Serum Folate but Not Vitamin B-12 Concentrations Are Positively Associated with Cognitive Test Scores in Children Aged 6–16 Years1,2

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Abstract

Folate and vitamin B-12 are important for nervous system functioning at all ages, with important roles in functions such as neurotransmitter synthesis. Although studies suggest a relation between folate and vitamin B-12 and cognitive function in the elderly population, there is relatively less evidence regarding these vitamins and children’s cognitive function. The purpose of the study was to examine the associations of serum folate and vitamin B-12 with cognitive performance in children 6–16 y old in the NHANES III, conducted from 1988 to 1994, prior to the implementation of folic acid fortification. A cross-sectional analysis was conducted using data on 5365 children 6–16 y old from NHANES III. Serum folate and vitamin B-12 concentrations were measured, along with performance, on the Wide Range Achievement Test-Revised and the Wechsler Intelligence Scale for Children-Revised. Associations of B vitamins with cognitive performance were assessed using linear regression models adjusted for various covariates. Higher serum concentrations of folate were associated with higher reading and block design scores after adjusting for various covariates. For example, compared with the lowest quartile of folate, children in the highest quartile scored 3.28 points or 0.19 SD units higher on the reading test (P < 0.05). Vitamin B-12 was not associated with any of the test scores. In the largest study to date, higher folate concentrations were associated with better reading and block design scores. These associations appear to be biologically plausible and merit further study. J. Nutr. 143: 500–504, 2013.

Introduction

Folate and vitamin B-12 are water-soluble vitamins that are important in neurological and psychological functions (1,2). Deficiencies in these B vitamins can influence multiple aspects of neuronal physiology, including neurotransmitter synthesis of dopamine, norepinephrine, and serotonin, and axon and myelin metabolism (3–5). As a result, deficiencies in folate and vitamin B-12 may have direct physiological effects on brain functions, with important implications for cognitive function.

Although studies have shown an association between increased concentrations of B vitamins, such as vitamin B-6, folate, and vitamin B-12, and higher test scores as well as lower cognitive decline in the elderly population (2,6–10), there is limited research regarding B vitamins and cognitive function in children. Three intervention studies suggested that increased concentrations of both folate and vitamin B-12 may improve cognitive performance. Jiang (11) examined normal children 9–11 y old in China and found that test scores were higher in the groups supplemented with the B vitamin compound (thiamin, riboflavin, niacin, and folate). Gewa et al. (3) examined first-grade children from 12 different primary schools in Kenya and found an association between higher digit span test scores and higher concentrations of vitamin B-12. Sen and Kanani (12) examined girls ages 9–13 y randomly selected from 4 schools in India and observed higher test scores for girls supplemented with both iron and folic acid. However, these studies had various limitations, such as small sample sizes (11,12); limited cognitive assessments (11); use of dietary recall data to assess intake (3); joint interventions with multiple supplements, thereby making it difficult to distinguish effects of B vitamins from other micronutrients (11); and uncertain generalizability to populations where micronutrient deficiencies are not as common (3,12).

To date, large population-based studies have not addressed potential associations of B vitamins and cognitive function in children. The present study examined the associations of serum folate and vitamin B-12 with cognitive performance in a U.S. nationally representative sample of children ages 6–16 y, NHANES III, prior to folic acid fortification.

Subjects and Methods

Sample explanation

A cross-sectional study was done based on the data presented in the NHANES III for children 6–16 y old. NHANES III is a nationally representative survey of the health and nutritional status of the civilian,

1 Author disclosures: C. T. Nguyen, E. J. Gracely, and B. K. Lee, no conflicts of interest.
2 Supplemental Figure 1 is available from the “Online Supporting Material” link in the online posting of the article and from the same link in the online table of contents at http://jn.nutrition.org.
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noninstitutionalized U.S. population and was conducted between 1988 and 1994 in 2 phases including 81 different counties. Data in NHANES III were obtained using a complex, multi-stage, probability sampling design. Details and documentations of NHANES III can be found at: http://www.cdc.gov/nchs/nhanes/nh3data.htm (13).

In total, there were 5365 children 6–16 y old in NHANES III. After excluding participants missing folate and vitamin B-12 measurements, cognitive test scores, or poverty-income ratio, sample sizes for folate and vitamin B-12 analyses were 3970 (74.1%) and 2014 (37.6%), respectively (Fig. 1).

**Specimen collection and laboratory measurement**

Blood specimens were collected at the mobile examination and home examination centers by medical technologists and phlebotomists. All collection materials were prescreened for contamination of lead, cadmium, and selenium. The vials were refrigerated or frozen until they were sent to the laboratories for analysis (14).

Detailed descriptions of the laboratory methodology are available on the CDC Web site (15). Briefly, folate and vitamin B-12 concentrations were measured using the Bio-Rad Laboratories Quantraphase Folate RIA kit. The limits of detection (LODs) for folate and vitamin B-12 were 0.2 ng/mL and 20 pg/mL, respectively. Of the 5365 children ages 6–16 y, 89.2% of the children had measured values for folate and 45.1% for vitamin B-12. The reduced sample size for vitamin B-12 analyses was due to the fact that vitamin B-12 measurements were conducted only during phase 2 of NHANES III (1991–1994) (14). Blood lead was measured by atomic absorption spectrometry with a LOD of 1 μg/dL. Serum concentrations of iron were measured by the colorimetric method with a LOD of 3.0 μg/dL. Serum concentrations of vitamins A, C, and E were measured by HPLC with LODs of 0.5 μg/dL, 0 mg/dL, and 20 μg/dL, respectively (15).

Cognitive tests

Trained interviewers measured cognitive function in children in a standardized fashion using the Wechsler Intelligence Scale for Children-Revised (WISC-R) (16) and the Wide Range Achievement Test-Revised (WRAT-R) (17), both well accepted as valid measures of children’s cognitive function (18,19). The outcomes of interest were the cognitive test scores on WRAT-R (reading and math) and WISC-R (block design and digit span). The detailed testing procedure is described elsewhere (20). Briefly, the reading test asks the child to recognize and name letters, pronounce words, spell words, and write single words. The math test asks the child to orally count the number of objects and to answer as many mathematical problems as he or she can in 10 min. In the block design test, the child is asked to arrange 9 blocks into particular shapes. Scores are assigned on succeeding within the given time limit. This test assesses perceptual reasoning index and executive functions (21). The digit span test has 2 parts: a digits forward and a digits backward. The child is asked to repeat a sequence of numbers within a given time limit. This test assesses working memory and attention span (21). Age-standardized scores were used in analyses.

Other covariates

Covariates considered for adjustment in models were based on a priori theory from likely or empirical associations with either folate/vitamin B-12 concentrations and/or cognitive performance. These covariates included: sex, age of the child at time of examination, height (cm) and weight (kg) of the child; race/ethnicity (non-Hispanic whites, non-Hispanic blacks, Mexican Americans, and other), income-poverty ratio (the ratio of the total family income over the federal poverty level/threshold); blood lead levels; and general nutrition status. To assess general nutrition status, iron and vitamins A, C, and E were combined to create a nutritional status variable. Indicator variables for iron and vitamins A, C, and E were created based on values above or equal to (1) or below (0) the median. The median concentrations for iron and vitamins A, C, and E were 78 μg/dL, 38 μg/dL, 0.96 mg/dL, and 749 μg/dL, respectively. The nutritional status variable is a sum of these 4 variables, ranging from 0 to 4.

**Statistical analyses**

**Power/sample size considerations.** The final sample size for the folate data was 3970. A univariate correlation analysis would have 80% power to detect a correlation magnitude of 0.045. The comparison of highest to lowest quartiles on a numeric measure (each quartile with ~900 participants) would have 80% power to detect a difference of ~0.13 SDs.

The final sample size for the vitamin B-12 data was 2014. A univariate correlation analysis would have 80% power to detect a correlation magnitude of 0.06. The comparison of highest to lowest quartiles on a numeric measure (each quartile with about 500 participants) would have 80% power to detect a difference of ~0.17 SD.

**Preliminary analyses.** Distributions of the test scores, folate, and vitamin B-12 were examined using univariate analysis and histograms. t tests and chi-square tests were used to compare means and percentages between groups. Spearman correlations were used to examine the associations of folate and vitamin B-12 with the test scores. Examinations of scatterplots suggested that the relations of folate and vitamin B-12 with test scores were nonlinear. Therefore, folate and vitamin B-12 were categorized into quartiles for further analysis ([Supplemental Fig. 1](#)). The unweighted means for serum folate and vitamin B-12 were 7.80 ng/mL and 671 pg/mL, respectively; the medians for serum folate and vitamin B-12 were 6.80 ng/mL and 627 pg/mL, respectively. The 10th and 90th percentiles for folate were 3.40 and 12.8 ng/mL and the 10th and 90th percentiles for vitamin B-12 were 377 and 999 pg/mL. Folate (ng/mL) quartile cutoff points were 5.10, 7.29, 10.5, and 143; vitamin B-12 (pg/mL) quartile cutoff points were 456, 578, 741, and 2014.

**Multivariate analyses.** Survey-weighted multiple linear regression models were used to examine adjusted differences in the 4 cognitive tests by folate and vitamin B-12 concentration quartiles. The cognitive test scores were treated as continuous variables. Covariates included age (continuous), sex, height (continuous), weight (continuous), race/ethnicity (4 categories), income-poverty ratio (quartiles), blood lead (quartiles), and nutritional status (5 categories). Three model specifications were examined. In model 1, unadjusted associations of the B vitamins with cognitive test scores were examined. Model 2 adjusted for age, race/ethnicity, sex, and income-poverty ratio. Model 3 adjusted for the covariates in Model 2 along with blood lead and nutritional status.

**Additional analyses.** Possible interactions of folate/vitamin B-12 quartiles with sex and age in their effect on cognitive test scores were separately assessed by adding cross-product interaction terms to the appropriate models, with statistical evidence determined through likelihood ratio tests.
In addition, models with continuous log folate and log vitamin B-12 were tested to determine whether results were sensitive to quartile parameterizations of folate and vitamin B-12.

All statistical analyses were performed using SAS software, version 9.2. Individual sampling weight, primary sampling cluster, and sampling strata variables were used to account for the complex survey sample design of NHANES III. For all statistical tests, $P < 0.05$ was considered significant.

### Results

The final sample sizes used for analyses for folate and vitamin B-12 were 3970 and 2014, respectively (Fig. 1). The survey weighted mean folate and vitamin B-12 concentrations for children ages 6–16 y were 8.5 ng/mL and 630.9 pg/mL, respectively. Table 1 shows the characteristics of the folate and vitamin B-12 samples. Note that the table shows the unweighted, actual numbers (which are heavily oversampled for some subgroups such as non-Hispanic blacks and Mexican Americans) but population weighted percentages. The respective survey weighted means for the 4 outcome variables, math, reading, block design, and digit span scores, for folate and vitamin B-12 are also shown in Table 1. The percent deficient values for folate and vitamin B-12 were 6.02 and 0.35%, respectively. There were small positive correlations between folate and math, block design, digit span test scores, and income-poverty ratio ($r = 0.10, 0.13, 0.08$, and $0.06$, respectively; $P < 0.001$). There were also small positive correlations between vitamin B-12 and math, and digit span test scores ($r = 0.06$ and 0.08, respectively; $P < 0.05$). There were modest negative correlations between folate and vitamin B-12 concentrations and age ($r = -0.38$ and $-0.39$, respectively; $P < 0.001$). Furthermore, there were modest positive correlations between income-poverty ratio and math, reading, block design, and digit span test scores ($r = 0.31, 0.35, 0.28$, and $0.24$, respectively; $P < 0.001$).

There were differences between sex, race/ethnicity, and income-poverty ratio quartiles for folate ($P < 0.0001$), with higher concentrations of folate observed in males, non-Hispanic whites compared with the other race/ethnicity groups, and those in the highest quartile of the income-poverty ratio compared with the lower 3 quartiles. There were also differences between race/ethnicity for vitamin B-12 ($P < 0.0001$), with higher concentrations of vitamin B-12 observed in non-Hispanic blacks compared with non-Hispanic whites.

### Multivariate analyses

In the unadjusted models (Table 2), there was a general increase in test scores as folate concentrations increased. For instance, there was a 4.54-point (0.27 SD units) increase in mean math score from the lowest to highest folate quartiles ($P < 0.001$). After adjusting for sex, age, race/ethnicity, and income-poverty ratio (model 2), compared with the lowest folate quartile, children in the highest folate quartile scored 3.67 points (0.22 SD units) higher on reading ($P < 0.001$) and 0.78 points (0.24 SD units) higher on mean block design ($P < 0.001$), whereas associations of folate with math and digit span scores were attenuated and no longer significant. Even after further adjustment for lead and nutritional status in model 3, higher levels of folate were still associated with higher mean scores in reading (3.28 points or 0.19 SD units; $P = 0.01$) and block design (0.64 points or 0.20 SD units; $P = 0.03$) scores compared with the lowest quartile of folate (Fig. 2).

In contrast to the results for folate, there was no statistical evidence that vitamin B-12 was associated with test scores in the unadjusted or adjusted regression models (Table 3).

### Discussion

The aim of this study was to examine the association between folate and vitamin B-12 concentrations and cognitive test scores. The results of these analyses suggest a positive association between folate concentrations and scores in reading and block design, with adjusted differences between the highest and lowest folate quartiles of ~0.20 SD units. In contrast, vitamin B-12 concentrations were not associated with cognitive test scores.
intervention studies, which used folic acid and other vitamins as well, in China and Kenya (11,12). In addition, the results for folic acid are consistent with the findings from a study done by Veena et al. (22). They found that maternal plasma folate was positively associated with cognitive function in children 9–10 years old in South India. Furthermore, they also did not find any evidence that higher vitamin B-12 concentrations were associated with higher cognitive test scores. However, our findings regarding vitamin B-12 are not consistent with other studies that focused on the primary school population in rural Kenya and cobalamin-deficient adolescents, respectively (3,23).

The study has several strengths and limitations. The present study sample is derived from NHANES III, a large, nationally representative survey of the U.S. population; therefore, findings from this study likely have high external validity. Of note, in 1996, the FDA published regulations for the addition of folic acid to enriched foods such as bread, flour, rice, and other grain products (24). As a result of food fortification, concentrations of serum folate in children today are higher than in NHANES III. Folate and vitamin B-12 measurements were collected through blood sampling, which is more reliable and accurate than if data were collected from dietary recall, which would be susceptible to recall and reporting bias. Moreover, cognitive assessments were more comprehensive than existing studies, with 4 cognitive domains included. However, this study was cross-sectional and observational in nature. As with any observational study, confounding due to unobserved covariates is possible. In addition, serum folate and vitamin B-12 concentrations were measured only once; therefore, it does not accurately represent long-term status. Intervention studies with longitudinal follow-up are needed to verify that higher folate concentrations lead to higher cognitive test scores.

Given that the prevalence of folic acid and vitamin B-12 deficiencies varies widely around the world (25), the generalizability of our results is uncertain. In our study, only 6.02 and 0.35% of the sample had serum concentrations of folate and vitamin B-12 indicating deficiency, respectively [for folate, <3 ng/mL for deficiency (26); for vitamin B-12, < 200 pg/mL for likely deficiency (27)]. The aforementioned study populations in India and Kenya were likely more deficient than those seen in NHANES III (3,12).

The association between higher folate concentrations and higher scores in the reading and block design tests may be due to the biological mechanisms in which folic acid and vitamin B-12 are involved. The hypomethylation hypothesis is a proposed neurochemical mechanism in which B vitamins influence cognitive function in children (2). In the hypomethylation hypothesis, folate directly affects the central nervous system. Folate is essential in 1-carbon metabolism, which is required for the synthesis of nucleic acids (28) as well as in methylation of DNA, a modification of the DNA after replication (29). Folate acts as a methyl group donor that adds a methyl group on to homocysteine, which is then converted to methionine and then to S-adenosylmethionine (SAM). SAM is important in gene transcription (30,31). Therefore, low concentrations of folate result in low concentrations of SAM, which will affect the metabolism of neurotransmitters.

### TABLE 2  Mean differences in cognitive test scores by serum folate quartiles in NHANES III participants 6–16 y old

<table>
<thead>
<tr>
<th></th>
<th>Unadjusted</th>
<th>Model 2</th>
<th>Model 3</th>
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<tbody>
<tr>
<td><strong>Math</strong></td>
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<tr>
<td>First quartile</td>
<td>Reference</td>
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<tr>
<td>Second quartile</td>
<td>2.15 ± 1.38</td>
<td>1.62 ± 1.21</td>
<td>1.88 ± 1.20</td>
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<td>Third quartile</td>
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<td>1.75 ± 1.30</td>
<td>1.81 ± 1.27</td>
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<td>Fourth quartile</td>
<td>4.54 ± 1.22***</td>
<td>2.54 ± 1.36</td>
<td>2.32 ± 1.40</td>
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<td><strong>Reading</strong></td>
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<tr>
<td>First quartile</td>
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<tr>
<td>Second quartile</td>
<td>−0.65 ± 1.16</td>
<td>0.72 ± 1.06</td>
<td>0.86 ± 1.07</td>
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<td>Third quartile</td>
<td>−0.89 ± 1.02</td>
<td>0.57 ± 0.90</td>
<td>0.46 ± 0.94</td>
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<tr>
<td>Fourth quartile</td>
<td>2.39 ± 1.18*</td>
<td>3.67 ± 1.53***</td>
<td>3.28 ± 1.26*</td>
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<td><strong>Block design</strong></td>
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<tr>
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<tr>
<td>Second quartile</td>
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<td>0.28 ± 0.23</td>
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<tr>
<td>Third quartile</td>
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<td>0.68 ± 0.23**</td>
<td>0.64 ± 0.24*</td>
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<tr>
<td>Fourth quartile</td>
<td>1.45 ± 0.27***</td>
<td>0.78 ± 0.27**</td>
<td>0.64 ± 0.28*</td>
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<td><strong>Digit span</strong></td>
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<tr>
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<td>Reference</td>
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<tr>
<td>Second quartile</td>
<td>0.46 ± 0.22*</td>
<td>0.32 ± 0.21</td>
<td>0.36 ± 0.21</td>
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<tr>
<td>Third quartile</td>
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<td>0.28 ± 0.22</td>
<td>0.31 ± 0.22</td>
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<td>Fourth quartile</td>
<td>0.74 ± 0.18***</td>
<td>0.35 ± 0.19</td>
<td>0.37 ± 0.22</td>
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</table>

1 Values are mean differences ± SE, n = 1216, 969, 1026, and 759 for folate quartiles, respectively. **P < 0.01; ***P < 0.001; *P < 0.05.
2 Data were analyzed using survey-weighted linear regression models.
3 Folate quartiles (ng/mL) = first: 0.700–5.10; second: 5.11–7.29; third: 7.30–10.5; fourth: 10.50–143.0.
4 Model 2 adjusted for sex, age, race/ethnicity, height, weight, and income-poverty ratio quartiles.
5 Model 3 adjusted for covariates in model 2, lead quartiles, and nutritional status.
6 To assess the magnitude of association, it may be helpful to compare against the associations of known predictors of cognitive performance. Model 3 estimated that for reading, females performed better than males by 1.90 points or 0.11 SD units ($P = 0.08$) and those in the highest quartile of blood lead performed worse than the lowest quartile by 5.55 points or 0.33 SD units ($P < 0.0001$). For block design, males performed better than females by 0.65 points or 0.20 SD units ($P < 0.0001$) and those in the highest quartile of blood lead performed worse than the lowest quartile by 0.96 points or 0.30 SD units ($P < 0.0005$).

### FIGURE 2  Reading scores (A) and block design scores (B) by folate quartiles in children 6–16 y old in NHANES III. Values are mean ± 95% CI. Scores are conditional on the mean values of the following covariates: sex, age, race/ethnicity, height, weight, income-poverty ratio quartiles, lead quartiles, and nutrition status. Folate quartiles (ng/mL) = first: 0.700–5.10; second: 5.11–7.29; third: 7.30–10.5; fourth: 10.50–143.0. n = 1216, 969, 1026, and 759 for folate quartiles, respectively.
TABLE 3  Mean differences in cognitive test scores by serum vitamin B-12 quartiles in NHANES III participants 6–16 y old1–3

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<td>−0.21 ± 0.36</td>
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<td>Fourth quartile</td>
<td>0.45 ± 0.27</td>
<td>0.46 ± 0.27</td>
<td>0.48 ± 0.27</td>
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</table>

<table>
<thead>
<tr>
<th>Digit span</th>
<th>Unadjusted</th>
<th>Model 24</th>
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<td>Second quartile</td>
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<td>Third quartile</td>
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<td>0.08 ± 0.33</td>
<td>0.09 ± 0.33</td>
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<tr>
<td>Fourth quartile</td>
<td>0.45 ± 0.27</td>
<td>0.46 ± 0.27</td>
<td>0.48 ± 0.27</td>
</tr>
</tbody>
</table>

TABLE 3  Mean differences in cognitive test scores by serum vitamin B-12 quartiles in NHANES III participants 6–16 y old1–3

1 Values are mean differences ± SE, n = 414, 417, 528, and 655 for vitamin B-12 quartiles, respectively. None of the associations were significant, P > 0.05.
2 Data were analyzed using survey-weighted linear regression models.
3 Vitamin B-12 quartiles (pg/mL) = first: 63.0–456; second: 456–578; third: 578–741; fourth: 741–5670.
4 Model 2 adjusted for sex, age, race/ethnicity, height, weight, and income-poverty ratio quartiles.
5 Model 3 adjusted for covariates in model 2, lead quartiles, and nutritional status.

In conclusion, higher serum folate concentrations were associated with better cognitive test scores for reading and block design in children. These associations appear to be biologically plausible and merit further study.

Acknowledgments
C.T.N. and B.K.L. designed research; C.T.N. conducted research; C.T.N., F.J.G., and B.K.L. analyzed data; C.T.N., E.J.G., and B.K.L. wrote the paper; and C.T.N. had primary responsibility for final content. All authors read and approved the final manuscript.

Literature Cited