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ORIGINAL ARTICLE

The effect of folic acid supplementation with ferrous sulfate on the linear and ponderal growth of children aged 6–24 months: a randomized controlled trial

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BACKGROUND/OBJECTIVES: Studies evaluating the effect of folic acid supplementation, either alone or in combination with iron, on the linear and ponderal growth of children are practically nonexistent. The aim of this study was to assess the effect of folic acid supplementation with ferrous sulfate on both linear growth and weight gain in anemic and nonanemic children attending Municipal Daycare Centers in Goiania, State of Goias, Brazil.

SUBJECTS/METHODS: A double-blind, randomized, controlled trial was conducted on 188 children aged 6–24 months. The effects of ferrous sulfate and folic acid supplementation were evaluated using the analysis of variance procedure, based on a double factorial model with two factors of fixed effects (folic acid supplementation and ferrous sulfate supplementation), adjusted for initial weight. The level of significance was 0.05.

RESULTS: The children who received folic acid supplementation showed greater weight gain than the monthly average weight gain of those not given the supplement ($P = 0.026$). This effect was independent of the dose of ferrous sulfate (P for interaction = 0.693). Folic acid supplementation increased the gain of weight-for-age Z-score when compared with the placebo group ($P = 0.018$), independent of the dose of ferrous sulfate.

CONCLUSION: Folic acid had no effect on linear growth. The use of folic acid supplementation increased the monthly average weight gain and the gain in weight-for-age Z-score compared with the placebo group. This effect was independent of the dose of ferrous sulfate.

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INTRODUCTION

Iron deficiency anemia (IDA) is a serious public health problem around the world; it is estimated that the overall prevalence of anemia in children aged 6–59 months is approximately 273 million.[1,2] This problem is closely related to the development and growth of children.[2] Data from the Brazilian Survey of Demographics and Health (PNDS, 2006), for instance, indicate that the prevalence of anemia in 6- to 59-month-old children is 20.9%.[3] However, more recent studies have reported higher values than those reported in the PNDS (2006), indicating an ongoing trend of an increasing prevalence of anemia in children.[4,5]

Children have a greater need for iron owing to the rapid expansion of tissues, which is proportional to the speed of growth and increased erythrocyte mass, resulting in an increased susceptibility to anemia.[6,7] The primary negative effects associated with iron deficiency anemia in childhood include deleterious consequences on cognitive and psychomotor development, impairments to immunity resulting in lower resistance to infections and deficits in both weight gain and stature growth.[1,2,8,9]

To treat anemia, some studies suggest that combined ferrous sulfate and folic acid supplementation would be more effective than ferrous sulfate supplementation alone.[10–12] In addition, a lack of folate may act as a limiting factor in weight gain and stature growth.[12–14] Studies also imply that iron plus folic acid supplementation can be justified based on the beneficial effects of these compounds on the cognitive and motor development in infants,[15–17] thereby reducing the risk of iron deficiency anemia.[18]

The benefits and disadvantages of using different dosages of iron either alone or in combination have been investigated, but only partially, in relation to linear growth and weight gain.[2,19] With regard to iron plus folic acid supplementation, the data are both rare and imprecise.[20,21] It is important to note that, in the Brazilian context, there are no recorded studies evaluating the effect of ferrous sulfate plus folic acid supplementation on growth in anemic and nonanemic infants.

In this study, we address the effect of iron and folic acid supplementation on infant growth. The aim is to evaluate the effect of folic acid supplementation with ferrous sulfate on linear growth and weight gain in anemic and nonanemic children enrolled in Municipal Daycare Centers (MDCs) in Goiania, State of Goias, Brazil.

SUBJECTS AND METHODS

This study used data obtained from a survey entitled 'Nutritional Anemia: Prevention and Treatment in Early Childhood', conducted in the city of Goiania, State of Goias, Brazil, from April 2005 to March 2006.[10] It is a randomized, double-blind, placebo-controlled clinical trial. The target

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population consisted of infants attending MDCs of the Goiania Municipal Department of Education.

Details of the study design have been previously reported.[10] Sampling occurred in two phases. In the first phase, all MDCs, 53 units, that had nurseries were selected (447 children). On the basis of these data, children were chosen through probabilistic sampling: 25 MDCs that presented at least four children aged 6–24 months were selected using a random numbers table in Microsoft Excel. In the second phase, after randomly selecting 25 MDCs with 268 children in total, 49 children were excluded for not meeting the predetermined criteria, leaving a total of 219 children. Among these, some additional children were excluded: eight children left the daycare centers; seven children stopped attending blood sample collections; the parents of eight children canceled the written informed consent; four children had hemoglobin $\geqslant 7.0$ and < 8.0 g/dl; one child became ill during the treatment; and three children did not have complete data in the anthropometric survey. Therefore, the final sample size was 188 infants (see Figure 1).

The inclusion criteria were as follows: all children aged 6–24 months who, at the time of the interview, attended the 25 MDCs with nurseries and whose parents or guardians agreed to participate in the research, giving written informed consent. Exclusion criteria included premature children, twins, children with special needs, low birth weights (< 2.500 g), neurological syndromes, cardiomyopathies and hemoglobinopathies, as well as children being treated for anemia.

For identification purposes, information was collected about the child, as well as the mother, including schooling, age and per capita income. In relation to the children, the birth weight, birth length and the type of feeding (that is, totally breastfed, formula fed or a combination of both) were all investigated. In addition, a clinical assessment was performed: standardized weight and length measurements were collected by properly

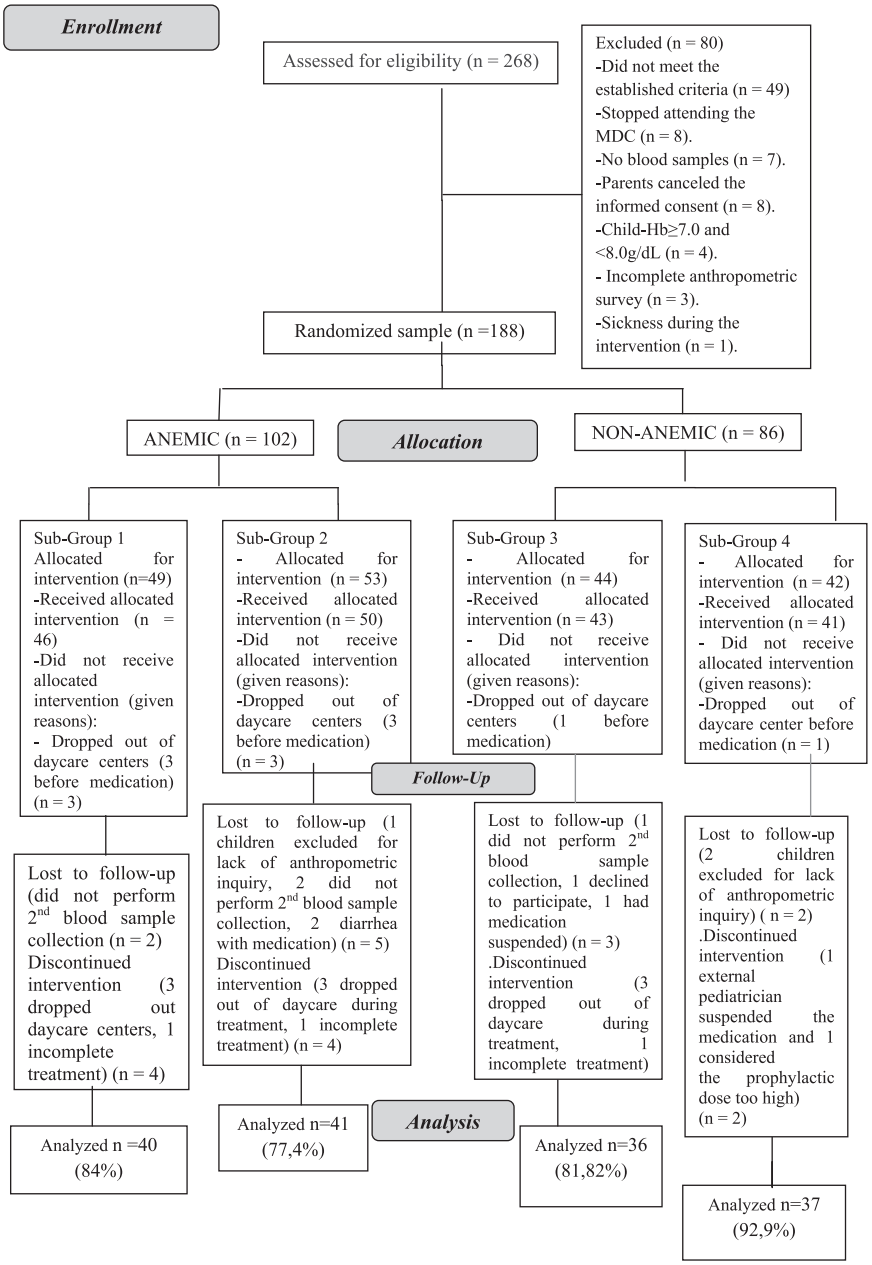

Figure 1. Logistic stages of the study. Group – anemic children, Subgroup 1: treatment with ferrous sulfate+folic acid. Subgroup 2: treatment with ferrous sulfate+placebo. Group – nonanemic children. Subgroup 3: treatment with ferrous sulfate+folic acid. Subgroup 4: treatment with ferrous sulfate+placebo. Anthropometric survey: weight-for-age Z-score; weight-for-length Z-score; length-for-age Z-score.

trained nutritionists and/or academics of nutrition, under the supervision of the research team.

At the time of the first and second blood sample collections, the children were weighed undressed on an electronic platform scale by Kratos (Kratos Cas, Taboão da Serra, Brazil), with a 150 -kg capacity, accurate to 0.05 kg. Length was measured using a length board, with the infant in a supine position.

Laboratory methods

Before and after the intervention, ~8 ml samples of venous blood were drawn from the infants after fasting. The samples were collected in two tubes: one containing EDTA for complete blood count analysis using the Cell-Dyn 3200 SL (Abbott Diagnostics, Mount View, CA, USA) and the other without anticoagulant, for biochemical analysis. With blood from the second tube, serum ferritin levels were assessed by chemiluminescence in the Abbott AxSym System Laboratory of Brazil Ltd. Children with hemoglobin levels < 11 g/dl were considered anemic.[22]

For the intervention, a pediatrician diagnosed the children as either anemic or nonanemic. The anemic group was composed of subgroups 1 and 2, and the nonanemic group was composed of subgroups 3 and 4 (described below). The MDCs were randomly allocated to receive folic acid or placebo. Some MDCs had groups 1 and 3, which received folic acid for the prevention and treatment of anemia, whereas other MDCs had groups 2 and 4, which received the placebo. The groups were divided by MDCs to avoid possible biases in the administration of the medicinal product within each MDC. Once divided into groups, the infants in each group received the following treatments from Monday to Friday:

Anemic children group

- Subgroup 1: daily treatment with 4.2 mg/kg/day ferrous sulfate+folic acid (50 μg/day);
- Subgroup 2: 4.2 mg/kg/day ferrous sulfate+placebo.

Nonanemic Children Group

- Sub-group 3: 1.4 mg/kg/day of ferrous sulfate + folic acid (50 μg/day);
- Sub-group 4: 1.4 mg/kg/day of ferrous sulfate + placebo.

The anemic infants received a treatment dose and the nonanemic patients received a prophylactic dose, according to the recommendation of the WHO.[22] The amount of folic acid given was in accordance with the recommendation of Stoltzfus and Dreyfuss.[23] As the children attended the MDCs during the weekdays, the daily doses were adjusted for Monday to Friday, in accordance with Hadler *et al.*[10] The children in each group received a median of 67 doses of medication and/or placebo.[10] To design the intervention with 40 children in each treatment arm, with the aim of detecting a minimum difference of 0.5 g/dl with a standard deviation of 0.7 and significance of 0.05, the test power was 0.89. This sample size is sufficient to detect differences of the same magnitude between the groups. The SAMPSI procedure from Stata 7.0 (Stata Corp., College Station, TX, USA) was used for calculation.[10]

Blinding was applied to the folic acid. Mothers, teachers or school staff, pediatrician, interviewers and the other participants were blinded after assignment to interventions. More details of blinding and use of placebo were previously reported.[10]

All drugs were administered by the teachers in the MDCs, after consultation and prescription by a pediatrician and authorization from those responsible for each child. The blood tests were repeated after all treatments were given, and new pediatric consultations were also performed. One of the two printed copies of the blood test results was provided to parents. The other copy was attached to the form of each infant.

The study was approved by the Research Ethics Committee of the Clinical Hospital of the Federal University of Goias, protocol no. 098/2004. All participants gave written informed consent. The study was registered with ClinicalTrials.gov, given the identifier NCT00701246, and is in accordance with the recommendations of the CONSORT (Consolidated Standards of Reporting Trials).[24]

Data analysis

The database was created after double typing, using the validation software Epi-info 6.04d to check and correct the data. We used the Windows Statistical Package for the Social Sciences – SPSS (version 18.0, SPSS Inc., Chicago, IL, USA) to analyze the database and the WHO Anthro[25] version 3.2.2, 2011 software to calculate the weight-for-age, length-for-age and weight-for-length Z-scores.

The Kolmogorov–Smirnov test was applied to assess adherence to the normal distribution of continuous variables and the nutritional status, where normal was defined as $P \geqslant 0.05$. Descriptive analyses (mean, s.d., median and percentiles 25° and 75°) were also used. Differences among proportions were tested by the χ^2-test. For variables that did not show normal distribution, the Mann–Whitney test was used for comparisons between the groups. In the variables with normal distribution, Student's *t*-test was used to evaluate independent samples in relation to anthropometric surveys. The homogeneity of variances was assessed by Levene's test. The averages for the groups, before and after the intervention, were assessed by the paired sample *t*-test. The evaluation of the effects of ferrous sulfate and folic acid supplementation was performed using analysis of variance based on a double factorial model, with two factors of fixed effects (folic acid supplementation and ferrous sulfate supplementation), with adjustment for the initial weight at baseline. The level of significance was 0.05 or 5%.

RESULTS

A total of 188 children were selected: 102 anemic patients (54.25%) and 86 nonanemic patients (45.75%). Among these, 154 patients (82%) completed the intervention and the anthropometric survey data. In Table 1, the characterization of each sample at baseline is described. The anemic and nonanemic groups of children did not differ in relation to gender, decimal age in months, birth weight, per capita income, maternal education, initial hemoglobin and ferritin, as well as the anthropometric indices data.

Within the group of anemic children, those who were supplemented with folic acid showed the highest mean weight at both the start ($P = 0.041$) and end ($P = 0.019$) of the study, compared with the patients who received the placebo (Table 2). For this reason, analysis of variance with a double factorial model was used with two factors of fixed effects, adjusted for the initial weight (Table 3). By comparing the mean values of the anthropometric indices of children before and after supplementation (Table 4), a significant increase was observed in the final average weight and the final average medium length in the anemic and nonanemic children groups, regardless of the type of supplement given. This result is consistent with the fact that this was a longitudinal study. For this reason, the use of Z-scores better reflects the growth data.

The final weight-for-age Z-score was higher in anemic children who received folic acid, compared with those who received the placebo ($P = 0.017$; Table 2); however, there were no statistically significant differences between these groups in relation to total weight gain, delta weight, total length gain, delta length, initial and final length-for-age Z-scores, initial and final weight-for-length Z-scores and weight-for-length Z-score gain.

In the nonanemic group supplemented with folic acid, a higher gain of weight-for-length Z-score was observed, compared with children who received the placebo ($P = 0.05$; Table 2). The weight-for-length Z-scores of nonanemic children who received the placebo were significantly reduced between the initial and final measurements ($P = 0.007$). However, the length-for-age Z-score in this group improved significantly between the evaluations ($P = 0.031$; Table 4).

The use of a prophylactic dose of ferrous sulfate in nonanemic children (Table 3) did not alter the rate of weight gain (g/month) or the total weight gain. However, the final weight-for-age Z-score had a tendency to be lower in children who received the prophylactic dose, compared with those who received the treatment dose of iron ($P = 0.052$) (Table 3). The use of folic acid,

Table 1. Socioeconomic, anthropometric and hematologic characteristics of children aged 6–24 months ($n = 188$) at baseline that were enrolled in Municipal Daycare Centers, Goiania, Goias, Brazil, 2005/2006

	Anemic (n = *102*)			*Nonanemic* (n = *86*)		
	Folic acid (n = *49*)	*Placebo* (n = *53*)	P-*value*	*Folic acid* (n = *44*)	*Placebo* (n = *42*)	P-*value*
Male	29 (50%)	29 (50%)	0.65[a]	25 (60.97%)	16 (39.03%)	0.08[a]
Female	20 (45.45%)	24 (54.55%)		19 (42.2%)	26 (57.8%)	
Age (months)[b]	13.02 ± 3.83	12.57 ± 4.7	0.592[c]	14.52 ± 4.3	15.76 ± 3.05	0.128[c]
Weight birth (g)[b]	3283 ± 393	3386 ± 448	0.222[c]	3316 ± 382	3417 ± 364	0.209[c]
Monthly per capita family income[d]	0.41 (0.28–0.70)	0.33 (0.21–0.54)	0.076[e]	0.52 (0.27–0.69)	0.54 (0.33–0.87)	0.424[e]
Maternal education (full years)[d]	7 (4.5–9.5)	8 (5–9.5)	0.546[e]	8 (5–11)	9.5 (6–11)	0.058[e]
Initial hemoglobin (g/dl)[b]	10.03 ± 0.76	9.89 ± 0.70	0.358[c]	11.74 ± 0.59	11.82 ± 0.59	0.515[c]
Initial ferritin (mg/l)[d]	6.1 (3.28–16.03)	6.32 (2.98–15.02)	0.987[e]	6.46 (11.71–21.31)	12.76 (5.32–25.40)	0.685[e]
Anthropometric indices						
Weight–for-age Z-score	0.31 ± 0.76	0.01 ± 0.87	0.073[c]	− 0.01 ± 0.87	0.26 ± 0.89	0.152[c]
Weight-for-length Z-score	0.46 ± 0.94	0.16 ± 0.88	0.100[c]	0.22 ± 1.04	0.47 ± 0.75	0.210[c]
Length-for-age Z-score	− 0.07 ± 0.83	− 0.19 ± 1.04	0.522[c]	− 0.38 ± 0.69	− 0.16 ± 1.21	0.302[c]

[a]χ^2-test. [b]Mean ± s.d. [c]Student's *t*-test for independent sample. [d]Median (percentiles 25 ° and 75 °). [e]Mann–Whitney test.

Table 2. Measures and anthropometric indices of anemic and nonanemic groups before and after the intervention

	Anemic[a]				*Nonanemic*[b]				
	Folic acid		*Placebo*		P-*value*[c]	*Folic acid*		*Placebo*		P-*value*[c]
	n	*Mean ± s.d.*	n	*Mean ± s.d.*		n	*Mean ± s.d.*	n	*Mean ± s.d.*	
Weight (kg)										
Initial	49	10.34 ± 1.30	53	9.81 ± 1.29	0.041	44	10.31 ± 1.27	42	10.65 ± 1.22	0.215
Final	40	12.05 ± 1.35	41	11.36 ± 1.24	0.019	36	11.75 ± 1.33	37	11.92 ± 1.33	0.593
Total weight gain	40	1.54 ± 0.54	41	1.35 ± 0.58	0.132	36	1.44 ± 0.59	37	1.30 ± 0.49	0.265
Delta weight (g/month)	40	221.4 ± 79.35	41	192.88 ± 80.23	0.112	36	216.33 ± 82.45	37	190.27 ± 71.41	0.153
Length (cm)										
Initial	49	77.79 ± 4.42	53	76.67 ± 5.62	0.265	44	78.66 ± 4.67	42	79.61 ± 4.17	0.325
Final	40	85.47 ± 4.18	41	84.05 ± 4.44	0.144	36	85.35 ± 4.18	37	85.92 ± 4.24	0.566
Final length gain	40	7.18 ± 1.82	41	6.5 ± 2.26	0.142	36	6.14 ± 2.37	37	6.50 ± 1.19	0.412
Delta length (cm/month)	40	1.03 ± 0.26	41	0.94 ± 0.33	0.196	36	0.93 ± 0.35	37	0.95 ± 0.17	0.722
Weight-for-age Z-score										
Initial	49	0.31 ± 0.76	53	0.01 ± 0.87	0.073	44	− 0.01 ± 0.87	42	0.26 ± 0.89	0.152
Final	40	0.38 ± 0.79	41	− 0.07 ± 0.88	0.017	36	− 0.03 ± 0.89	37	0.06 ± 0.79	0.638
Weight-for-age gain	40	0.003 ± 0.40	41	− 0.11 ± 0.43	0.208	36	0.06 ± 0.42	37	− 0.10 ± 0.36	0.074
Length-for-age Z-score										
Initial	49	− 0.07 ± 0.83	53	− 0.19 ± 1.04	0.522	44	− 0.38 ± 0.69	42	− 0.16 ± 1.21	0.302
Final	40	0.07 ± 0.99	41	− 0.24 ± 0.86	0.133	36	− 0.27 ± 0.97	37	− 0.24 ± 0.95	0.878
Length-for-age gain	40	0.07 ± 0.66	41	− 0.10 ± 0.80	0.31	36	0.05 ± 0.70	37	0.14 ± 0.38	0.708
Weight-for-length Z-score										
Initial	49	0.46 ± 0.94	51	0.16 ± 0.88	0.1	44	0.22 ± 1.02	42	0.47 ± 0.75	0.210
Final	40	0.41 ± 0.89	41	0.05 ± 0.89	0.073	36	0.11 ± 0.90	37	0.20 ± 0.70	0.660
Weight-for-length gain	40	− 0.09 ± 0.60	41	− 0.11 ± 0.71	0.89	36	0.04 ± 0.70	37	− 0.26 ± 0.54	0.050

[a]Treatment dose of iron supplement in anemic children. [b]Prophylactic dose of iron in nonanemic children. [c]Student's *t*-test.

when compared with placebo, produced a significant effect on the variable delta weight values (g/month; $P = 0.026$). This effect was independent of the ferrous sulfate dose (P for interaction = 0.693; Table 3).

Children supplemented with folic acid also showed a trend of higher total weight gain and higher final weight than those not given the supplement ($P = 0.050$). This effect was independent of the dose of ferrous sulfate (P for interaction = 0.539). A positive effect of folic acid supplementation was observed both in the gain of weight-for-length Z-score ($P = 0.075$) and the gain of weight-for-age Z-score ($P = 0.018$), independent of the use of ferrous sulfate (P for interaction = 0.538 and P for interaction = 0.716, respectively). These results indicate that folic acid is responsible for the highest monthly average weight gain (g/month) and gain of weight-for-age Z-score (Table 3).

Table 3. Analysis of variance of the effect and interaction of ferrous sulfate and folic acid supplementation on growth parameters and anthropometric indices

Variable	*Ferrous sulfate supplementation group*			*Folic acid supplementation group*			*Interaction*
	Treatment dose[a] (n = *81*)	*Prophylactic dose*[a] (n = *73*)	P-*value*[b]	*Acid folic* (n = *76*)[a]	*Placebo* (n = *78*)[a]	P-*value*[b]	P-*value*[b]
Weight (kg)[c]							
Final weight	11.70 ± 1.33	11.83 ± 1.33	0.528	11.91 ± 1.34	11.62 ± 1.31	0.05	0.539
Delta weight (g/month)	206.96 ± 80.58	203.12 ± 77.63	0.875	219.0 ± 80.33	191.64 ± 75.70	0.026	0.693
Total weight gain	1.44 ± 0.57	1.37 ± 0.54	0.528	1.49 ± 0.56	1.33 ± 0.54	0.05	0.539
Length (cm)							
Final length	84.76 ± 4.35	85.64 ± 4.19	0.205	85.41 ± 4.15	84.94 ± 4.42	0.539	0.151
Length gain	6.83 ± 2.07	6.32 ± 1.86	0.105	6.69 ± 2.15	6.50 ± 1.82	0.622	0.103
Delta length (cm/month)	0.98 ± 0.30	0.94 ± 0.27	0.321	0.98 ± 0.31	0.94 ± 0.26	0.496	0.239
Weight-for-length Z-score[c]							
Final weight-for-length Z-score	0.23 ± 0.91	0.16 ± 0.80	0.305	0.27 ± 0.90	0.12 ± 0.81	0.373	0.391
Weight-for-length Z-score gain	− 0.10 ± 0.66	− 0.11 ± 0.64	0.76	− 0.03 ± 0.65	− 0.18 ± 0.64	0.075	0.538
Weight-for-age Z-score[c]							
Final weight-for-age Z-score	0.15 ± 0.86	0.02 ± 0.84	0.052	0.19 ± 0.86	− 0.007 ± 0.83	0.195	0.288
Weight-for-age Z-score gain	− 0.06 ± 0.41	− 0.02 ± 0.40	0.389	0.03 ± 0.40	− 0.11 ± 0.40	0.018	0.716
Length-for-age Z-score							
Final length-for-age Z-score	− 0.09 ± 0.93	− 0.25 ± 0.95	0.279	− 0.09 ± 0.98	− 0.24 ± 0.90	0.362	0.255

[a]Mean ± s.d. [b]Analysis of variance of the general linear model using two fixed factors. [c]The variables were adjusted in relation to the initial weight.

Table 4. Comparison of growth parameters and anthropometric indices in anemic and nonanemic children who completed the treatment or prophylaxis proposed by the study

Variables	*Anemic*[a]		*Nonanemic*[b]	
	Folic acid (n = *40*)[c]	*Placebo* (n = *41*)[c]	*Folic acid* (n = *36*)[c]	*Placebo* (n = *37*)[c]
Weight, kg				
Initial	10.51 ± 1.30	10.01 ± 1.25	10.30 ± 1.36	10.61 ± 1.26
Final	12.05 ± 1.35	11.36 ± 1.24	11.75 ± 1.33	11.91 ± 1.33
P-value[d]	< *0.001*[d,e]	< *0.001*[d,e]	< *0.001*[d,e]	< *0.001*[d,e]
Length, cm				
Initial	78.29 ± 4.05	77.60 ± 5.25	79.28 ± 4.82	79.42 ± 4.28
Final	85.47 ± 4.18	84.05 ± 4.44	85.35 ± 4.17	85.92 ± 4.24
P-value[d]	< *0.001*[d,e]	< *0.001*[d,e]	< *0.001*[d,e]	< *0.001*[d,e]
Weight-for-age Z-score				
Initial	0.38 ± 0.80	0.04 ± 0.89	− 0.09 ± 0.87	0.16 ± 0.82
Final	0.38 ± 0.79	− 0.07 ± 0.87	− 0.03 ± 0.89	0.06 ± 0.79
P-value[d]	*0.965*[d]	0.097[d]	0.356[d]	0.100[d]
Length-for-age Z-score				
Initial	0.005 ± 0.85	− 0.14 ± 0.91	− 0.32 ± 0.70	− 0.38 ± 0.88
Final	0.067 ± 0.99	− 0.24 ± 086	− 0.27 ± 0.96	− 0.24 ± 0.95
P-value[d]	*0.528*[d]	0.428[d]	0.659[d]	0.031[d,e]
Weight-for-length Z-score				
Initial	0.50 ± 1.00	0.16 ± 093	0.08 ± 1.03	0.45 ± 0.75
Final	0.41 ± 0.89	0.05 ± 089	0.11 ± 0.90	0.19 ±0.70
P-value[d]	*0.332*[d]	0.312[d]	0.765[d]	0.007[d,e]

[a]Treatment dose of iron supplement in anemic children. [b]Prophylactic dose of iron in nonanemic children. [c]Mean ± s.d. [d]Paired sample *t*-test. [e]Significant difference (*P*-value < 0.05).

DISCUSSION

To our knowledge, this is the first study to demonstrate that folic acid is responsible for increasing the monthly average weight gain and leads to a great gain in weight-for-age Z-score at the end of the intervention in both anemic and nonanemic infants. The use of folic acid tended to both increase the total weight gain and result in higher final weights. These results are independent of the use of ferrous sulfate. In other words, it is the folic acid and not iron that has led to these increases. This occurred regardless of the initial weight of the child, because the initial weight was used as a covariate adjusted in the analysis. The results are useful, in particular, for guiding public health actions to decrease the deficits in both linear growth and weight gain[19] by strengthening the efficacy of iron supplementation programs.

It is worth emphasizing that previously published randomized clinical trials did not observe any effect of iron and folic acid treatments on changes in length-for-age Z-scores in anemic and nonanemic infants,[16] a result that is confirmed in the present study. However, nonanemic children given prophylactic doses of iron and placebo showed better length-for-age Z-scores at the end of the study. Nonanemic children supplemented with folic acid did not differ in weight-for-length Z-scores at the end of intervention, whereas the placebo group showed a significant reduction in Z-score.

In the Brazilian literature, only data that evaluated the growth by means of iron supplementation are found. Silva *et al.*[26] compared nonanemic infants supplemented with different prophylactic doses of iron and found that there were no differences in relation to weight gain, length gain and increments in anthropometric indices. In disagreement with these results, other studies have found negative effects of ferrous sulfate supplementation on the growth in nonanemic infants. A randomized, controlled study of Swedish children and Honduran breastfed children showed that iron supplementation affected the linear growth in nonanemic children.[27] Similarly, in a randomized, controlled study with placebo among nonanemic

($n = 100$) and anemic ($n = 50$) Indian children aged 6–24 months, the researchers reported that iron therapy, in comparison with the placebo, significantly decreased both weight gain and linear length among the nonanemic children.[28] In addition, a cohort study with nonanemic Italian babies found that a group of breastfed babies receiving oral iron supplementation were significantly shorter in length, compared with babies not receiving the supplement.[29]

Another important contribution of the present study is that the prophylactic iron supplementation did not differ from the treatment supplementation in regard to the effects on linear growth and weight gain. Therefore, the data show that there is no risk to the growth of nonanemic children given prophylactic doses of ferrous sulfate.

A methodological limitation owing to ethical issues does exist, as it is impossible to have a placebo group with anemic children not receiving ferrous sulfate supplementation. This fact prevents us from drawing precise conclusions about the effect of iron supplementation with or without folic acid on the nutritional status of anemic children. Nevertheless, the data from this study are coming from a randomized, double-blind, placebo-controlled clinical trial with small sample loss (18%). It is also important to mention that the detection of effects on the growth and nutritional status usually requires a homogeneous sample, which was the case with this study. In particular, the socioeconomic characteristics, hematological parameters and anthropometric indices did not differ at baseline. The initial weight differences among the anemic children were adjusted for in the analysis of variance with two fixed factors. In addition, we used the anthropometric indices Z-scores on growth to allow for the proper analysis of the children in the longitudinal study. In relation to the generalizability of the findings, the folic acid supplementation with iron can be used in Daycare Centers and by mothers in nonendemic areas of malaria and high prevalence of anemia in developing countries. The folic acid can be used for children with normal weight or with weight deficit.

We conclude that the use of folic acid supplementation increased the overall weight gain, the monthly average weight gain and weight-for-age Z-score gain when compared with the placebo. Furthermore, folic acid tended to increase the total weight gain and result in higher final weights. These effects were independent of the use of ferrous sulfate both in the form of treatment and prophylactic doses. Ferrous sulfate improved the linear gain in nonanemic children. Nevertheless, folic acid had no effect on linear growth. Moreover, prophylactic iron supplementation did not differ from the treatment supplementation in regard to the effects on linear growth and weight gain.

It is necessary to carry out new studies that address the relationship between iron supplementation, with or without folic acid, and the growth of children, aiming to confirm these findings and further contribute to the nutritional support programs related to deficits in infant growth.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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AUTHOR CONTRIBUTIONS

DAM and MCCMH designed research, analyzed data and wrote the article; AS wrote the article. VT prescribed the medication, conducted the clinical evaluation and revision of the article.

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