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Serum folate and vitamin B_{12} status in young Brazilian children

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Abstract

Objective: To assess the nutritional status of folate and vitamin B_{12} with anaemia in young children.
Design: A cross-sectional study was conducted at the primary health-care centres of four Brazilian cities. Folate and vitamin B_{12} were assessed by fluoroimmunoassay. Multilevel Poisson regression models were used to explore the association of folate and vitamin B_{12} status in relation to anaemia in young children.
Setting: Brazil.
Participants: Children (n 460) aged 11 to 15 months.
Results: The median (interquartile range) of serum folate was 39·7 (28·8–55·3) nmol/l and only four children presented with folate deficiency (<10 nmol/l). Surprisingly, 30·9 % of children presented with serum folate concentrations above the upper limit of detectable values by the commercial kit used for analysis. The frequency of vitamin B_{12} deficiency (<148 pmol/l) was 15 % and it was inversely associated with the highest tertile of serum folate concentrations ($P < 0·001$). Having high serum folate concentration (≥50·1 nmol/l) and vitamin $B_{12} \geq 148$ pmol/l was associated with lower frequency of anaemia in these children (prevalence ratio = 0·53; 95% CI 0·30, 0·92).
Conclusions: High frequency of elevated serum concentration of folate was found among young Brazilian children and 15 % of them had vitamin B_{12} deficiency. The combination of high serum folate and normal vitamin B_{12} status was associated with a lower frequency of anaemia in these children. Improvements in the current strategies to promote healthy food-based complementary feeding along with prevention and control of micronutrient deficiencies are recommended to improve children's health.

Keywords
Folate status
Vitamin B_{12} status
Nutritional anaemia
Child health
Nutritional status

Folate and vitamin B_{12} are both part of the B-complex group of vitamins and are required for biological methylation and DNA synthesis. Deficiency in these vitamins can lead to several adverse effects on health, including anaemia, neuropathy and birth defects[1,2].

There are several known causes of folate and vitamin B_{12} deficiencies. The most common cause of folate deficiency is low intake of sources rich in this vitamin, such as green leafy vegetables and legumes, but it can also be caused by alcoholism and lactation. Vitamin B_{12} deficiency, in turn, can be caused by low intake of this vitamin which is naturally present in animal-source foods, malabsorption, parasitic infections, medications and polymorphisms[3].

To prevent folic acid-sensitive neural tube defects, several countries have adopted mandatory fortification of wheat flour and maize meal with folic acid, a synthetic form of folate, as a public health intervention to improve the folate status of women of reproductive age[4]. In Brazil, this intervention became mandatory in 2004 and consists of the addition of 140 to 220 μg of folic acid per 100 g of maize and wheat flours marketed in the country.

Epidemiological studies have shown a low prevalence of folate deficiency in children from countries where folic acid fortification is in place[5–7]; however, the same might not be observed for vitamin B_{12} deficiency, which still varies greatly according to the child's age and eating practices[8].

In Nepal, for example, the prevalence of vitamin B_{12} deficiency in children aged 6–23 months was 30·2 %, while no children presented with folate deficiency[5]. In Mexico,

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7·7 and 3·2 % of children aged 1–6 years had vitamin B_{12} and folate deficiencies, respectively[6]; while in Colombia, 1·6 % of school-age children presented with vitamin B_{12} deficiency and less than 1 % with folate deficiency[7]. In Brazil, a study conducted with 106 children (≤6 years old) attending at a health-care centre in a south-western city of Brazil identified that no children had folate or vitamin B_{12} deficiency[9]; however, a low prevalence of folate deficiency (4·1 %) with 13·6 % of vitamin B_{12} deficiency was found in a study conducted with 169 Amazonian children younger than 24 months[10].

The mandatory flour fortification with folic acid in Brazil has proved to be effective in increasing serum folate concentrations in children, adolescents and healthy adults[11]. However, some international researchers raise questions about the harmful effects of excessive folic acid intake on one's health, especially in people with low vitamin B_{12} status[12,13].

Among older adults, some evidence has shown that the imbalance of serum folate status and low vitamin B_{12} status was associated with increased prevalence of anaemia and cognitive impairment. During pregnancy, this combination may increase women's predisposition to gestational diabetes and may cause insulin resistance, adiposity and low birth weight in their offspring[13]. In school-age children, a cohort conducted in Bogotá, Colombia identified that Hb concentration was inversely associated with red blood cell (RBC) folate, especially in children with marginal and low vitamin B_{12} status[14]. The mechanisms that explain this association are unclear. Some researchers speculate that excess folate may interfere with vitamin B_{12} metabolism and may worsen the functional consequences of impaired vitamin B_{12} deficiency[13].

To our knowledge, few studies have assessed the distribution of folate and vitamin B_{12} nutritional status in young children; they tend to focus on rates of deficiency instead. In addition, no studies have assessed the association between high folate and low vitamin B_{12} status in this age group. Thus, the objectives of the present study were to assess the nutritional status of folate and vitamin B_{12} and to explore their combined association with anaemia in young Brazilian children.

Methods

Study design, settings and participants

The present cross-sectional study is part of the Estudo Nacional de Fortificação caseira da Alimentação Complementar (ENFAC Study). This study was a pragmatic controlled clinical trial designed to assess the impact of multiple micronutrient powder on anaemia and nutritional deficiencies of infants seen at primary health-care centres in four Brazilian cities. A detailed description of the study design, sampling methodology and field procedures can be found elsewhere[15].

A sample size of at least 105 children in each control and intervention group by study city was deemed minimally necessary for the trial and then increased by 30 % to account for dropouts. This resulted in an anticipated 540 children in each group. Eligibility criteria comprised parental approval to participate and absence of current treatment for anaemia. Exclusion criteria included premature births (<37 weeks' gestation), twins, reported cases of HIV infection, malaria, tuberculosis or genetic Hb disorders, as well as fever (>39°C) on the day of blood sampling.

All children receiving routine paediatric care at primary health-care units during data collection who met the inclusion and exclusion criteria of the study were invited to participate as a convenience study population. For the present analysis, the study sample was composed of all children from the ENFAC control group, aged 11–15 months. Overall, 543 children were invited to participate; parents of twenty-two children (4·1 %) declined participation; one child did not provide a blood sample and the blood samples of sixty children were insufficient for folate and vitamin B_{12} laboratory analysis, resulting in a final sample of 460 children.

Data collection and laboratory procedures

From June 2012 to January 2013, caregivers of children attending at primary health-care centres were invited to participate in the present study. At enrolment, trained fieldworkers applied a structured questionnaire through a face-to-face interview with each child's caregiver, to collect data on socio-economic, environmental, demographic and maternal variables, as well as to gain information on the child's birth, infant feeding practices, supplementation and morbidities.

Each child's anthropometric measurements were obtained in duplicate directly by trained research assistants following standardized procedures and using calibrated equipment[16]. Weight was measured with an electronic scale (model UM-061; Tanita, Tokyo, Japan), accurate to 10 g, and recumbent length was ascertained to the nearest 0·1 cm on a flat surface with a portable infant measuring board (model ES-2000; Sanny, Los Angeles, CA, USA). The mean value of both measurements was analysed. BMI was computed as [weight (kg)]/[length (m)]^2. *Z*-scores for length-for-age and BMI-for-age were calculated according to WHO guidelines[17]. The cut-off defined for stunting was length-for-age *Z*-score < -2 and that for overweight was BMI-for-age *Z*-score >1[18].

Venous blood samples were collected by trained technicians up to a week after the interview. They were collected in the morning on a day previously scheduled with the caregivers, after at least 3 h of fasting. At the field laboratory, Hb concentrations were determined on portable haemoglobinometers (Hb301; HemoCue®, Angelholm, Sweden). A separate blood sample was protected from light and centrifuged within 1 h of collection; serum and plasma samples were separated in micro tubes and frozen at −20°C before being shipped to São Paulo on dry ice and maintained at −70°C until further analysis.

Serum folate and vitamin B_{12} concentrations were measured by fluoroimmunoassay (PerkinElmer; Wallac Oy, Turku, Finland). Plasma ferritin and soluble transferrin receptor concentrations were measured using commercially available enzyme immunoassays (Ramco, Houston, TX, USA) and serum concentrations of retinol by HPLC methods (HP-1100 HPLC system; Hewlett Packard, Palo Alto, CA, USA) as previously described[15]. C-reactive protein and α_1-acid glycoprotein were determined using an IMMAGE Immunochemistry System (Beckman Coulter, Brea, CA, USA). The laboratory assayed internal and external blinded quality control specimens in each run. Based on the control specimens, the accuracy and inter-assay CV for these analyses were within 7 %.

Folate deficiency was defined as serum folate concentration <10 nmol/l[19] and high folate concentration as serum folate ≥50·1 nmol/l (lower bound of highest tertile). Vitamin B_{12} status was defined as normal (>221 pmol/l), marginal (221–148 pmol/l) or low (<148 pmol/l)[20]. Anaemia was defined as Hb concentration <110 g/l as established by WHO[21]. Iron deficiency was defined as plasma ferritin concentration <12 μg/l and/or soluble transferrin receptor concentration >8·3 mg/l[22]. Children with serum retinol concentration <1·05 μmol/l were considered as having marginal vitamin A status. C-reactive protein concentration >5 mg/l and α_1-acid glycoprotein concentration >1 g/l were considered as presence of inflammation[23].

Statistical analysis

Descriptive statistics were calculated as numbers and percentages for the categorical variables and as means and standard deviations, or medians and interquartile ranges, for the continuous variables. The differences in the prevalence rates between groups were assessed using Pearson's χ^2 and Fisher's exact tests and the differences in the medians of the continuous variables were assessed using the Kruskal–Wallis test.

To evaluate the combined association of vitamin B_{12} status and serum folate in relation to anaemia frequency, prevalence ratios (PR) were estimated using random multilevel mixed-effects Poisson regression models adjusted for city as clustering variable. For the present study, a hierarchical conceptual framework model with three levels of determination was used for selecting independent variables, as proposed by Cardoso *et al.*[24]. The most distal level included demographic, socio-economic, environmental and maternal characteristics; the intermediate level comprised birth variables, infant feeding practices and use of supplements; and the proximal level comprised anthropometric variables, reported morbidity and biochemical indicators. First, in model 1, analyses were conducted for selecting variables to be tested in multiple models ($P < 0·20$). Then, at each level of determination, variables were retained in the model if they were associated with the outcome at $P < 0·10$ (model 2).

In further analyses, PR were also estimated to assess the factors associated with high concentration of serum folate using random multilevel mixed-effects Poisson regression models adjusted for city as clustering variable, following the same hierarchical approach for selection of independent variables. The main dichotomous dependent variable was high serum folate and the independent variables were: child's sex and age; child's ethnicity (classified according to skin colour as used in the Brazilian census); maternal schooling, age and marital status; sanitary sewer (categorized as no public sewage or public sewage); number of children aged less than 5 years living in the child's house; child's birth weight; duration of breastfeeding; timing of introduction of fruits and/or vegetables, meat, beans and cereals, defined as early (<6 months), adequate (≥6– < 8 months) or late (≥8 months); iron, vitamin A plus D and multivitamin supplementation; stunting; overweight; hospitalization at least once in life; presence of diarrhoea, fever and wheezing in the past 15 d; and presence of vitamin B_{12} deficiency, inflammation, anaemia, iron deficiency and marginal vitamin A status. The predictors of high serum folate were considered if, after adjustment for potential factors at the same level and hierarchically superior levels, they presented $P < 0·05$ in the final model.

Missing observations were included in the multiple models by creating missing-value categories. All analyses were performed using the statistical software package Stata version 12.0.

Results

Of the 460 children, the mean age was 13·51 (SD 1·03) months and 47·6 % were female. More than half of children's mothers had more than 9 years of education. About 65 % of children were born with adequate weight and about a third of them were weaned before age 6 months. Few children were stunted (4·7 %) but 37·1 % already were overweight. Further characteristics are presented in Table 1.

Overall, the median of serum folate was 39·7 (IQR 28·8–55·3) nmol/l and only four children (0·9 %) presented with folate deficiency (<10 nmol/l; Table 2). Surprisingly, 142 (30·9 %) children presented with serum folate concentrations above the upper limit of detectable values by the commercial kit used for biochemical analysis. In total, 15·0 % of children had vitamin B_{12} deficiency (<148 pmol/l) and it was inversely associated with the highest tertile of serum folate concentrations ($P < 0·001$). The same was noted for anaemia: the overall frequency of anaemia (Hb < 110 g/l) was 23·0 % and it decreased in the highest tertile of folate concentrations ($P < 0·001$). Six children

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Table 1 Characteristics of the young Brazilian children (*n* 460) included in the study, June 2012–January 2013

	Total*	
Variable	Mean or *n*	SD or %
Age (months), mean and SD	13·51	1·03
Female sex, *n* and %	219	47·6
Skin colour, *n* and %		
White	78	17·4
Brown	345	77·2
Black	24	5·4
Maternal education ≥9 years, *n* and %	273	60·8
Maternal age ≤25 years, *n* and %	223	48·5
Birth weight, *n* and %		
< 2500 g	25	5·5
≥ 2500– < 3500 g	291	64·1
≥ 3500 g	138	30·4
Breastfeeding < 6 months, *n* and %	132	28·9
Stunting†, *n* and %	21	4·7
Overweight‡, *n* and %	161	37·1
Iron deficiency§, *n* and %	173	37·9
Marginal vitamin A status‖, *n* and %	162	35·2
Presence of inflammation¶, *n* and %	61	14·2

*Totals differ from the total number of study children due to missing values.
†Defined as length-for-age *Z*-score < −2.
‡Overweight was defined as BMI-for-age *Z*-score > 1 (including 'at risk of overweight', overweight and obesity).
§Defined as plasma ferritin < 12 μg/l and/or soluble transferrin receptor > 8·3 mg/l.
‖Defined as vitamin A < 1·05 μmol/l.
¶Defined as α_1-acid glycoprotein > 1 g/l and C-reactive protein > 5 mg/l.

(3·9 %) presented with an imbalance of high folate status (lower bound of highest tertile) and low vitamin B_{12} status (<148 pmol/l; Table 2).

Table 3 summarizes the vitamin B_{12} status, serum folate status and the combined association between these two vitamins in relation to frequency of anaemia. It was observed that having low vitamin B_{12} alone was significantly associated with higher frequency of anaemia in these children (PR = 1·78; 95 % CI 1·10, 2·88), while having high serum folate concentration alone was significantly associated with lower frequency of this condition (PR = 0·53; 95 % CI 0·31, 0·88). In the combined analysis, compared with having normal status for both vitamins, having high serum folate status alone was significantly associated with lower frequency of anaemia (PR = 0·53; 95 % CI 0·30, 0·92). No significant association was found for a combination of low vitamin B_{12} status and normal serum folate with anaemia (PR = 1·48; 95 % CI 0·89, 2·46), or for low vitamin B_{12} status and high serum folate with anaemia (PR = 1·83; 95% CI 0·42, 7·92; data not shown in tables), probably due to the low number of observations.

The factors associated with high serum folate are presented in Table 4. In the multiple analysis, vitamin B_{12} deficiency was associated with lower frequency of high folate concentration.

Discussion

In the present study, 15 % of children had vitamin B_{12} deficiency and one-third of them had marginal vitamin B_{12} status. Comparing these findings with other studies, similar prevalence to this deficiency was reported among Amazonian children aged 6–24 months (13·6 % defined as vitamin B_{12} < 150 pmol/l)[10] and among 1-year-old Mexican children (9·1 % deficiency defined as vitamin B_{12} < 203 pg/ml (equivalent to 150 pmol/l))[6]. A higher prevalence of vitamin B_{12} deficiency was found in Nepalese children aged 6–23 months (30·2 %)[5].

In infants and young children, vitamin B_{12} deficiency is mainly due to maternal low vitamin B_{12} status during pregnancy and lactation that influences the infant's stores of this vitamin and causes a lower vitamin B_{12} concentration from breast milk. In addition, low intake of animal-source foods in complementary feeding and malabsorption of vitamin B_{12} due to intestinal parasite infection can also cause deficiency in young children[8,25].

In contrast to the observations of vitamin B_{12} deficiency in the present study, less than 1 % of children had folate deficiency. This low frequency of folate deficiency is consistent with other studies carried out in Brazil[9,10] and in other developing countries, such as Nepal, Mexico and Colombia[5–7]. The lack of folate deficiency in these studies could be related for the most part to the folic acid fortification of staple foods in place in these countries. Several studies have shown that this strategy has the potential to increase folate intake in the entire population[11,26] and, consequently, serum folate concentrations[11]. A review of forty-seven Brazilian studies showed that serum folate concentrations increased by 57 % in healthy children and adolescents and by 174 % in adults after the implementation of flour fortification with folic acid in Brazil[11].

Surprisingly, the median of serum folate concentration found in the present study (39·7 nmol/l) was much higher than that found in other studies: 22·7 nmol/l in Amazonian children below 24 months of age[10] and 12·4 ng/ml (28·1 nmol/l) in children younger than 6 years[9]. In addition, almost one-third of children from the present study presented serum folate concentrations above the upper limit of detectable values by the commercial kit used for biochemical analysis.

We also found that early introduction of cereals (<8 months of age) was positively associated with having high folate concentration in these children, although this finding was only marginally significant (P = 0·073). Although fortification of wheat and corn flours with folic acid is mandatory in Brazil and is effective for increasing serum folate concentrations in the Brazilian population[11], this association was undesired because of the children's age. At 1 year, children should not consume a considerable amount of farinaceous products that supposedly are increasing the folate concentrations in this population. This hypothesis corroborates a study conducted among 446 children aged 2–3 years in Brazil, which identified that 88·1% of children consumed fortified foods and the percentage of folate intake provided by consuming

Table 2 Vitamin B_{12} and Hb status by serum folate concentration in young Brazilian children (*n* 460), June 2012–January 2013

	Total (*n* 460)		Serum folate status: 1st tertile (*n* 152)		2nd tertile (*n* 152)		3rd tertile (*n* 156)		
	Median or *n*	IQR or %	Median or *n*	IQR or %	Median or *n*	IQR or %	Median or *n*	IQR or %	*P* value*
Serum folate (nmol/l)									
Median and IQR	39·7	28·8–55·3	25·4	20·6–28·7	39·4	35·8–43·6	55·3	55·3–55·3	< 0·001
Deficiency (<10 nmol/l), *n* and %	4	0·9	–	–	–	–	–	–	
Serum vitamin B_{12} (pmol/l)									
Median and IQR	274·2	184·5–403·5	245·7	153·5–387·3	226·6	163·8–371·4	325·1	238·2–419·2	< 0·001
Category, *n* and %									< 0·001
Deficient (<148 pmol/l)	69	15·0	33	21·7	30	19·7	6	3·9	
Marginal (148–221 pmol/l)	92	20·0	29	19·1	41	27·0	22	14·1	
Normal (>221 pmol/l)	299	65·0	90	59·2	81	53·3	128	82·0	
Haemoglobin (g/l)									
Median and IQR	118	110–126	113·5	106–122	118	110–124	122	115–128	< 0·001
< 110 g/l, *n* and %	106	23·0	54	35·5	33	21·7	19	12·2	< 0·001

*Kruskal–Wallis or Pearson χ^2 tests were used to examine differences in continuous variables and in proportions, respectively, between serum folate status groups.

Table 3 Combined association of vitamin B_{12} status and serum folate in relation to anaemia risk in young Brazilian children (*n* 460), June 2012–January 2013

			Frequency of anaemia†,‡		Model 1§		Model 2‖	
Vitamin status*	*n*	%	*n*	%	PR	95% CI	PR	95% CI
Low B_{12} alone	69	15·0	25	36·2	1·90	1·21, 2·98	1·78	1·10, 2·88
High folate alone	156	33·9	19	12·2	0·47	0·29, 0·79	0·53	0·31, 0·88
Combined conditions								
Normal B_{12} + normal folate	241	52·4	64	26·6	1·00	Ref.	1·00	Ref.
Normal B_{12} + high folate	150	32·6	17	11·3	0·49	0·29, 0·85	0·53	0·30, 0·92
Low B_{12} + normal folate	63	13·7	23	36·5	1·56	0·96, 2·54	1·48	0·89, 2·46

PR, prevalence ratio; ref., reference category.
*Normal B_{12} (vitamin $B_{12} \geq 148$ pmol/l); low B_{12} (vitamin $B_{12} < 148$ pmol/l); normal folate (serum folate < 50·1 nmol/l); high folate (serum folate ≥ 50·1 nmol/l, representing the lower bound of highest tertile of folate concentration).
†Defined as Hb concentration < 110 g/l.
‡Pearson's χ^2 or Fisher's exact tests.
§Multilevel Poisson regression models adjusted for city as clustering variable.
‖Multilevel Poisson regression models adjusted for city (clustering variable), maternal schooling, more than one child aged <5 years in the house, age of introduction of fruits and/or vegetables, age of introduction of grains, use of multivitamin supplements, stunting, previous hospitalization, inflammation, iron deficiency and marginal vitamin A status (vitamin A < 1·05 μmol/l).

Table 4 Factors associated with high concentration of serum folate (≥50·1 nmol/l) among young Brazilian children (*n* 460), June 2012–January 2013

			Model 1†			Model 2‡		
Independent variable	*n**	%	PR	95% CI	*P* value	PR	95% CI	*P* value
Children aged <5 years in the house								
1 child	340	73·9	1·00	Ref.	–	–	–	–
> 1 child	120	26·1	0·77	0·53, 1·14	0·195	–	–	–
Duration of breast-feeding								
< 6 months	132	28·9	1·42	1·02, 1·98	0·039	–	–	–
≥ 6 months	325	71·1	1·00	Ref.	–	–	–	–
Age of introduction of cereals								
< 6 months	95	20·7	1·68	1·02, 2·76	0·040	1·54	0·92, 2·57	0·098
6– < 8 months	253	55·1	1·46	0·95, 2·23	0·081	1·48	0·96, 2·27	0·073
≥ 8 months	111	24·2	1·00	Ref.	–	1·00	Ref.	–
Iron supplementation								
Never used it	269	59·0	1·00	Ref.	–	–	–	–
Has used or uses it	187	41·0	0·75	0·51, 1·08	0·124	–	–	–
Vitamin A plus D supplementation								
Never used it	343	75·0	1·00	Ref.	–	–	–	–
Has used or uses it	114	25·0	0·63	0·33, 1·18	0·149	–	–	–
Diarrhoea in the last 15 d								
No	345	75·2	1·00	Ref.	–	–	–	–
Yes	114	24·8	0·64	0·42, 0·96	0·031	–	–	–
Wheezing in the last 15 d								
No	289	62·8	1·00	Ref.	–	–	–	–
Yes	171	37·2	0·77	0·54, 1·09	0·141	–	–	–
Anaemia§								
No	354	77·0	1·00	Ref.	–	1·00	Ref.	–
Yes	106	23·0	0·49	0·30, 0·81	0·005	0·62	0·37, 1·02	0·061
Marginal vitamin A status‖								
No	298	64·8	1·00	Ref.	–	–	–	–
Yes	162	35·2	0·72	0·50, 1·02	0·063	–	–	–
Vitamin B_{12} deficiency¶								
No	391	85·0	1·00	Ref.	–	1·00	Ref.	–
Yes	69	15·0	0·22	0·10, 0·49	< 0·001	0·25	0·11, 0·57	0·001

PR, prevalence ratio; ref., reference category.
*Totals differ from the total number of study children due to missing values.
†Multilevel Poisson regression model adjusted for city (clustering variable).
‡Multilevel Poisson regression model adjusted for city (clustering variable) and variables that were associated with the outcome at $P < 0·10$ following a hierarchical conceptual framework model.
§Defined as Hb < 110 g/l.
‖Defined as vitamin A < 1·05 μmol/l.
¶Defined as vitamin $B_{12} < 148$ pmol/l.

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ultra-processed foods was 11·3 %. Moreover, 70·2 % of the children presented with consumption above the RDA value for this vitamin[27].

Despite the known beneficial effects of high acid folic intake in reducing the risk of neural tube defects and other congenital anomalies, and (less conclusively) reducing the risk of CVD and cancer[28], an increasing amount of evidence has raised concerns regarding the adverse effects of high folate concentrations on one's health, especially in individuals with marginal or deficient vitamin B_{12} status[13].

In school-age children, two studies conducted in Colombia and Guatemala have found an inverse association between Hb concentration and folate status[14,29]. In addition, the Colombian study showed that Hb concentration was inversely associated with RBC folate, especially in children with marginal or deficient vitamin B_{12} status[14]. In our study, contrary to these studies, Hb concentration was positively associated with serum folate concentration and, unfortunately, our data have insufficient power to assess the interaction between low vitamin B_{12} and high folate status in relation to anaemia risk. Arsenault *et al.*[14] discussed in their paper that those studies were characterized by the absence of folate deficiency in the children studied, but this explanation does not apply to our study because the frequency of folate deficiency in our children was less than 1 %. However, a comparison of these findings should be made with caution for some reasons: (i) the children in the present study are younger than those in previous studies; (ii) different indicators to assess folate status were used; and (iii) a higher frequency of anaemia was found in the present study (23 % *v.* <7 % in those studies).

In our analysis, compared with having normal status for both vitamins, high serum folate status alone was significantly associated with lower frequency of anaemia. The literature clearly presents the mechanisms by which low concentrations of folate as well as vitamin B_{12} lead to anaemia. These vitamins are metabolically linked and the deficiency of any of these vitamins results in an inhibition of DNA synthesis within RBC production leading to megaloblastic anaemia[1].

It is important to mention that vitamin B_{12} status can influence folate metabolism. This vitamin serves as a coenzyme in the methionine synthase reaction required for the conversion of 5-methyltetrahydrofolate to tetrahydrofolate. Although Bruyn *et al.*[30] found in their study a positive correlation between RBC or serum folate and serum vitamin B_{12}, there was an exception in cases where vitamin B_{12} values were lower than 200 ng/l or 150 pmol/l, when a negative correlation with serum folate was observed, a phenomenon known as folate trapping[30].

Our study has limitations that deserve mention. First, the cross-sectional nature of the study is a potential limitation because this kind of design does not allow one to infer causality; the directions of the relationship between variables are unclear and, for this reason, results must thus be interpreted carefully. Second, the present study lacked a sufficient number of individuals with high folate levels and low vitamin B_{12} status to have statistical power to detect a difference between the groups. Despite that, the present study is one of the most comprehensive ones on folate and vitamin B_{12} in young children carried out in Brazil. It is important to highlight that folate status can be assessed by serum concentration or RBC folate. Both indicators are recommended by WHO[31] as an accurate biomarker for large-scale assessment of folate status. In our study, we chose to use serum folate concentrations because is cheaper and faster to perform than RBC folate. Although RBC folate reflects the long-term folate status, since we assessed children around 1 year old, the use of serum folate could be considered a good proxy for folate status.

We suggest more studies assessing the distribution of folate status, rather than focusing on rates of deficiency, in young children and further research to clarify the short- and long-term effects of high intake of folic acid in infants and young children to elucidate these complex relationships with potential adverse effects.

Conclusion

A high serum folate concentration was found among 1-year-old children in Brazil and 15 % of them had vitamin B_{12} deficiency. The combination of high serum folate status and normal vitamin B_{12} status was associated with lower frequency of anaemia. Adequate complementary feeding and periodic monitoring of nutritional status of these vitamins in this age group are recommended to avoid possible side-effects of these conditions in the future.

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Conflict of interest: None. *Authorship:* L.L.S.S. contributed to the data collection, conducted data analyses, participated in data interpretation, and wrote the initial draft of the article; W.W.F. participated in data analysis and interpretation, and was involved in the review of the article; M.A.C. supervised all study protocols, was responsible for project management, participated in data analysis and interpretation, and was involved in the writing of the article. All authors have critically reviewed the manuscript content and have approved the final version submitted for publication. *Ethics of human subject participation:* This study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects were approved by the Human Ethical Review Board of the School of Public Health, University of São Paulo, Brazil. Written informed consent was obtained from the caregivers of all children after they had been informed of the objectives, benefits and possible risks of this study. Children who were anaemic or had any nutritional deficiency diagnosed during the study were referred for treatment in the health centres. This study was registered at www.ensaiosclinicos.gov.br as RBR-5ktv6b.

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Appendix

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