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ARTICLE

Serum folate levels and cognitive performance in the ELSA-Brasil baseline assessment

Níveis séricos de folato e desempenho cognitivo na avaliação de linha de base do ELSA-Brasil

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ABSTRACT

Background: Most studies that analyze the association between serum folate levels and cognitive function either restrict their assessments to specific clinical scenarios or do not include middle-aged individuals, to whom strategies for preventing cognitive impairment may be more feasible. **Objective:** To examine the association between serum folate levels and cognitive function in the Brazilian Longitudinal Study of Adult Health (ELSA-Brasil) baseline assessment. **Methods:** Data from 4,571 ELSA-Brasil participants who live in the state of São Paulo, aged 35–74 years, were analyzed. The word list learning, delayed recall, word recognition, verbal fluency, and Trail Making Test Part B consisted in the cognitive tests. For each test, age, sex, and education-specific standardized scores and a global cognitive score were calculated. Crude and adjusted linear regression models were used to examine the associations of serum folate levels with cognitive test scores. **Results:** In multivariable-adjusted models, serum folate was not associated with global cognitive score (β=-0.043; 95% confidence interval [95%CI] -0.135 to 0.050 for lowest *vs.* highest quintile group), nor with any cognitive test performance. We did not find associations between serum folate and global cognitive scores in subgroups stratified by age, sex, or use of vitamin supplements either. **Conclusions:** We did not find significant associations between serum folate and cognitive performance in this large sample, which is characterized by a context of food fortification policies and a consequent low frequency of folate deficiency. Positive results from previous studies may not apply to the increasingly common contexts in which food fortification is implemented, or to younger individuals.

Keywords: Cognition; Dietary Supplements; Epidemiology; Folic Acid; Memory.

RESUMO

Introdução: A maioria dos estudos que analisam a associação entre os níveis séricos de folato e a função cognitiva restringem suas avaliações a cenários clínicos específicos ou não incluem indivíduos de meia idade, nos quais estratégias preventivas para a função cognitiva podem ser mais viáveis. **Objetivo:** Examinar a associação entre os níveis séricos de folato e a função cognitiva na avaliação inicial do Estudo Longitudinal da Saúde do Adulto (ELSA-Brasil). **Métodos:** Foram analisados dados de 4.571 participantes do ELSA-Brasil em São Paulo, com idades entre 35 e 74 anos. Os testes cognitivos foram aprendizagem, recordatório tardio e reconhecimento de lista de palavras; fluência verbal e teste de trilhas parte B. Calculamos, para cada teste e globalmente, escores padronizados para idade, sexo e educação. Foram utilizados modelos de regressão linear para examinar as associações dos níveis séricos de folato com o desempenho nos testes cognitivos. **Resultados:** Em modelos ajustados para múltiplas variáveis, o folato sérico não esteve associado ao escore cognitivo global (β=-0,043; intervalo de confiança de 95%: [IC95%] -0,135 a 0,050 para 1º *vs.* 5º quintil), ou desempenho em qualquer teste cognitivo. Também não encontramos associações entre folato sérico e escores cognitivos globais em subgrupos estratificados por idade, sexo ou uso de suplementos vitamínicos. **Conclusões:** Não encontramos associações significativas entre folato sérico e desempenho cognitivo

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nesta grande amostra, caracterizada por um cenário sob políticas de fortificação alimentar e consequente baixa frequência de deficiência de folato. Resultados positivos de estudos anteriores podem não se aplicar às situações cada vez mais comuns em que a fortificação de alimentos é implementada, ou a indivíduos mais jovens.

Palavras-chave: Cognição; Suplementos Nutricionais; Epidemiologia; Ácido Fólico; Memória.

It is still controversial whether serum folate levels (not necessarily as for the folate deficiency range) influence the cognitive performance. The specific pathways linking folate levels to brain functioning (including cognitive performance) in adult life is not fully understood yet[1]. Besides its major role during fetal development, there is experimental evidence that folates influence neuronal structure and function, including vesicular transport, cell polarity, and plasticity[2], all potentially influencing cognition.

Most clinical studies that assess the association between serum folate levels and cognitive function restrict their assessments to the Mini-Mental State Examination (MMSE) score or the clinical dementia diagnosis[3,4,5], or only evaluate individuals with specific medical conditions such as stroke survivors or individuals with multiple sclerosis[6,7].

Observational studies that use a broader set of tests to analyze the association between serum folate levels and cognitive performance in less specific samples have found mixed results[8,9,10,11]. In addition, most of these studies are focused on older adults, and there are scarce data about this association in younger individuals, to whom strategies for preventing cognitive decline may be more feasible. According to one of the few studies that included individuals younger than 55 years of age, Horvat et al.[10] analyzed cross-sectional (4,166 subjects) and prospective (2,739 subjects, with a mean follow-up of 3.8 years) data from the Health, Alcohol and Psychosocial factors in Eastern Europe (HAPIEE) study, using four cognitive tests (word list immediate recall, word list delayed recall, verbal fluency, and letter search). The authors found a positive association between folate levels and cognitive performance only for the letter search (in cross-sectional analyses) and verbal fluency (in longitudinal analyses) tests.

Studies analyzing the influence of folate supplementation on cognition in nonpregnant adults have found mixed results as well. Ma et al. randomized a sample of 180 individuals with mild cognitive impairment (19 with folate levels below 4 ng/mL) and verified that, in this case, folate supplementation was associated with better performance in two (information and digital span tests) out of the 11 cognition tests from the Chinese version of the Wechsler Adult Intelligence Scale[12]. A double-blind randomized clinical trial performed by Ford et al. found no benefits for cognition in the group of participants under folate supplementation[13]. Durga et al. randomly assessed 818 participants with high homocysteine levels (up to 26 μmol/L, in order to exclude causes other than suboptimal folate concentrations) in Netherlands. They found that folate supplementation was associated with higher global cognitive function scores after three years[14].

The Brazilian Longitudinal Study of Adult Health (ELSA-Brasil) is a multicenter cohort study on 15,105 participants, aged 35 to 74 years, from six Brazilian cities (Belo Horizonte, Porto Alegre, Rio de Janeiro, Salvador, São Paulo, and Vitória). The investigation site in São Paulo is the largest one, with 5,061 participants. The baseline assessment included the application of five cognitive tests and, for the sample from São Paulo, serum folate levels at baseline were also determined. This allows studying the association between serum folate levels and cognitive performance in middle-aged adults, to whom available data are very scarce. The aim of this study is to analyze the association between serum folate levels and cognitive performance in the ELSA-Brasil baseline assessment.

METHODS

Study design

The ELSA-Brasil design and cohort profile have been described elsewhere[15,16]. The baseline assessment was conducted from August 2008 to December 2010 and included a detailed protocol with validated questionnaires and laboratory, clinical, and imaging examinations. Blood samples were cryopreserved in liquid nitrogen for future analyses. In the present study, data on participants from the São Paulo investigation site (n=5,061) were used, and serum folate levels at baseline were assessed. ELSA-Brasil study protocol was approved by the Institutional Review Board of Hospital Universitário — Universidade de São Paulo (approval number 659/06). All participants provided a written informed consent form.

Study sample

Of the 5,061 potentially eligible subjects, the following were excluded: 63 (1.2%) due to previous stroke; 180 (3.6%) who took medication that could alter cognition (antipsychotics, antiparkinsonian agents, and anticonvulsants) at the time of the study; 7 (0.1%) due to lack of information on medication that could alter cognition or on previous stroke; 88 (1.7%) who did not have valid serum folate measurements; and 152 (3.0%) who did not complete the cognitive evaluation. The 4,571 remaining subjects were included in the main analyses.

Cognitive tests

The cognitive tests conducted in the ELSA-Brasil baseline assessment have been previously described[17,18]. We used the Brazilian version of the Consortium to Establish a Registry for Alzheimer's Disease (CERAD)[19], which included three parts, namely: (a) word list learning — the subjects were asked to read and memorize 10 words after three exposures to the list. The final score was defined by the sum of the words recalled each time; (b) delayed word recall — the subjects were asked to recall the list of words about five minutes after performing other activities; and (c) word recognition — a card was presented containing the 10 previous words along with another 10 distractor words, and participants were asked to select the 10 words that were in the initial list. The final score for the recognition test was the number of words that were correctly recalled minus the number of words that were incorrectly recalled. The semantic verbal fluency test (VFT) evaluates language, semantic memory, and executive function. In VFT, the subjects were asked to name animals for 60 seconds. In the Trail Making Test Part B (TMT-B), a test on executive function, processing speed, concentration, and attention, the subjects were asked to draw lines connecting letters and numbers in ascending order (alphabetical) and alternating between them (1 - A - 2 - B - 3 etc.). The time taken to perform the task was recorded, in seconds, as the measure of performance. Therefore, for the TMT-B, higher scores indicated poorer performance.

The scores for each cognitive test were presented in crude form only for the study sample description. For the main analyses, the subjects were classified into 32 groups according to sex (men/women), age (using 10-year intervals) and education level (<8, 8–10, 11–14, and >14 years of formal education), similar to the procedure adopted in a previous publication[20]. Within each group, scores for each cognitive test were standardized to a mean of zero and a standard deviation of one. For the TMT-B, positive standardized scores indicated that the time taken to perform the task was below the mean of the group. The global cognitive score is equal to the mean of the five standardized test scores, rescaled to a mean of zero and a standard deviation of one.

Serum folate levels

Serum folate levels were determined in cryopreserved (using liquid nitrogen, -196°C) blood samples[21]. The long-term stability of folate levels is very high at temperatures below -70°C[22]. Measurements were performed by electrochemiluminescence (Roche™ Cobas® E 601™ automated equipment). As reference levels for some of the analyses, the World Health Organization (WHO) cutoffs for folate deficiency (<3 ng/mL) and possible folate deficiency (<6 ng/mL) were used[23].

Other variables

Age, sex, education level, family income, and smoking status were self-reported and classified accordingly. Ethnicity was self-reported based on the question from the Population Census of the Brazilian Institution for Geography and Statistics (IBGE) as White, Mixed, Black, Asian, and Native. Asian and Native groups were reclassified as "Other" due to their low number. Excessive alcohol use was defined as alcohol consumption >210 g/week for men and >140 g/week for women. The use of vitamin supplements was defined as self-reported use of multivitamin, vitamin B, or folic acid supplements in the past 12 months. Anthropometric measurements were obtained using standard procedures[24], and body mass index was calculated as the weight (in kilograms) divided by the square of height (in meters). Anemia was defined according to the World Health Organization criteria (<13 g/dL for men and <12 g/dL for women). Thyroid function, hypertension, diabetes, and dyslipidemia statuses were defined as previously adopted in other ELSA-Brasil articles[25,26].

Statistical analysis

Categorical variables are presented as counts and proportions. Continuous variables are presented as means and standard deviations. Bivariate associations with serum folate levels in quintiles with categorical and continuous variables were assessed by using the chi-square or Jonckheere-Terpstra trend tests, respectively.

Crude and adjusted linear regression models were built to analyze the association between serum folate levels in quintiles and (a) global cognitive scores and (b) standardized scores for each cognitive test. Adjusted models were adjusted for ethnicity, family income, smoking status, hypertension, diabetes, dyslipidemia, thyroid function, and excessive alcohol use. The sample size of the study was adequate to detect a 0.15 difference in standardized scores between the 1st and 5th quintile groups, with 90% power and at a significance level of 0.05.

Models were carried out to analyze the influence of sex, age, and the use of vitamin supplements on the association between serum folate levels and global cognitive scores. First, in models using the global cognitive scores as the dependent variable, it was analyzed whether there was any significant interaction between serum folate levels and those variables in the adjusted models. Then, crude and adjusted linear regression models were carried out to analyze the association between serum folate levels and global cognitive scores in subgroups according to sex, age, and use of vitamin supplements. In some subgroup analyses, serum folate levels were categorized into tertiles (instead of quintiles) considering that the number of individuals was relatively small. Upon finding a significant sole association between TMT-B performance and serum folate levels, a *post-hoc* analysis was performed with further adjustment for age, sex, and education level to reduce residual confounding. The significance level was set at 0.05. The R software version 3.5.1 was used for all analyses.

RESULTS

In our sample, 2,931 (64.1%) individuals aged under 55 years and 2,467 (54.0%) were women. Only one participant (<0.1%) had serum folate levels below 3 ng/mL, and 13 (0.3%) had serum folate levels below 6 ng/mL. Table 1 shows the study sample characteristics according to folic acid levels at baseline categorized into quintiles. Trend tests showed that women, older age, White ethnicity, >14 years of formal education, monthly income ≥USD 3,320 (all p<0.001), and diagnosis

Table 1. Characteristics of the study sample, according to serum folate levels in quintiles.

	Serum folate groups					Total (n=4,571)
	1st quintile (≤12.6 ng/dL) (n=922)	2nd quintile (12.7–15.0 ng/mL) (n=919)	3rd quintile (15.1–17.5 ng/mL) (n=902)	4th quintile (17.6–21.3 ng/mL) (n=928)	5th quintile (≥21.4 ng/mL) (n=900)	
Women (n [%])	409 (44.4)	473 (51.5)	477 (52.9)	549 (59.2)	559 (62.1)	2,467 (54.0)
Age (mean±SD)	49.9±8.5	50.4±8.6	50.6±8.9	51.7±8.9	54.6±9.6	51.4±9.0
Formal education (n [%])						
<8 years	71 (7.7)	60 (6.5)	45 (5.0)	53 (5.7)	49 (5.4)	278 (6.1)
8–10 years	88 (9.5)	78 (8.5)	75 (8.3)	63 (6.8)	42 (4.7)	346 (7.6)
11–14 years	422 (45.8)	393 (42.8)	356 (39.5)	352 (37.9)	315 (35.0)	1,838 (40.2)
>14 years	341 (37.0)	388 (42.2)	426 (47.2)	460 (49.6)	494 (54.9)	2,109 (46.1)
Ethnicity (n [%])						
White	495 (54.4)	505 (55.7)	529 (59.6)	594 (64.6)	588 (66.1)	2,711 (60.1)
Mixed	201 (22.1)	236 (26.0)	187 (21.1)	164 (17.8)	150 (16.9)	938 (20.8)
Black	178 (19.6)	130 (14.3)	116 (13.1)	105 (11.4)	83 (9.3)	612 (13.6)
Other	36 (4.0)	35 (3.9)	56 (6.3)	56 (6.1)	68 (7.6)	251 (5.6)
Family income (n [%])						
<USD 1,245	342 (37.3)	309 (33.6)	277 (31.0)	242 (26.2)	198 (22.0)	1,368 (30.0)
USD 1245–3319	392 (42.7)	392 (42.7)	379 (42.4)	401 (43.4)	391 (43.5)	1,955 (42.9)
≥USD 3320	184 (20.0)	218 (23.7)	238 (26.6)	282 (30.5)	309 (34.4)	1,231 (27.0)
Excessive alcohol use (n [%])	58 (6.3)	50 (5.4)	58 (6.4)	45 (4.8)	38 (4.2)	249 (5.4)
Body mass index (mean±SD)	27.5±5.1	27.3±4.7	27.7±5.1	27.1±4.9	27.1±4.5	27.4±4.9
Thyroid function (n [%])						
Normal	795 (86.3)	756 (82.4)	782 (86.8)	791 (85.4)	761 (84.6)	3,885 (85.1)
Hypothyroidism	110 (11.9)	148 (16.1)	105 (11.7)	116 (12.5)	129 (14.3)	608 (13.3)
Hyperthyroidism	16 (1.7)	13 (1.4)	14 (1.6)	19 (2.1)	10 (1.1)	72 (1.6)
Smoking status (n [%])						
Never smoked (n [%])	449 (48.7)	494 (53.8)	496 (55.0)	501 (54.0)	485 (53.9)	2,425 (53.1)
Former smoker (n [%])	272 (29.5)	269 (29.3)	272 (30.2)	296 (31.9)	308 (34.2)	1,417 (31.0)
Current smoker (n [%])	201 (21.8)	156 (17.0)	134 (14.9)	131 (14.1)	107 (11.9)	729 (15.9)
Hypertension (n [%])	293 (31.8)	276 (30.0)	275 (30.5)	278 (30.0)	320 (35.6)	1,442 (31.6)
Diabetes (n [%])	167 (18.1)	169 (18.4)	182 (20.2)	192 (20.7)	213 (23.7)	923 (20.2)
Dyslipidemia (n [%])	519 (56.3)	507 (55.2)	505 (56.0)	534 (57.5)	548 (60.9)	2,613 (57.2)
Anemia (n [%])	45 (4.9)	51 (5.5)	25 (2.8)	32 (3.5)	34 (3.8)	187 (4.1)
Use of vitamin supplements (n [%])	71 (7.7)	83 (9.0)	74 (8.2)	125 (13.5)	180 (20.0)	533 (11.7)
Cognition test performances (mean±SD)						
Word list learning	19.78±3.94	19.97±3.78	20.11±3.73	20.32±3.78	20.29±3.75	20.09±3.80
Delayed recall	6.44±1.99	6.50±2.00	6.56±1.96	6.60±1.96	6.62±1.92	6.54±1.97
Word recognition	9.52±0.83	9.46±1.01	9.51±0.87	9.55±0.81	9.51±0.91	9.51±0.89
Verbal fluency test	17.76±4.98	18.03±5.04	18.24±5.07	18.37±4.99	18.50±4.89	18.18±5.00
Trail Making Test Part B	129.51±91.34	129.09±95.41	123.03±85.53	124.14±87.58	120.50±79.31	125.28±88.08

SD: standard deviation; USD: US dollars.

of diabetes (p=0.001) were associated with higher folic acid levels, whereas current smokers (p<0.001) and individuals with anemia (p=0.037) had lower folic acid levels. Regarding the performance in cognition tests, the trend tests showed that individuals with higher serum folate level in quintiles had better performance in the word list learning (p=0.001), delayed recall (p=0.044), and verbal fluency tests (p<0.001), but not in word recognition (p=0.32) or TMT-B (p=0.12).

Table 2 shows the beta coefficients for the association between serum folate levels in quintiles and performance (according to standardized scores) in the cognitive tests. Serum folate levels were not associated with global cognitive score in the crude (p=0.66 for 1st *vs.* 5th quintile comparison) and adjusted (p=0.37 for 1st *vs.* 5th quintile comparison) models. When analyzing each cognition test separately, we did not find significant associations either, except for a poorer performance in TMT-B in the highest quintile group (p=0.037 in the adjusted model). However, when we further adjusted the model for age, sex, and education level to reduce residual confounding, this association vanished (β:-0.050; 95% confidence interval [95%CI] -0.143 to 0.042; p=0.29).

Interaction terms between serum folate quintiles and sex (p=0.52 to p=0.88), age (p=0.58 to p=0.92), and use of vitamin supplements (p=0.20 to p=0.69) were not significant. Table 3 shows the beta coefficients for the association between serum folate quintile groups and global cognitive scores in subgroups. Due to the smaller subsample size of individuals using vitamin supplements (n=533), in this subgroup the serum folate levels were analyzed in tertiles. In all cases, the results were consistent with the findings for the

Table 2. Beta coefficients for the association between serum folate levels in quintiles and standardized scores in the cognitive tests.

	Serum folate group	Crude models	Adjusted models
Global cognitive score	1st quintile	0 (Reference)	0 (Reference)
	2nd quintile	-0.022 (-0.113 to 0.069)	-0.042 (-0.133 to 0.049)
	3rd quintile	-0.009 (-0.101 to 0.083)	-0.041 (-0.133 to 0.051)
	4th quintile	0.018 (-0.073 to 0.109)	-0.039 (-0.131 to 0.052)
	5th quintile	0.021 (-0.071 to 0.113)	-0.043 (-0.135 to 0.050)
Word list learning	1st quintile	0 (Reference)	0 (Reference)
	2nd quintile	-0.004 (-0.095 to 0.087)	-0.027 (-0.119 to 0.064)
	3rd quintile	0.006 (-0.086 to 0.097)	-0.019 (-0.112 to 0.073)
	4th quintile	0.040 (-0.051 to 0.131)	-0.002 (-0.094 to 0.089)
	5th quintile	0.043 (-0.048 to 0.135)	0.000 (-0.093 to 0.093)
Delayed recall	1st quintile	0 (Reference)	0 (Reference)
	2nd quintile	-0.015 (-0.106 to 0.076)	-0.031 (-0.122 to 0.061)
	3rd quintile	-0.010 (-0.101 to 0.082)	-0.034 (-0.126 to 0.059)
	4th quintile	-0.004 (-0.095 to 0.086)	-0.040 (-0.132 to 0.052)
	5th quintile	0.029 (-0.063 to 0.120)	-0.009 (-0.102 to 0.084)
Word recognition	1st quintile	0 (Reference)	0 (Reference)
	2nd quintile	-0.059 (-0.150 to 0.032)	-0.068 (-0.160 to 0.024)
	3rd quintile	-0.028 (-0.120 to 0.063)	-0.042 (-0.135 to 0.050)
	4th quintile	0.013 (-0.078 to 0.104)	-0.005 (-0.097 to 0.088)
	5th quintile	-0.025 (-0.117 to 0.066)	-0.033 (-0.126 to 0.061)
Verbal fluency test	1st quintile	0 (Reference)	0 (Reference)
	2nd quintile	0.022 (-0.069 to 0.113)	0.021 (-0.071 to 0.113)
	3rd quintile	0.017 (-0.074 to 0.109)	0.006 (-0.087 to 0.098)
	4th quintile	0.042 (-0.049 to 0.133)	0.015 (-0.077 to 0.107)
	5th quintile	0.044 (-0.047 to 0.136)	0.005 (-0.088 to 0.099)
Trail Making Test Part B	1st quintile	0 (Reference)	0 (Reference)
	2nd quintile	-0.013 (-0.104 to 0.078)	-0.026 (-0.117 to 0.064)
	3rd quintile	-0.013 (-0.105 to 0.078)	-0.039 (-0.130 to 0.052)
	4th quintile	-0.033 (-0.124 to 0.057)	-0.091 (-0.182 to 0.000)
	5th quintile	-0.026 (-0.117 to 0.066)	-0.098 (-0.190 to -0.006)

Models are adjusted for ethnicity, family income, smoking status, hypertension, diabetes, dyslipidemia, thyroid function, and excessive alcohol use.

Table 3. Beta coefficients for the association between serum folate levels in quintiles and global cognitive score in prespecified subgroups.

	Serum folate group	Serum folate levels	Crude models	Adjusted models
Men (n=2,104)	1st quintile	≤12.2 ng/dL	0 (Reference)	0 (Reference)
	2nd quintile	12.3–14.3 ng/dL	0.089 (-0.044 to 0.223)	0.055 (-0.078 to 0.189)
	3rd quintile	14.4–16.8 ng/dL	0.026 (-0.107 to 0.158)	-0.017 (-0.150 to 0.116)
	4th quintile	16.9–20.4 ng/dL	0.097 (-0.036 to 0.230)	0.023 (-0.111 to 0.157)
	5th quintile	≥20.5 ng/dL	0.089 (-0.044 to 0.223)	0.010 (-0.125 to 0.145)
Women (n=2,467)	1st quintile	≤13.2 ng/dL	0 (Reference)	0 (Reference)
	2nd quintile	13.3–15.6 ng/dL	-0.048 (-0.174 to 0.077)	-0.063 (-0.188 to 0.062)
	3rd quintile	15.7–18.2 ng/dL	-0.041 (-0.167 to 0.085)	-0.068 (-0.193 to 0.058)
	4th quintile	18.3–22.0 ng/dL	0.008 (-0.117 to 0.134)	-0.029 (-0.154 to 0.096)
	5th quintile	≥22.1 ng/dL	-0.007 (-0.132 to 0.119)	-0.063 (-0.190 to 0.063)
Age <55 years (n=2,931)	1st quintile	≤12.4 ng/dL	0 (Reference)	0 (Reference)
	2nd quintile	12.5–14.5 ng/dL	0.007 (-0.107 to 0.121)	-0.004 (-0.117 to 0.110)
	3rd quintile	14.6–16.9 ng/dL	0.020 (-0.093 to 0.132)	-0.011 (-0.122 to 0.101)
	4th quintile	17.0–20.1 ng/dL	0.014 (-0.100 to 0.128)	-0.030 (-0.143 to 0.083)
	5th quintile	≥20.2 ng/dL	0.036 (-0.078 to 0.150)	-0.005 (-0.119 to 0.109)
Age ≥55 years (n=1,640)	1st quintile	≤13.2 ng/dL	0 (Reference)	0 (Reference)
	2nd quintile	13.3–16.1 ng/dL	-0.041 (-0.195 to 0.113)	-0.033 (-0.189 to 0.124)
	3rd quintile	16.2–19.0 ng/dL	0.012 (-0.143 to 0.166)	-0.039 (-0.196 to 0.119)
	4th quintile	19.1–23.4 ng/dL	-0.002 (-0.155 to 0.152)	-0.045 (-0.201 to 0.112)
	5th quintile	≥23.5 ng/dL	0.008 (-0.146 to 0.162)	-0.032 (-0.189 to 0.125)
No reported use of vitamin supplements (N=4,038)	1st quintile	≤12.5 ng/dL	0 (Reference)	0 (Reference)
	2nd quintile	12.6–14.8 ng/dL	-0.013 (-0.110 to 0.084)	-0.028 (-0.125 to 0.068)
	3rd quintile	14.9–17.2 ng/dL	0.017 (-0.080 to 0.114)	-0.016 (-0.113 to 0.081)
	4th quintile	17.3–20.9 ng/dL	0.004 (-0.094 to 0.102)	-0.049 (-0.147 to 0.049)
	5th quintile	≥21.0 ng/dL	0.008 (-0.089 to 0.106)	-0.043 (-0.141 to 0.055)
Reported use of vitamin supplements (N=533)	1st tertile	≤15.9 ng/dL	0 (Reference)	0 (Reference)
	2nd tertile	16.0–21.4 ng/dL	0.028 (-0.179 to 0.236)	-0.010 (-0.221 to 0.202)
	3rd tertile	≥21.5 ng/dL	0.040 (-0.168 to 0.248)	-0.018 (-0.235 to 0.198)

Adjusted models are adjusted for ethnicity, family income, smoking status, hypertension, diabetes, dyslipidemia, thyroid function, and excessive alcohol use. Considering that the subgroup of individuals who use vitamin supplements is comparably small, in this case serum folate levels were categorized into tertiles.

entire sample, and we did not find any significant associations between serum folate and global cognitive scores in these subgroups.

DISCUSSION

We did not find any association between serum folate levels and global performance in the cognitive tests at the ELSA-Brasil baseline. When analyzing the cognitive tests separately, we observed poorer performance in TMT-B among those in the quintile of highest serum folate level. However, further adjustment for age, sex, and education level demonstrated that this association was probably due to residual confounding. Analyses stratified by age or sex did not show any significant associations between serum folate and global performance in the cognitive tests either.

Previous observational studies (mainly focused on older adults) found mixed results. Hooshmand et al.[9] analyzed 274 subjects from the Cardiovascular Risk Factors, Aging and Dementia (CAIDE) study (mean age of 70.1 years) who underwent five cognitive tests (MMSE, immediate word recall test, Stroop test, a category fluency test, and a combination of the bimanual Purdue Pegboard Test and the Letter Digit Substitution Test). The main results, for the entire sample did not demonstrate evidence of a significant association between serum folate levels and cognitive performance. After excluding individuals with dementia (at baseline or follow-up), increased serum folate concentrations at baseline were related to higher scores in global cognition and verbal

expression tests seven years later. However, this association was not consistent for all quartile groups, with lack of a dose-response pattern. Additionally, when individuals with serum folate <2.3 ng/mL were excluded, this association vanished as well. This is consistent with our findings, suggesting that although folate deficiency is involved in cognitive impairment[27,28], serum folate levels outside the deficiency range may not influence the cognitive performance.

On the other hand, de Lau et al.[11] analyzed cross-sectional data from 1,033 participants of the Rotterdam Scan Study, aged 60 to 90 years (mean age of 72.2 years) to verify whether serum folate levels were associated with performance in a composition of several tests to assess psychomotor speed, memory function, and a composite global cognitive score. They found that an increase of one standard deviation in serum folate was associated with better global cognition and psychomotor speed, but not with memory functions after adjustment for major confounders. In addition, Doets et al.[8] analyzed data from 2,203 individuals with a mean age of 72.5 years from Norway, who were evaluated with six cognitive tests (MMSE, digit symbol substitution test, block design, Kendrick Object Learning Test, Controlled Oral Word Association Test, and Trail Making Test Part A. They also weighted a summary score using principal component analysis. The authors found that higher folate levels were cross-sectionally associated with better cognitive performance. Nevertheless, this finding was due to the association between cognitive performance and folate levels in two particular subgroups: participants in the lowest quantiles of the cognitive score and/or those with low vitamin B12 levels, as evidenced by a significant interaction between folate and vitamin B12 levels. It is worth emphasizing two important differences between these two aforementioned studies and ours. First, the subjects' mean ages are very different from the mean age of our study (51.4 years). Second, mean serum folate levels in the study conducted by de Lau et al. (6.3 ng/dL) and median serum folate levels in the study of Doets et al.[8] (7.0 ng/dL) were well below the cutoff for the first quintile group in our sample (12.6 ng/dL). The sample characteristics in these two studies are comparable to what would be expected for individuals not exposed to food fortification. Currently, more than 80 countries have adopted folic acid fortification policies[29]. In these countries, including Brazil[30], the prevalence of inadequacy in folate intake has decreased. This new scenario demands new studies focusing on clinical conditions determined or influenced by low folate levels. In this context, significantly different conclusions may be drawn[31,32,33], as demonstrated by the contrasts between these studies and ours. Based on our results, we hypothesize that these positive results from previous studies cannot be extrapolated to populations in which food fortification is implemented (with rarer cases of folate deficiency), or for younger populations.

Results from clinical trials also support the interpretation that, in contexts with food fortification policies and low folate deficiency prevalence, folate levels are not associated with cognitive performance. The aforementioned study conducted by Durga et al.[14] was focused on individuals with high homocysteine levels, a subset of individuals with high probability of folate deficiency. In addition, at the time of their study, folic acid fortification of foods was prohibited in the Netherlands. Other trials, according to which folate supplementation was beneficial to cognition in adults, were mainly focused on individuals with low folate[34,35] levels or high homocysteine levels, whereas studies on samples without this characteristic mostly found negative results[36,37,38].

Neuropathological lesions, which ultimately lead to cognitive impairment, may be present several years before the symptoms of clinical dementia[39]. Therefore, it is worth studying the determining factors of cognitive function in younger individuals, to whom preventive strategies may be more feasible. As previously stated, there have been few studies on the association between serum folate levels and cognitive performance in young individuals. The aforementioned article by Horvat et al.[10], a rare study including individuals aged 55 or less, had a mean age of 64.5 years at baseline. This is slightly higher than the mean age of our sample. In addition, the high proportion of individuals aged 55 years or less in our sample (64.1%) enabled us to separately analyze this subset of middle-aged adults. Our results, therefore, extend the findings of non-association between folate levels and cognitive performance to a younger population.

Our study has some strengths. We analyzed data from a large cohort, with strict protocol and cognitive tests applied by trained professionals. Our sample includes a significant proportion of individuals aged 55 years or less, subjects to whom potential preventive strategies may be more feasible. In addition, we analyzed data from a remarkably diverse sample, outside the USA and Europe. In addition, our study must be understood within its context. This is a cross-sectional analysis, and it is not possible to completely exclude reverse causation. A possibility for reverse causality in this case is that individuals with advanced dementia could have nutritional deficiency (and consequent low folate levels) due to the disease. However, at ELSA-Brasil enrollment, individuals with severe cognitive or communication impairments were excluded[15], making our sample less exposed to this potential reverse causation. The ELSA-Brasil sample has higher socioeconomic status compared with the general Brazilian population, and there must be caution when generalizing our results to the entire country. Our sample does not enable to study the effects of folate deficiency on cognitive performance. Very few participants had serum folate levels below the WHO cutoffs for folate deficiency[23]. However, this is the current scenario in most countries where food fortification was adopted[40,41], leading to increasing interest in whether serum folate levels outside the folate deficiency range may be associated with health outcomes. Our protocol did not include red cell folate and homocysteine[42], which might have resulted in more subtle

cases of folate deficiency. Nevertheless, considering the very low number of individuals with possible folate deficiency in our sample, it is implausible that we would be able to identify a considerable number of individuals with folate deficiency using this strategy. We also performed a single determination of folate levels for each participant, which may not reflect in long-term folate levels and may have reduced our power to detect positive associations due to non-systematic (random) error. We had high (90%) power to detect a difference in standardized scores of 0.15. Probably, larger samples would be able to detect differences smaller than that with the same study power. However, we consider that such small differences would be of little clinical significance.

In conclusion, we did not find significant associations between serum folate levels and cognitive performance in this large sample, characterized by a very low frequency of folate deficiency and a context of food fortification policies. Our results can also be extrapolated to adults aged 35 to 54 years, an age stratum more prone to preventive strategies for cognitive impairment and poorly studied in previous reports.

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