilities, as well as the use of orange juice as an iron carrier in a study by de Almeida *et al.* (2003) [17]. Although the pH of the solutions in both studies was not reported, a laboratory analysis conducted prior to both studies showed that water pH ranged from 4.1 to 5.1 after addition of iron and ascorbic acid [18]. Supervisors from the 8-daycare reported an adhesion of 100% since all children consumed the fortified drinking water (both types) without any complaints and/or negative side effects during the 8 m trial.

Estimation of Nutrient Intake

A two-day, nonconsecutive mean dietary evaluation of the quantity of nutrients offered to preschool children attending 5-daycare is shown in Table 6. Hypothetically, if all nutrients offered were consumed by preschoolers, mean total energy and iron consumption would be considered below the RDA allowances established for 1989 and 1997 for groups 1 to 3, and 4 to 8 years of age. Mean quantity of energy offered to age group 1 to 3 years was 746.98 ± 177.22 Kcal/d. This would amount to 58% and 61% of the recommended daily allowances for 1989 and 1997 RDAs, respectively. The quantity of energy (943.47 ± 173.42 Kcal/d) for age group 4 to 8 years of age was also below 1989 and 1997 RDA allowances of 1,800 and 1,660 Kcal/d, respectively. Mean quantity of iron offered in diet amounted to 50% of daily allowances (RDA 1989) and 71% of (RDA 1997) daily allowances for age group 1 to 3, and 70% (RDA 1989 and 1997) of daily allowances for age group 4 to 8 years. Percentages of 1989 RDA allowances were greater than 100%, and more than 200% (1997 RDAs) for protein and vitamin C for both age groups, respectively. Finally, mean total consumption of iron-fortified drinking water, estimated in milliliters per child/day, was 503.3 ± 64.8 mL/d. This represents an additional iron-intake of approximately 6.04 mg Fe/d.

DISCUSSION

The relatively simple method of adding concentrated iron solution to drinking water on a daily basis was effective in improving the iron-nutritional status of pre-school children. There are only two studies on iron-fortified drinking water with results comparable to ours [4,8]. These studies showed a substantial reduction of anemia prevalence in social-economically poor populations whose initial prevalence of iron deficiency was greater than 40%. It was demonstrated that incorporating 20 mg of element iron per liter of drinking water during 8 m in a small study involving 31 children at daycare reduced anemia prevalence in more than 48%, at a cost intake per child similar to our study [8]. Mean hemoglobin (Hb) level at baseline was 10.7 ± 0.7 g/dL and 13.0 ± 1.1 g/dL at 8 m. The second study on iron-fortified drinking was also conducted by Dutra-de-Oliveira *et al.* (1996) [4]. During 4 m, iron was added to drinking water and consumed by family members at home. Mean Hb levels at baseline in adults (12.9 g/dL) and in children (10.9 g/dL) increased significantly ($p < 0.01$) to 13.7 g/dL and 11.7 g/dL, respectively. Hemoglobin levels in control group families decreased, contrary to experimental group families. In both studies, iron-solution was added to clay-earthen water filter pots contrary to the use of mineral-water plastic jugs (20L), which were easier to maintain and clean in our study.

Table 5. Analysis of Iron-Fortified Drinking Water: Color and Turbidity

Solutions	Concentration (mg/L)		Color (mg Pt/L)[1] Days after preparation				Turbidity (mg SiO_2)[2] Days after preparation			
	Iron	Ascorbic acid	0	1	3	7	0	1	3	7
Ferrous sulphate	12	—	70	70	70	70	9.3	24.0	26.0	27.0
Ferrous sulphate + ascorbic acid	12	90	5	5	30	55	0.6	0.5	0.4	1.2

Quality control.
[1] Color: recommended up to 10 mg Pt/L, tolerable up to 20 mg Pt/L.
[2] Turbidity: recommended up to 2 mg SiO_2, tolerable up to 5 mg SiO_2 [32].

Table 6. Two-day Nonconsecutive Dietary Evaluation of Quantity of Nutrients Offered to Pre-Schoolers by Age Group in Day-Care according to Recommended Daily Allowances for Energy, Protein, Vitamin C and Iron

Age (yr)	Nutrients	Values	RDA[1] 1989	(%)	RDA[2] 1997	(%)
1–3	Energy (Kcal/d)	746.98 ± 177.22	1,300	58	1,225	61
	Protein (g/d)	26.67 ± 8.05	16	167	13	205
	Vitamin C (mg/d)	60.16 ± 32.82	40	150	15	401
	Fe (mg/d)	5.00 ± 1.44	10	50	7	71
4–8	Energy (Kcal)	943.47 ± 173.42	1,800	52	1,660	57
	Protein (g/d)	47.56 ± 12.77	24	198	19	250
	Vitamin C (mg/d)	60.96 ± 32.78	45	136	25	244
	Fe (mg/d)	7.0 ± 1.90	10	70	10	70

[1] Recommended Daily Allowances (RDA) (NRC, 1989) [35].
[2] Dietary Reference Intakes (DRI) for RDAs, 2002 [34].

The authors from the first study added 20 mg iron/L in drinking water during 8 m compared to 12 mg iron/L in our study, almost double. We considered 20 mg iron/L strongly affected taste, and therefore, limited concentration to 12 mg iron/L. There were mean Hb increases observed from post-intervention results in the second study (0.80 g/dL) when compared to our study (0.60 g/dL). The number of children, their social status, anthropometry and daily food-intake measures were not taken in the first nor second studies, thus limiting overall comparison to hematological results.

For an iron-fortification program to be successful, it is important to select food vehicles that are consumed daily, to select an iron compound that is well absorbed, and to have the ability to control enrichment [9,19]. Preparation, dispensation and management of water fortification presented no difficulty for daycare personnel, who had less than five years of school instruction. This simple procedure requires less then one hour each week to prepare the iron-concentrate, however, this may vary on the number of children to be targeted. The iron-fortified drinking water from the present study indicated that it was acceptable to children aged 6 to 59 m, and RDA allowance of iron increased, given less than one liter of iron-fortified drinking water was consumed daily.

After 8 m intervention, ~23% of children remained anemic. There are many possible explanations for this observation. Endemic infections such as gastroenteritis and respiratory infections are common in the region due to poor hygienic practices and the lack of a citywide sewage treatment facility. Infections and inflammatory diseases are referred to collectively as the anemia of chronic disease (ACD) and are known to interfere with utilization of absorbed iron [20]. Another possible reason for the lack of response to iron was a subclinical vitamin A deficiency because children in the Valley region are known to be at risk of this deficiency [21]. Studies have shown that vitamin A deficiency and anemia often coexist [22]. It has been shown that responses to iron fortification are limited in children with marginal vitamin A status [23] and that supplementation with iron is more effective when iron is given in conjunction with vitamin A [24]. Thus, it is plausible that those children who did not respond to the intervention during 8 m did not suffer from an iron deficiency, but had other causes of anemia.

Our findings suggest important policy and program implications for the treatment and prevention of anemia in preschool children populations using an inexpensively available iron-carrier such as water. Our data suggest that compliance was sufficient at improving anemia in subjects even though 38 (23%) failed to show improvements at end of study. Compliance was 100% while monthly absenteeism was low when compared to before intervention (2.5 ± 1.1 days) (Table 2). Mean Hb increased significantly in the three groups aged 7 to 47.9 m, but not significantly in children aged 48 m or older (Table 4). A probable reason is that the younger age groups started the intervention not only more affected by anemia (initial prevalence of 50%, compared with 31.1% in older children), but also with a higher proportion of children with low hemoglobin levels. For this reason, the magnitude of the catch-up needed to "cure" anemia in younger children was obviously much higher than that in older aged children.

Iron deficiency was common largely in part due to a high cereal-based diet, one that contains little animal protein, as the latter is generally too costly for families in this region. At the start of this study, we evaluated the mean quantity of foods offered to children in daycare. Results revealed that non-hem iron sources consisted of 5 mg iron/d, and only 2 mg iron/d was in the form of animal-derived heme-iron, for a total daily iron-intake of 7 mg iron/d. This would amount to 71% of 1997 RDA allowances for children 1 to 3 years, and 70% of 1997 RDA allowances for children 4 to 8 years of age (Table 6). Available total vitamin C offered in foods was high (>200% of 1997 RDA) in both age groups, which is a plus, as it will increase absorption of non-heme iron foods [25].

No further evaluation was done to measure possible increase in food consumption during study. The quantity and quality of foods served to children was limited, as there was not enough for repeated servings. Children attended daycare from 7 a.m. to 5 p.m. Monday thru Friday, and received four meals (breakfast, lunch, snack and dinner) per day. Dietary consumption by children at home was not evaluated. If appetite did increase, as was reported by daycare workers, there may have been an increase consumption of foods at home, particularly energy, contributing to an increase growth response.

It has been estimated that iron absorption can vary 1% to 40%, but this however, will depend on ones dietary habits, i.e. the mix of enhancers and inhibitors in the meal [26]. Children chronically anemic will possibly absorb a greater percentage of supplemental iron, for example, 35%, this according to double isotope techniques to determine the absorption of iron from ferrous sulfate when delivered via drinking water in an attempt to restore iron stocks, and in turn, restore hemoglobin levels [27]. Anemic children in daycare 1 to 3 years and 4 to 5 years consumed 5 mg iron/d and 7 mg iron/d, respectively. Another 6.04 mg iron/d was available when children consumed a mean of 503 mL of iron-fortified drinking water, for a total of 11.04 mg iron/d and 13.04 mg iron/d in both younger and older age groups. Theoretically, anemic children from the younger age group would absorb 3.9 mg iron/d (11.04 mg iron/d $\times$ .35), and the older age group would absorb 4.6 mg iron/d (13.04 mg iron/d $\times$ .35). Concerns regarding risks of iron overload from iron-fortified drinking water are very remote, as the quantity of iron/L (12 mg iron/L) is small, and iron overload disorders and hemochromatosis are mostly prevalent in European populations [28]. Importantly was that children attending daycare, especially at-risk of iron deficiency, had *ad libitum* to iron-fortified drinking water five days per week.

Parasitic infection, as demonstrated from fecal exams in 109 of the 160 children participating in the intervention study, brings forth the urgent need to improve overall hygienic conditions in areas were the poor inhabit. This region possesses one of the lowest Human Develop Indexes (0.49) according to UNICEF [10], and lacks proper sewage disposal and treatment systems as was earlier mentioned. The relatively high percentage of children infected with parasites can be attributed to the contaminated nearby streams and springs that often receive discharged human feces. Although *G. lamblia* is the most prevalent parasite infection in the region, municipal health centers do not distribute free medication, which is otherwise unaffordable by most. This explains the insignificant decrease (39.1% to 37.4%) in *G. lamblia* cases after reevaluation was mostly due to the family's inability to afford anti-Giardia medication and the practice of basic, hygienic measures.

During the fortification period, weight and height increased as would be expected for growing children. Effects of iron-fortified drinking water may have possibly influenced this increase, as there were expressive, statistically significant increases seen in Z-score values and these results could not have happened by chance alone. Correlation between anemic children (69) and mean WAZ Z-score (-1.02 ± 0.81) was statistically significant ($p < 0.05$) at baseline, however as there were less anemic children (34) at study end, this was not longer observed ($p = 0.87$), and final WAZ Z-score was 0.04 ± 1.02. Daycare workers reported increased appetite in children during study, but the extent to which this may be true was not measured. During the study, children were less absent than before, which may indicate that they were less prone to disease. Many children who were stunted (11.3%) or severely underweight (8.1%) at baseline were less so to be after 8 m of intervention as percentage of children with a Z-score of <-2 for HAZ and WAZ decreased from 11.3% and 8.1%, to 1.3% and 1.9% respectively.

Two shortcomings of the study design included the use of one hematological indicator and the ethical consideration of depriving an anemic control group of receiving iron. Since hemoglobin is the most readily measured essential iron compound, an increase in Hb concentration in response to iron administration can be regarded as presumptive evidence of prior iron deficiency [29]. Hemoglobin measurement to detect iron-deficiency anemia is suitable in field circumstances where other methods are less suitable and make analysis complex. Furthermore, hemoglobin measurements give a satisfactory estimate of the prevalence of iron deficiency when prevalence is high [30,31].

Despite the limiting factors of a control group and use of one hematological measure in assessing iron deficiency in children, we feel confident that the high prevalence of anemia from this child population was real, and thus justified a creative, low-cost, effective intervention model. In fact, the mean daily quantity of foods available to children was below the 1997 RDA allowances of 7 mg iron/d and 10 mg iron/d for age groups 1 to 3 and 4 to 8 years, respectively (Table 6).

There is a great need for alternative, feasible, effective and practical ways to distribute iron. The role of nutrition education as a long-term solution should, however, not be overlooked. Should a program such as iron-fortified drinking water be implemented in schools, it is strongly recommended that it be accompanied by a relevant nutritional message, which would put fortified drinking water into perspective. Finally, advocacy and national programs should no longer be constrained by erroneous perceptions that effective, practical interventions are not available [33].

We conclude that consumption of drinking water fortified with iron contributed to increasing iron-intake to meet minimal daily-recommended allowance of bioavailable iron acceptable to pre-school children aged 6 to 59 m attending daycare. Other then adding iron-concentrate to the appropriate number of liters of drinking water, it is easy to distribute and can be easily monitored. This program was offered to all children ($\approx$400) attending daycare and will continue on a permanent daily basis, and plans are to multiply it to other regions with a high prevalence of anemia. Future design replication using controls and additional hematological parameters could add to the argument for increased fortification of non-fortified foods in an attempt to reduce iron deficiency anemia in developing countries.

ACKNOWLEDGMENTS

We would like to thank Fabíola Adriane Cardoso Santos and Juliana Mambrini for their statistical analysis support.

Special thanks to Dr. José Garrofe Dórea for his redactorial suggestions. Support for this project was especially appreciated in the form of a scholarship of the Brazilian National Council of Scientific and Technological Development (CNPq).

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Received July 31, 2003; revision accepted December 5, 2004