The American Journal of Clinical Nutrition 118 (2023) 162–173

The American Journal of CLINICAL NUTRITION

journal homepage: https://ajcn.nutrition.org/

Original Research Article

Association of development quotient with nutritional status of vitamins B6, B12, and folate in 6–59-month-old children: Results from the Brazilian National Survey on Child Nutrition (ENANI-2019)

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A B S T R A C T

Background: Vitamins B6, B12, and folate are essential for the formation and maintenance of the human brain, but studies evaluating these vitamins with early childhood development (ECD) in children under 5 y are limited and controversial.

Objectives: To evaluate the association between vitamins B6, B12, and folate concentrations/status and ECD.

Methods: Data regarding 6520 children aged 6–59 mo from the ENANI-2019 (the Brazilian National Survey on Child Nutrition) were analyzed. ECD was assessed using the Survey of Well-being of Young Children's milestones questionnaire. Vitamin B6 concentration (nmol/L) was classified according to the tertile of the distribution and with the cutoff <20 nmol/L. Folate concentrations >45.3 nmol/L were classified as high, and vitamin B12 <150 pmol/L was deficient. The graded response model was used to estimate developmental age, and the developmental quotient (DQ) was calculated as the developmental age divided by chronological age. Multiple linear regression models were adjusted for confounders.

Results: The DQ mean (95% confidence interval) for Brazilian children was 0.99 (0.97–1.01). Children aged 6–23 mo [1.13 (1.10–1.16)] had a higher DQ mean than those aged 24–35 [0.99 (0.95–1.03)] and 36–59 mo [0.89 (0.86–0.92)]. Child age was inversely associated with DQ ($\beta = -0.007$; $P < 0.001$). An interaction between child age and vitamin B12 deficiency in the DQ ($\beta = -0.005$; $P < 0.001$) indicated that, in children aged 36–59 mo, the DQ was markedly lower in children with vitamin B12 deficiency than in those without vitamin B12 deficiency. Vitamin B6 concentrations were directly associated with the DQ ($\beta = 0.0004$; $P = 0.031$) among children aged 24–59 mo in the adjusted model. No association was observed between folate status and DQ.

Conclusions: In Brazil, the DQ is lower among older children and those with vitamin B12 deficiency. Vitamin B6 status was directly associated with the DQ in children aged 24–59 mo.

Keywords: early child development, B vitamins, vitamin B6, folate, cobalamin, vitamin B12, neurodevelopment, SWCY, children, low-income countries

Introduction

Early childhood development (ECD) encompasses cognitive, physical, language, temperament, socio-environmental, and motor development, which begins at conception and extends to 8 y of age [1]. Achieving the full potential of development is a human right and a requirement for sustainable development [1].

Coping with malnourished children under 5 y of age has been a considerable public health challenge worldwide. Data from UNICEF indicate that, after major global efforts, from 2000–2020, the prevalence of stunting (*z*-score of height for age < –2) has decreased by a third, but it still affects 55 million children [2]. Despite this improvement, the micronutrient status of children remains a problem. In 2020, it was estimated that 1 in 2 children suffered from deficiencies of

Abbreviations: DAG, directed acyclic graph; DQ, developmental quotient; ECD, early childhood development; ENANI-2019, Brazilian National Survey on Child Nutrition; ICC, item characteristic curve; SWYC, survey of well-being of young children; β, beta coefficient.

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https://doi.org/10.1016/j.ajcnut.2023.04.026
Received 7 January 2023; Received in revised form 28 March 2023; Accepted 19 April 2023
Available online 25 April 2023

vitamins and minerals, including vitamin A and nutrients related to anemia, such as iron, vitamin B12, and folate [3], especially in low- and middle-income countries, such as Brazil [2].

Micronutrient inadequacy is associated with stunting and poor cognitive performance, limiting short- and long-term ECD [4]. Most of the central nervous system (CNS) formation happens in the prenatal period; however, in the postnatal period and during childhood, part of the brain formation continues, e.g., the proliferation and migration of glial cells, an essential process for the functional organization of neuronal circuits [5]. B vitamins and nutritional biomarkers participating in 1-carbon metabolism have been investigated as critical limiting factors in fetal and infant development. In addition, 1-carbon metabolism involves multiple physiological processes, including methylation of nucleic acids, myelination, and synthesis of neurotransmitters, purines, thymidine, and phospholipids [6,7]. Therefore, the 1-carbon metabolism is essential for forming and maintaining the human brain. These metabolic reactions are dependent on the dietary supply of methyl donors, including folate (vitamin B9) and methionine [8], as well as essential cofactors such as vitamins B12, B6, and riboflavin [9].

Folate and vitamin B12, supplementation during pregnancy, has short- and long-term effects on ECD. Most studies indicate a positive effect on ECD [10–13], although some have found no effect [14,15], whereas others suggest adverse effects of high-dose folate (>1000 μg/d) [16,17]. Vitamins B6, B12, and folate inadequacy in early childhood can adversely affect CNS homeostasis [5]. This can limit long-term child development, as this is a continuous process in which previous stages limit the following phases. Studies regarding the association between vitamins B6, B12, and folate status in children under 5 y of age and ECD are limited, and their results remain controversial [18–25]. Some authors have observed a direct association [18,19,21–23], whereas others have not observed or described an inverse association between these vitamins and ECD [20,24,25]. Furthermore, no known epidemiological studies have assessed the relationship between these biomarkers and ECD in Brazilian children under 5 y of age or in other countries experiencing a similar nutritional transition process.

This study evaluated the association between vitamins B6, B12, and folate status and ECD in Brazilian children. We also assessed this association in different age groups to identify different behaviors, as the extent of brain development varies throughout early childhood.

Methods

Study design and participants

This study used data from the Brazilian National Survey on Child Nutrition (ENANI-2019). The ENANI-2019 study population consisted of households with at least 1 child under 5 y of age. The following households were not eligible: *1*) indigenous people who lived in villages; *2*) foreigners residing in households where Portuguese was not spoken; *3*) children with some condition that would disable them for anthropometric measurement; and *4*) children living in hotels, pensions, orphanages, and hospitals.

The ENANI-2019 sample was calculated as 15,000 households in 123 municipalities and 26 states, and the Federal district. A sample size of 3000 households per Brazilian macro-region was defined, considering a minimum proportion of 2%, a relative error estimate of 35%, a confidence level of 95%, and a sampling and design effect set at 2. More details on the sample design, the selection process of the households, and other methodological aspects can be found elsewhere [26,27].

The ENANI-2019 is a population-based household survey conducted in a probability sample of 12,524 households between February 2019 and March 2020. A total of 14,558 children under 5 y of age were evaluated. In the study protocol, only children ≥6 mo of age were eligible for drawing blood samples (n = 12,598 children). Among these, 8829 (70.1%) had their blood samples collected. Adjustments to sample weights were made considering micronutrient analyses referring to a subset of the population surveyed by ENANI-2019 and the occurrence of nonresponse (lack of measures for 1 or more biomarkers) [26,28]. The subset used for analysis consisted of all children with complete vitamins B6, B12, and folate data (n = 6532). The study sample comprised 6520 children with information on vitamins B6, B12, folate, and ECD assessment (Figure 1).

ECD assessment

ECD was assessed using the Survey of Well-being of Young Children (SWYC) milestones questionnaire, developed and validated by Sheldrick and Perrin (2013). A version of the SWYC has been translated, adapted, and validated for use in Brazilian children [29]. We evaluated the internal consistency of the SWYC milestones questionnaire using the ENANI-2019 data and Cronbach's alpha. The results showed adequate performance (0.965; 95% CI: 0.963–0.968). We also used confirmatory factor analysis to assess the unidimensionality of the questionnaire. The 2-factor model showed a better fit than the 1- and 3-factor models. This result was consistent with the findings of Sheldrick and Perrin (2013).

The SWYC milestones questionnaire consists of 54 different developmental milestones that form 12 different sets of 10 age-specific items for the following age ranges: 0–3, 4–5, 6–8, 9–11, 12–14, 15–17, 18–22, 23–28, 29–34, 35–46, 47–58, and 59–65 mo. It has quick and easy-to-read questions about daily activities for each age group, indicating the progress of 1 or more dimensions of child development. For example, in the 0–3 mo age group, the development milestones include: "Follows a moving toy with his or her eyes" and "Looks when you call his or her name." For children aged 35–46 mo, some examples comprise: "Talks so other people can understand him or her most of the time" and "Tells you a story from a book or television." There were 3 response options: not at all, somewhat, or very much.

The ENANI-2019 data collection system automatically selected the appropriate set of developmental milestones according to the child's age, which was previously registered at the beginning of the interview. In cases where a child was under 2 y of age at the assessment and was born preterm (<37 weeks of gestation), the corrected age was considered in selecting the appropriate questionnaire. The corrected age was calculated by subtracting the child's gestational age at birth from 40 wk (full-term) and then subtracting the result from the child's chronological age at the time of testing. For example, for a child born at 32 weeks of gestation who had a chronological age of 4 mo and 10 d (132 d), it was subtracted from the child's chronological age of 8 wk (56 d), and the set of items was established according to the corrected age (2 mo and 16 d). Using a corrected age is a recommendation from the scientists that developed the SWYC. The main rationale argues that preterm children have a shorter development time in the uterus than those born at term. The expected differences tend to reduce over the first 2 y of life and are no longer critical from 2 y onward, so the correction is performed only for children under 2 y. The interviewers administered the questionnaire, and the respondent was the child's mother or caregiver with or without the help of other people, such as nannies.

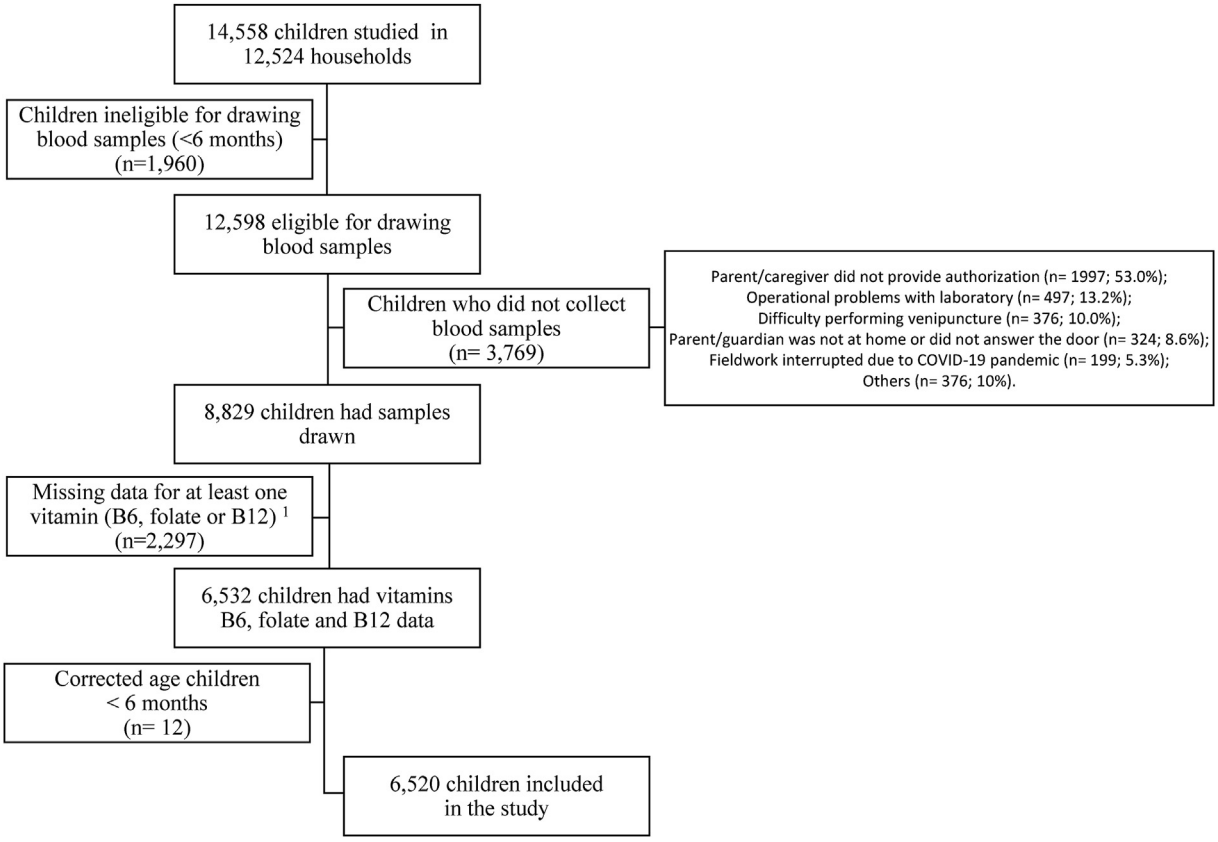

FIGURE 1. Flow diagram of children with blood samples and early childhood development data evaluated in ENANI-2019 (the Brazilian National Survey on Child Nutrition). COVID-19, coronavirus disease 2019. [1]Children without complete data for vitamins B6, B12, and folate blood/serum biomarkers. The difference is because of the different matrices (whole blood, serum, or plasma) used for nutritional status assessment and the prioritization of the material collected for some biomarkers, considering the main objectives of ENANI-2019.

Although we intended to study children aged up to 59 mo, 3 children were older than 59 mo on the day the questionnaire was administered. This was because of the differences between the date the household was selected and the day the SWYC questionnaire was administered. Therefore, we chose not to exclude these children to avoid bias, as the sample weight was adjusted based on these observations.

Blood collection and vitamin analyses

A trained professional performed children's blood collection at the household, and no fasting was needed. More details on procedures adopted for blood collection and processing and laboratory analyses are described in Castro et al. [28].

Briefly, 8 mL of blood was collected and distributed in a trace tube (6 mL) and an EDTA tube (2 mL). The trace tube was wrapped in aluminum foil to stabilize the sample for photosensitive analyses. The samples were transported under refrigeration (2–8°C) to a local laboratory, where the trace tubes were centrifuged for serum separation. Whole blood (0.5 mL) was transferred from an EDTA tube to an empty amber tube. All samples were stored at freezing temperature (–20°C) until laboratory analyses were performed in a central clinical test laboratory.

Vitamin B6 status (PLP) was analyzed using whole blood and HPLC with fluorescence detection equipment (HPLC; Chromsystems). In addition, serum folate and vitamin B12 concentrations were analyzed using chemiluminescence enzyme immunoassays (DxI 800; Beckman Coulter). Folate deficiency was classified as concentrations $<$10 nmol/L, high folate status was classified as serum concentrations $>$45.3 nmol/L [30], and vitamin B12 deficiency was classified as concentrations $<$150 pmol/L [31]. Vitamin B6 was assessed using the cutoff for insufficiency ($<$20 nmol/L) [32,33] and the following tertile distribution: first: 10.92–93.06; second: 93.06–144.03; and third: 144.04–1531.41 nmol/L.

Co-variables

Dietary intake was assessed using a 24-h recall (R24h). The R24h was applied only once because of logistical and cost constraints. The data was collected using the 24-h Food Recall Application (AppR24h) and the Infant Food Quantification Photographic Manual with photos of 101 foods with portions designed specifically for infant feeding assessment [34], both developed by the ENANI-2019 research team.

The R24h administration followed the approach of the multiple pass technique recommended by the USDA [35]. This approach consists of 5 steps in the following order: *1*) reporting the list of foods consumed by the child on the previous day; *2*) questioning the list of commonly forgotten foods (soda, juice, breast milk, candy, cookies, bread, sugar, honey, and among others); *3*) filling the time and meal type for each food mentioned in the previous steps; *4*) detailing of each food (type of preparation, place where the meal was consumed, and quantity/unit consumed); *5*) Review of information provided by the mother/caregiver.

The Brazilian Food Composition Table (TBCA) was used to define the nutritional value of consumed foods [36]. The TBCA has 34 dietary components of $>$4600 food items, including natural foods, culinary

preparations, and industrialized foods. The reliability of the TBCA data is ensured by its compliance with the rules of the International Network of Food Data Systems (Infoods) and the FAO of the United Nations, which determine guidelines and criteria to be used in the generation, compilation, and use of food composition data [37]. The TBCA is freely available online at: http://www.fcf.usp.br/tbca.

The conversion of home measurements and units of food consumed into units of mass (g, mg, μg) was performed using the Table of Referenced Measurements for Food Consumption in Brazil [38] and the Table of Evaluation of Food Consumption in Home Measurements [39]. If some measurements were not identified in these references, the ENANI-2019 team weighed the food according to the reported home measurement. Finally, consistency analysis of the variables and database cleaning were performed to identify missing data and determine the cases to be corrected.

The vitamins dietary intake was classified as adequate and inadequate according to the Recommended Dietary Allowances and Adequate Intakes for each age group [40]. The following cutoffs were used: vitamin B6 (0.3, 0.5, and 0.6 mg for 6–11, 12–47, and 48–59 mo, respectively); vitamin B12 (0.5, 0.9, and 1.2 μg for 6–11, 12–47, and 48–59 mo, respectively), and folate (80, 150, and 200 μg for 6–11, 12–47, and 48–59 mo, respectively).

The use of micronutrient supplements (yes or no) at the time of the study and in the previous 6 mo was investigated. We identified those that contained vitamins B6, B12, and folate. Details regarding supplement use for children in ENANI-2019 are available elsewhere [41]. In addition, BMI (in kg/m^2)/age and length/height-for-age *z*-scores were calculated considering the sex and age (days) of the child on the day of the anthropometric assessment according to WHO recommendations [42].

Chronological child age (months), attendance at daycare centers or schools (public, private, or no daycare/school), maternal/caregiver education (0–7, 8–10, or ≥11 y), and per-capita family income categories (0–62.2, 62.2–124.4, 124.4–248.7, or >248.7–33.0) were estimated from the Brazilian minimum wage (R$ 998.00) and converted to the United States dollar exchange rate (R$ 4.013 = USD 1) in 2019.

Developmental quotient

Developmental age refers to the child's age of achievement of specific developmental milestones. This variable was estimated using the item response theory and graded response models [43], using the Mplus software version 7 (Los Angeles, CA: Muthén & Muthén), with the full information method and incorporating the complex sample design [44]. This method allows the evaluation of each responding item and not only the final score, as the item set can be biased—that is, there may be a disproportion in the number of activities more or less commonly achieved among the specified items. Therefore, reaching the maximum score on the scale may be easier in some age groups.

This procedure allowed estimation in all the 12 age groups listed before, including milestones that did not apply and using chronological age as 1 of the estimators. The SWYC comprises 54 developmental milestones; each child's mother/caregiver answered 10; thus, there are 44 milestones because of nonapplication for each age group. The estimated model allowed the construction of an item characteristic curve (ICC) for each milestone, representing the change in the probability of a given response (sometimes or always) from birth to 62 mo of age and estimating the age of development. In addition, this method enables the analysis of outcomes as a continuous variable. The item response theory model has been previously used to assess ECD with the SWCY [45,46] and Denver test [47].

The graded response model generated ICC and 2 parameters (α and β). α1 and α2 refer to the probable age at which children reach each developmental milestone, performing them sometimes (α1) or always (α2), respectively. β refers to the discrimination of each milestone. It describes the curve slope, indicating the probability of reaching the developmental milestone, which increases with the child's chronological age. ICC, α1, α2, and β made it possible to estimate developmental age according to the developmental milestones reached by each child.

The developmental quotient (DQ) was calculated by dividing developmental age by chronological age [45]. The expected age milestones are attained when DQ = 1. Values <1 and >1 suggest attaining age milestones below and above expectations, respectively.

Statistical analyses

The descriptive analysis comprised the estimation of the mean of DQ with 95% CI. The CV was used to assess the data homogeneity. CV is a dispersion measure obtained from the ratio between the SE and the estimated value for each indicator. CV <30% was established as an adequate precision level for the indicators and variables evaluated in the ENANI-2019. Otherwise, the results should be interpreted with caution. All estimates presented in this manuscript show a CV <30%. For descriptive analyses, the child's chronological age was categorized as 6–23, 24–35, and 36–59 mo. After graphical visualization of the different behaviors of the DQ in the age groups, we combined this variable in the multiple analyses into 2 categories: 6–23 and 24–59 mo.

A Directed Acyclic Graph (DAG) [48] was used to define better the operating model for vitamins associated with ECD. The DAG was constructed considering factors related to exposure (vitamin status) and outcome (ECD) based on the literature. The variables suggested by the DAG for adjustment of the models were maternal vitamin status during pregnancy and postpartum, breastfeeding practices, maternal/caregiver education, and dietary vitamin sources (Figure 2). Maternal vitamin status was not assessed, and indicators of breastfeeding practice data were collected only for children aged 0–23 mo. The dietary vitamin sources were obtained from the 24-h recall. However, the regression models did not consider these estimates because only one R24h was administered. Therefore, all models were adjusted to maternal/caregiver education and age group.

Linear regression analysis was performed to evaluate the association between chronological age and DQ. The independent interactions of chronological age in months and vitamins B6, B12, and folate status with the DQ were also evaluated. Multiple linear regression was used to assess the association between vitamins B6, B12, folate, and DQ adjusting for confounders identified through the DAG. Because of the importance of chronological age in child development, the interaction of vitamin concentration and child chronological age (6–23 and 24–59 mo) was also evaluated using vitamins B6, B12, and DQ regression. The regression results were presented as the beta coefficient (β), SE, and *P* value.

Pearson's correlation was used to assess the relationship between blood/serum concentration and dietary vitamins B6, B12, and folate intake. The analyses were performed considering the complex sample design of the study in the R language using "srvyr" and "survey" packages. The differences between the mean DQ and frequencies according to selected variables were considered statistically significant when there was no overlap between 95% CI. In addition, statistical significance was considered when $P < 0.05$ for all other analyses.

Ethical approval

The ENANI-2019 was approved by the research ethics committee of the Clementino Fraga Filho University Hospital of the Federal

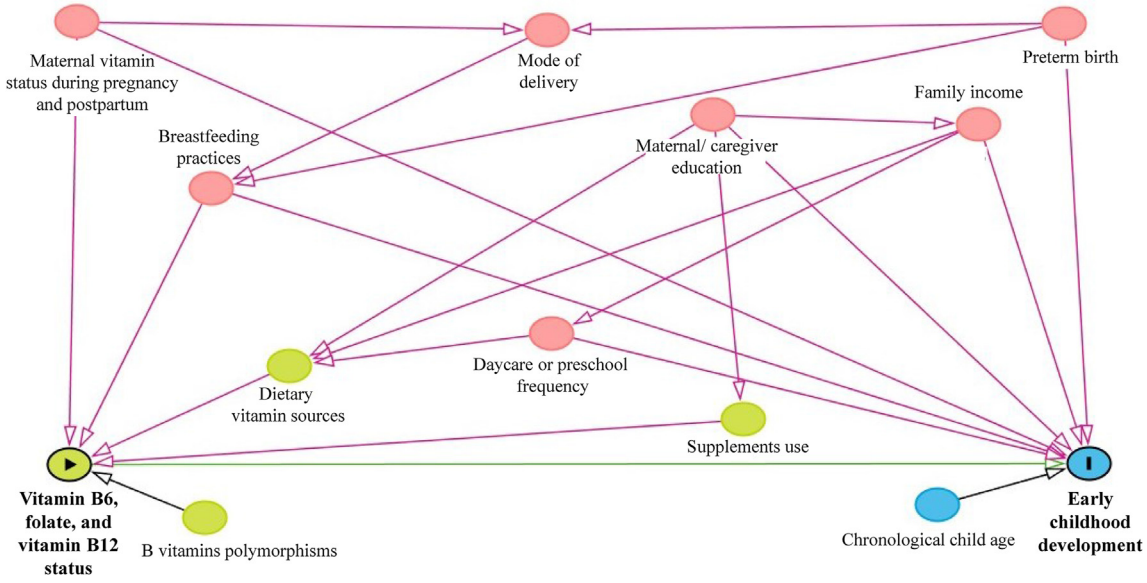

FIGURE 2. Directed acyclic graph of the association between vitamin B6, folate, and vitamin B12 status and early childhood development. DAG, directed acyclic graph. Notes: Breastfeeding practices refer to breastfeeding in the first hour of life, exclusive until 6 mo and/or complemented until 2 y. All breastfeeding practices are associated with vitamin B status and early childhood development. Minimum adjustments suggested by the DAG: maternal vitamin status during pregnancy and postpartum, breastfeeding practices, maternal/caregiver education, and dietary vitamin sources. Green circle: ancestor of the exposure; blue circle: ancestor of the outcome; pink circle: ancestor of exposure and outcome.

University of Rio de Janeiro (CAAE:89798718.7.0000.5257). Research participation occurred after signing the free and informed consent form by the child's parents or caregivers and after hearing an explanation of all the ethical issues of the study.

Results

Six thousand five hundred and 20 children were evaluated in this analysis. The majority were aged between 36–59 (44.5%), followed by the 6–23 mo age group (33.3%). Fifty-one percent were boys. Eighteen percent of the children were at risk of being overweight, and 9.9% presented excessive weight. The prevalence of stunting was 6.1%. Most children lived in households with a per-capita monthly income of USD 62.2–124.4 (35.3%), and their maternal/caregiver had ≥11 y of education (54.1%) (Table 1).

The prevalence of inadequate vitamins B6, B12, and folate dietary intake was 53.5, 14.5, and 20.6%, respectively. The prevalence of supplement use was 12.6% for vitamin B6, 8.1% for B12, and 5% for folate. The prevalence of vitamin B6 blood insufficiency was 2.6%, and vitamin B12 deficiency was 14.1%. We observed a prevalence of folate deficiency of 1.1% and high folate of 37.0% (Table 1). Weak correlations were observed between the dietary intake of vitamins B6, B12, and folate and serum/blood concentrations of vitamins B6, B12, and folate (Supplemental Table 1).

The DQ mean (95% CI) for Brazilian children was 0.99 (0.97–1.01). Children aged 6–23 mo [1.13 (1.10–1.16)] had a higher DQ mean than those aged 24–35 [0.99 (0.95–1.03)] and 36–59 mo [0.89 (0.86–0.92)] (Table 2). The child's chronological age (months) was inversely associated with the DQ ($\beta = -0.007$; SE = 0.0006; $P < 0.001$) (Figure 3).

No differences were observed in the mean DQ between children with and those without adequate dietary intake for the 3 micronutrients evaluated. No statistical differences were observed in the DQ mean according to vitamin B6 blood status (tertiles or <20 nmol/L) and age groups. Children with vitamin B12 deficiency had a higher DQ mean than those without deficiency [1.06 (1.01–1.12); 0.98 (0.96–1.00)] when all age groups were evaluated, but no differences were observed for the age groups. No statistical differences were found in folate status and chronological age groups (Table 2).

Associations between vitamin B12 and folate serum concentrations and DQ were not observed (Table 3). However, an interaction between chronological child age and DQ was observed when vitamin B12 groups were considered ($\beta = -0.005$; SE = 0.001; $P < 0.001$), indicating that among children with vitamin B12 deficiency, the DQ decreased more markedly with increasing chronological age compared with those without vitamin B12 deficiency (Figure 4).

The interaction between chronological child age and the vitamin B6 <20 nmol/L ($\beta = 0.00157$; SE = 0.00418; $P = 0.707$); and tertiles (second tertile: $\beta = 0.001$; SE = 0.001; $P = 0.437$); (third tertile: $\beta = 0.001$; SE = 0.001; $P = 0.575$), and folate status groups ($\beta = -0.001$; SE: 0.0011; $P = 0.482$) with DQ was not statistically significant (Supplemental Figures 1A,B and 2). Vitamin B6 blood concentration (nmol/L) was not associated with DQ when analyzed as a main independent factor. This variable needed interaction with the child's age (24–59 mo) to express its association. The interaction indicated that vitamin B6 blood concentrations are directly associated with DQ ($\beta = 0.0004$; SE = 0.002; $P = 0.031$) in the adjusted models for this group. Among children aged 6–23 mo, we did not observe a statistically significant association, indicating that vitamin B6 concentration is a DQ determinant only in the 24–59 mo age group (Figure 5, Table 3).

Discussion

We found that the mean DQ was inversely associated with chronological age, indicating a potential delay in ECD (DQ <1) when Brazilian children reach 36–59 mo. This association was even more intense among children with vitamin B12 deficiency in this age group. However, vitamin B6 status was directly associated with DQ in

TABLE 1
Characteristics of children 6–59 mo evaluated in ENANI-2019 (the Brazilian National Survey on Child Nutrition) ($n = 6520$)

Variables	Frequency (%)	95% CI
Age group (mo)		
6–23	33.3	33.2, 33.4
24–35	22.2	22.2, 22.2
36–59	44.5	44.4, 44.6
Sex		
Male	51.1	51.0; 51.2
Female	48.9	48.8; 49.0
BMI for age (z-score)		
Underweight ($z < -2$)	1.9	1.2, 2.7
Normal ($-2 \leq z \leq 1$)	69.4	66.7, 71.7
Overweight risk ($1 < z \leq 2$)	18.8	17.0, 20.5
Excessive weight ($z > 2$)	9.9	8.3, 11.5
Length/height-for-age (z-score < -2)		
Yes	6.1	4.8, 7.3
No	93.9	92.4, 95.0
Per-capita family income (USD)[1]		
<62.2	27.5	23.7, 31.4
62.2–124.4	35.3	32.6, 37.9
124.5–248.7	24.3	21.5, 27.0
>248.7	12.9	10.7, 15.2
Maternal/caregiver education (y)		
0–7	23.9	21.8; 26.0
8–10	22.0	19.7; 24.2
≥11	54.1	51.4; 56.9
Vitamin B6 dietary intake[2]		
Adequate	46.5	43.3, 49.7
Inadequate	53.5	50.3, 56.7
Vitamin B6 supplement use		
Yes	12.6	10.3, 15.0
No	87.4	85.0, 89.7
Vitamin B6 blood insufficiency[3]		
Yes	2.6	0.9, 4.3
No	97.4	95.7, 99.1
Vitamin B6 blood (tertile)[3]		
First	33.3	29.9, 36.6
Second	33.3	30.9, 35.8
Third	33.4	30.2, 36.7
Vitamin B12 dietary intake[2]		
Adequate	85.5	83.5, 87.6
Inadequate	14.5	12.4, 16.5
Vitamin B12 supplement use		
Yes	8.1	6.4, 9.9
No	91.9	90.1; 93.6
Vitamin B12 serum deficiency[4]		
Yes	14.1	11.9, 16.3
No	85.9	83.7, 88.1
Folate dietary intake[2]		
Adequate	79.4	77.1, 81.6
Inadequate	20.6	18.4, 22.9
Folic acid supplement use		
Yes	5.0	3.4, 6.6
No	95.0	93.4, 96.6
Folate serum deficiency[5]		
Yes	1.1	0.5, 1.7
No	98.9	98.3, 99.5
Folate serum (high)[5]		
Yes	37.0	33.4, 40.5
No	63.0	59.5, 66.6

CI, confidence interval; USD, United States dollar.

[1] Estimated from the Brazilian minimum wage (R$ 998.00) and converted to the USD exchange rate (R$ 4.013 = USD 1) in 2019.

[2] Vitamin B6, folate, and B12 dietary intake adequacy were defined according to Recommended Dietary Allowances and Adequate Intakes: vitamin B6 (0.3, 0.5, and 0.6 mg for 6–11, 12–47, and 48–59 mo, respectively); vitamin B12 (0.5, 0.9, and 1.2 μg for 6–11, 12–47, and 48–59 mo, respectively), and folate (80, 150, and 200 μg for 6–11, 12–47, and 48–59 mo, respectively.

[3] Vitamin B6 blood insufficiency was defined as <20 nmol/L and vitamin B6 blood tertiles were defined as: First ≤93.1; second = 93.1–144.03; and third ≥144.04 nmol/L.

[4] Vitamin B12 serum deficiency was defined as <150 pmol/L.

[5] Folate serum deficiency and high folate concentrations were defined as <10 and >45.3 nmol/L, respectively.

children aged 24–59 mo, but no statistically significant association was observed between folate status and DQ.

Our findings are consistent with Brazilian studies showing lower ECD status among older children [49–51]. Venancio et al. [50] showed a lower prevalence of the attainment of expected age-specific developmental milestones among children aged 36–59 mo compared with younger children (0–36 mo) in a cross-sectional study in 16 municipalities in Ceará State (Brazil), with 6447 children aged 0–59 mo. Correia et al. [51] conducted a population-based survey in Ceará State (Brazil) that included 3566 children aged 0–6 y and observed a higher prevalence of ECD delay in communication, fine and gross motor skills, problem resolution, and personal-social domains among children aged 36−66 mo than among those aged 2–35 mo.

The difference in the attainment of developmental milestones according to age can be explained by different determinants, including a longer time of harmful exposure to development determinants, such as micronutrient inadequacy [52], and environmental aspects, such as access to health, opportunities for early learning, stressful situations, such as violence and poverty, and adequate food and cultural habits [53]. The achievement of milestones in the first 12 mo appeared to be less influenced by these factors. A previous study showed that children aged 0–42 mo from Argentina, India, South Africa, and Turkey presented similar ages when reaching milestones until the first year of life [53]. Subsequently, differences in attaining the milestones were observed between countries, especially for children older than 36 mo, a period in which the environment and cultural factors seem to have a stronger influence on ECD.

Vitamin B12 is essential for the formation and maintenance of the CNS. A systematic literature review found a direct association between vitamin B12 status, mental and cognitive development, and school performance in school-aged children (≥5 y) and adolescents [54]. Our results revealed that vitamin B12 deficiency was associated with a worse ECD status. Studies in India, Norway, China, and Nepal also observed a direct association between vitamin B12 status and ECD in children under 5 y of age [18,21,23,25,55]. In contrast, some studies have shown inverse associations between vitamin B12 markers, such as homocysteine and MMA, with development [19,22] or did not find a statistically significant relationship [20,24]. Notably, these studies are still limited to populations with specific characteristics, such as restricted consumption of dietary vitamin B12 sources [21,22], hospitalized or very young children (6 mo) [19,24], and limited sample size (<200 children) [18,19].

We observed a direct association between vitamin B6 concentrations and DQ among children aged 24–59 mo but not in those aged 6–23 mo. The absence of a significant association in the younger age group can be explained by the variation in vitamin B6 (PLP) concentrations in this period; vitamin B6 concentrations are higher in the first few months after birth and decrease after 6 mo of life [56]. In addition, the development of older children is more influenced by environmental factors, such as health and adequate nutrition, than that of younger children. To the best of our knowledge, a unique cross-sectional study carried out with 321 children aged 5 y in Nepal

TABLE 2
Developmental quotient means and 95% CI according to vitamins B6, B12, and folate variables, and age group for children 6–59 mo evaluated in ENANI-2019 (the Brazilian National Survey on Child Nutrition) (n = 6520).

Variables	All			Child chronological age (months)								
				6–23			24–35			36–59		
	Mean	95% CI		Mean	95% CI		Mean	95% CI		Mean	95% CI	
Brazil	0.99	0.97	1.01	1.13[ab]	1.10	1.16	0.99[ac]	0.95	1.03	0.89[bc]	0.86	0.92
Vitamin B6 dietary intake[1]												
Adequate	1.01	0.98	1.03	1.16	1.11	1.21	1.01	0.95	1.07	0.91	0.87	0.95
Inadequate	0.98	0.95	1.01	1.11	1.07	1.15	0.97	0.92	1.02	0.87	0.83	0.90
Vitamin B6 supplement use												
Yes	1.00	0.94	1.05	1.20	1.08	1.31	0.99	0.91	1.08	0.90	0.85	0.96
No	0.99	0.97	1.01	1.13	1.10	1.15	0.99	0.95	1.03	0.89	0.86	0.91
Vitamin B6 serum insufficiency[2]												
Yes	1.03	0.96	1.09	1.19	0.96	1.41	0.90	0.78	1.03	0.93	0.80	1.05
No	0.99	0.97	1.01	1.13	1.10	1.16	0.99	0.95	1.03	0.89	0.86	0.91
Vitamin B6 blood (tertile)[2]												
First	0.97	0.94	1.00	1.14	1.09	1.19	0.96	0.89	1.02	0.86	0.82	0.90
Second	0.98	0.96	1.01	1.11	1.06	1.17	1.01	0.94	1.07	0.90	0.86	0.93
Third	1.02	0.98	1.06	1.14	1.08	1.20	1.01	0.95	1.06	0.91	0.87	0.94
Vitamin B12 dietary intake[1]												
Adequate	0.99	0.97	1.00	1.12	1.09	1.15	1.00	0.95	1.04	0.89	0.86	0.91
Inadequate	1.03	0.98	1.09	1.20	1.12	1.27	0.94	0.86	1.03	0.90	0.84	0.95
Vitamin B12 supplement use												
Yes	0.98	0.93	1.03	1.14	1.05	1.24	0.96	0.87	1.05	0.89	0.83	0.95
No	0.99	0.97	1.01	1.13	1.10	1.16	0.99	0.95	1.03	0.89	0.86	0.92
Vitamin B12 serum deficiency[3]												
Yes	1.06[a]	1.01	1.12	1.20	1.14	1.25	0.88	0.78	0.99	0.86	0.79	0.93
No	0.98[a]	0.96	1.00	1.11	1.08	1.14	1.00	0.96	1.04	0.89	0.86	0.92
Folate dietary intake[1]												
Adequate	0.99	0.97	1.02	1.15	1.12	1.18	0.99	0.94	1.04	0.88	0.85	0.91
Inadequate	0.99	0.95	1.02	1.07	1.00	1.14	0.99	0.91	1.06	0.92	0.88	0.96
Folic acid supplement use												
Yes	0.99	0.91	1.07	1.10	0.96	1.23	0.91	0.83	1.00	0.96	0.86	1.05
No	0.99	0.97	1.01	1.13	1.10	1.16	0.99	0.95	1.03	0.88	0.86	0.91
Folate serum deficiency[4]												
Yes	0.92	0.83	1.00	1.12	1.03	1.21	0.95	0.80	1.11	0.86	0.74	0.98
No	0.99	0.97	1.01	1.13	1.10	1.16	0.99	0.95	1.03	0.89	0.86	0.92
Folate serum (high)[4]												
Yes	1.01	0.98	1.04	1.14	1.09	1.18	0.98	0.92	1.03	0.88	0.85	0.90
No	0.98	0.95	1.01	1.13	1.09	1.16	0.99	0.94	1.05	0.89	0.86	0.93

CI, confidence interval.
[a,b,c]Superscript letters represent differences between mean development quotient (DQ) between the categories of the variables using a lack of overlapping 95% CI. The DQ was calculated using the Survey of Well-being of Young Children - Brazilian version milestones questionnaire. Developmental age was estimated considering the child's age, and developmental milestones were achieved. DQ = developmental age ÷ chronological age [45]. DQ = 1 indicates that expected age milestones have been attained; DQ <1 and >1 suggest that the attainment of specific age milestones occurred below or above expectations, respectively.
[1] Vitamin B6, folate, and B12 dietary intake adequacy were defined according to Recommended Dietary Allowances and Adequate Intakes: vitamin B6 (0.3, 0.5, and 0.6 mg for 6–11, 12–47, and 48–59 mo, respectively); vitamin B12 (0.5, 0.9, and 1.2 μg for 6–11, 12–47, and 48–59 mo, respectively), and folate (80, 150, and 200 μg for 6–11, 12–47, and 48–59 mo, respectively.
[2] Vitamin B6 blood insufficiency was defined as <20 nmol/L and vitamin B6 blood tertiles were defined as: first = <93.1; second = 93.1–144.03; and third = ≥144.04 nmol/L.
[3] Vitamin B12 serum deficiency was defined as <150 pmol/L.
[4] Folate serum deficiency and high folate concentrations were defined as <10 and >45.3 nmol/L, respectively.

observed a direct association between plasma vitamin B6 concentration and ECD test results [25]. The relationship between vitamin B6 and ECD has also been demonstrated in studies that evaluated vitamin B6 concentration in breast milk [57,58]. Breast milk is the main dietary source of vitamin B6 in infants and is directly related to vitamin B6 status [59].

Folate plays a vital role in brain development. During pregnancy, folate deficiency has been established as a cause of neural tube defects [60]. Although the data are sparse during childhood, low folate status is associated with lower linear growth rates [61]. A positive effect on ECD was observed when children aged 5–9 mo with malaria were supplemented with folate/iron/zinc [62], and healthy children aged 6–30 mo received vitamin B12 supplementation [63]. No statistically significant association was found between folate status and ECD in children under 5 y of age in Taiwan [24] and Nepal [25]. This result is consistent with our finding of no association between folate nutritional status and ECD assessed by the DQ. In contrast, in an Indian cohort of 650 children aged 12–18 mo, folate status was directly associated with ECD only in the group with serum vitamin B12 concentrations below the 25th percentile (>145 pmol/L) [23]. In our study, no statistically

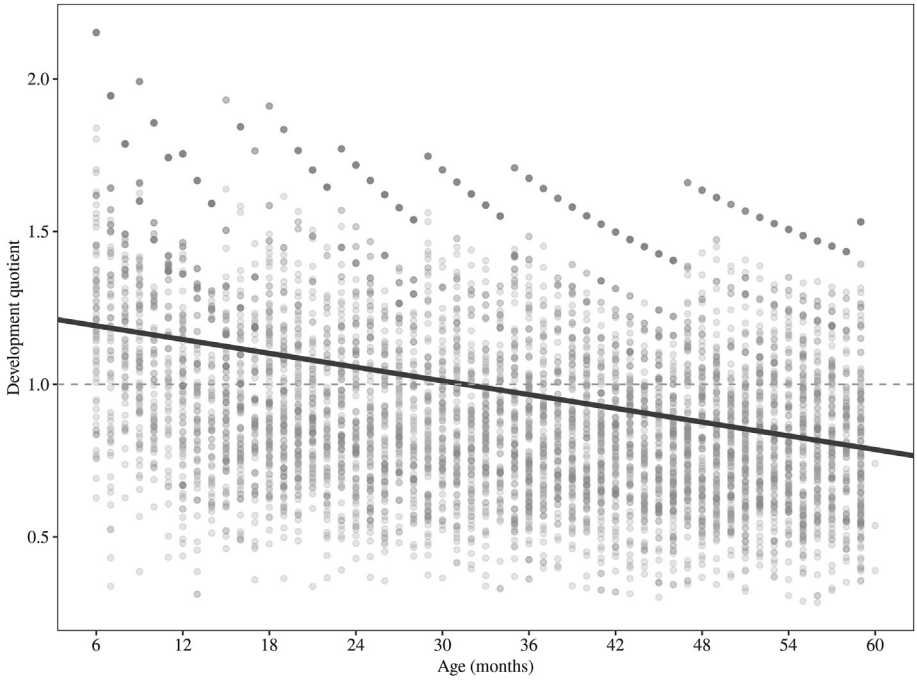

FIGURE 3. Simple linear regression between developmental quotient (DQ) and age (months) for children 6–59 mo evaluated in ENANI-2019 (the Brazilian National Survey on Child Nutrition) ($n = 6520$). Notes: The dashed line indicates DQ = 1. The DQ was calculated using the Survey of Well-being of Young Children - Brazilian version milestones questionnaire. Developmental age was estimated considering the child's age, and developmental milestones were achieved. DQ = developmental age ÷ chronological age [45]. DQ = 1 indicates that expected age milestones have been attained; DQ <1 and >1 suggest that the attainment of specific age milestones occurred below or above expectations, respectively.

TABLE 3
Association between developmental quotient and vitamins B6, B12, and folate with chronological age interaction for children 6–59 mo evaluated in ENANI-2019 (the Brazilian National Survey on Child Nutrition) ($n = 6520$)[1]

Variables	Coefficient	SE	P value
Vitamin B6 blood concentrations (nmol/L)			
Vitamin B6 (nmol/L)	–0.0002	0.0002	0.334
Vitamin B6 (nmol/L): age, 24–59 mo[2]	0.0004	0.0002	0.031
Vitamin B12 serum concentrations (pmol/L)			
Vitamin B12 (pmol/L)	–0.0001	0.0001	0.240
Vitamin B12 (pmol/L): age, 24–59 mo[2]	0.0001	0.0001	0.116
Folate serum concentrations (nmol/L)			
Folate (nmol/L)	0.0005	0.0006	0.382
Folate (nmol/L): age, 24–59 mo[2]	–0.0008	0.0007	0.243

SE, standard error.

[1] Multiple linear regression adjusted for maternal/caregiver education and age group. The developmental quotient (DQ) was calculated using the Survey of Well-being of Young Children - Brazilian version milestones questionnaire. Developmental age was estimated considering the child's age, and developmental milestones were achieved. DQ = developmental age ÷ chronological age [45]. DQ = 1 indicates that expected age milestones have been attained; DQ <1 and >1 suggest that the attainment of specific age milestones occurred below or above expectations, respectively.

[2] Interaction term between vitamin concentration and age group.

significant difference was found in the means of the DQ between children with or without high folate concentrations. We are unaware of other studies that evaluated high folate concentrations and ECD in this age group.

We found that vitamin B12 sufficiency and higher vitamin B6 concentrations were related to improved ECD. This indicates that interventions that improve nutrient concentrations are essential to achieve full child development. Furthermore, interventions in ECD have short- and long-term benefits for learning, productivity, health, and social cohesion [1]. From this perspective, Sustainable Development Goal 4.2 proposes to ensure that all children have access to quality care and preprimary education for ECD [64]. Similarly, the Lancet series "Advancing ECD: from science to scale" coined the term "nurturing care," which deals with a stable environment for child development; adequate nutrition is a component of this care [65].

This study had some limitations. The study was cross-sectional, which prevents us from making temporal conclusions regarding the effects of vitamin B12 deficiency and vitamin B6 concentration on ECD. In addition, we measured serum folate that reflects the recent status and not chronic nutritional status, in contrast to the erythrocyte folate assessment. We also did not have access to the data on the maternal nutritional status during pregnancy. Moreover, the breastfeeding practices to which children were exposed were available only for children under 2 y of age. These are essential variables that impact both the nutritional status of these nutrients and ECD in the short- and long-term [66].

Among the study's strengths is using the DQ to assess ECD, a continuous parameter that considers each developmental milestone response and the child's chronological age at its achievement, and not a summed score and cutoff points, like most child development scales and studies. This allowed us to capture more variability in the outcome occurrence. In addition, this is a population-based survey of children under 5 y of age, pioneering the collection of biological samples to assess the nutritional status of this population in Brazil. Unlike most studies that have evaluated the association between the nutritional

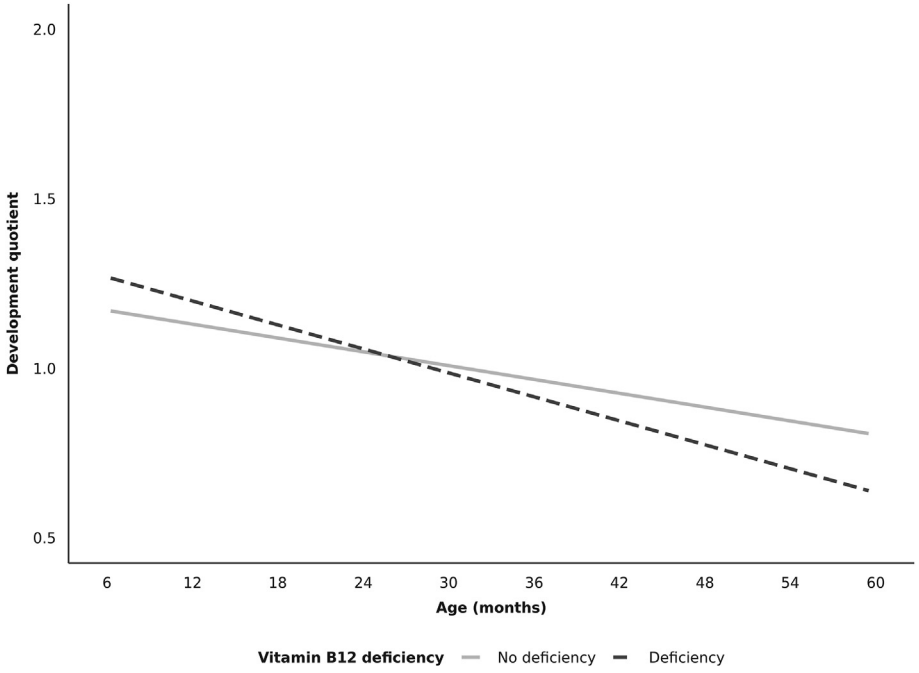

FIGURE 4. Association between developmental quotient (DQ) and chronological age with vitamin B12 deficiency interaction for children 6–59 mo evaluated in ENANI-2019 (the Brazilian National Survey on Child Nutrition) ($n = 6520$). SE, standard error; β, beta coefficient. Notes: Simple linear regression with vitamin B12 deficiency interaction: β: –0.005; SE = 0.001; $P < 0.001$. The DQ was calculated using the Survey of Well-being of Young Children - Brazilian version milestones questionnaire. Developmental age was estimated considering the child's age, and developmental milestones were achieved. DQ = developmental age ÷ chronological age [45]. DQ = 1 indicates that expected age milestones have been attained; DQ <1 and >1 suggest that the attainment of specific age milestones occurred below or above expectations, respectively. Vitamin B12 deficiency was defined as <150 pmol/L.

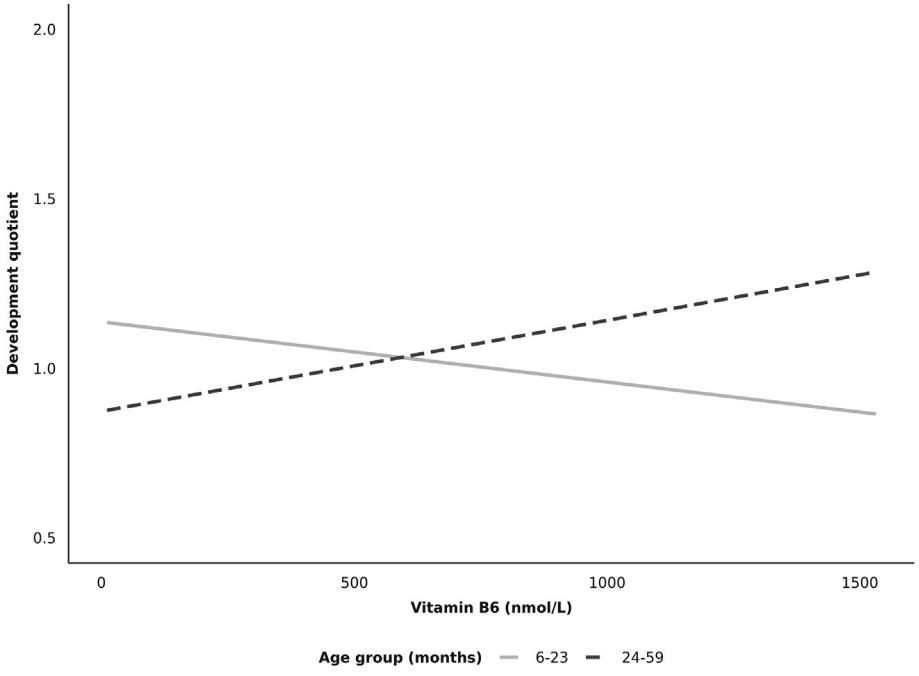

FIGURE 5. Association between developmental quotient (DQ) and vitamin B6 with chronological age interaction for children 6–59 mo evaluated in ENANI-2019 (the Brazilian National Survey on Child Nutrition) ($n = 6520$). SE, standard error; β, beta coefficient. Notes: Multiple linear regression adjusted for maternal/caregiver education and age group. Vitamin B6 (nmol/L): age, 6–23 mo (reference); age, 24–59 mo – β = 0.0004; SE = 0.002; $P = 0.031$. The DQ was calculated using the Survey of Well-being of Young Children - Brazilian version milestones questionnaire. Developmental age was estimated considering the child's age, and developmental milestones were achieved. DQ = developmental age ÷ chronological age [45]. DQ = 1 indicates that expected age milestones have been attained; DQ <1 and >1 suggest that the attainment of specific age milestones occurred below or above expectations, respectively.

status of vitamins B6, B12, folate, and ECD, this is the first such study in Latin America. Our study reinforces the need to integrate public policies on nutrition and child development, as suggested by the WHO [1]. Therefore, they serve as vital evidence to support public policies in Brazil and other countries undergoing the same stage of nutritional transition.

In summary, the ECD of Brazilian children is lower among older children, and this association is even more intense among those with vitamin B12 deficiency. Furthermore, vitamin B6 status is directly associated with the DQ in children aged 24–59 mo. Children are at risk of nutritional deficiencies in early childhood, and public policies must focus on this age group as these deficiencies tend to impede full development.

Acknowledgments

We thank all the participants of the Brazilian National Survey on Child Nutrition (ENANI-2019) and the Brazilian Ministry of Health/ Brazilian National Research Council (CPNq) – process: 440890/2017-9.

Author contributions

The authors' responsibilities were as follows– NCF-C: analyzed and interpreted the data and wrote the manuscript, with input from all authors; PGA and CER: analyzed and interpreted the data and revised the manuscript; PN, KSSN, IRRC, EMAL, and DRF: contributed to the study conception and design, and the interpretation of the data and revision of the manuscript; GK: the coordinator of the Brazilian National Survey on Child Nutrition (ENANI-2019) and participated in all phases of analysis and interpretation of the data and writing of the manuscript and all authors: read and approved the final manuscript.

Conflict of interest

The authors report no conflicts of interest.

Funding

This study was supported by the Brazilian Ministry of Health and the Brazilian National Research Council (CPNq) - process: 440890/ 2017-9, and Coordination of Improvement of Higher Education Personnel (CAPES, PhD's fellowship for NCF-C).

Data availability

Data described and the code book in the manuscript will be made publicly and freely available without restriction at https://enani.nutricao.ufrj.br. Analytic code will be made available via the corresponding author's email upon request.

Author disclosures

The authors report no conflicts of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ajcnut.2023.04.026.

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