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## Research Article

# The Status of Folate, Vitamin B-12 and Homocysteine among Australian Vegetarian and Non-Vegetarian Teenagers

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**Keywords**

- Folate
- Vitamin B-12
- Homocysteine
- Vegetarians
- Adolescents

**Abstract**

**Background/Aims:** Vegetarians have a high risk of abnormal vitamin B-12 (B-12), and homocysteine (Hcy), status. The objectives included assessment of: 1) folate, B-12, and Hcy status; 2) incidence rate of abnormal folate, B-12, and Hcy; and 3) associations between folate and B-12 with Hcy status among vegetarian and non-vegetarian adolescents.

**Methods:** A cross-sectional plasma folate, B-12, and Hcy assessment in 49 vegetarian and 639 non-vegetarian, 14-17 year-old, participants from New South Wales, Australia.

**Results:** Mean (range) folate (nmol/L), B-12 (pmol/L), and Hcy ( $\mu$ mol/L), were: 33.4 (9.57-101) vs. 27.7 (2.7-86),  $p=0.033$ ; 287.81 (134-702) vs. 392.22 (119-1300),  $p<0.001$ ; and 8.82 (3.1-28.7) vs. 8.19 (2.9-30.8),  $p=0.33$ , in vegetarians and non-vegetarians, respectively. A higher percentage of vegetarians were in lower serum B-12 categories, 8.2% vs. 0.9%, for  $<148$  pmol/L,  $p=0.003$ ; 22.4% vs. 9.4%, for B-12 between 148 and 222 pmol/L,  $p<0.001$ ; and 36.7% vs. 22.5%, for B-12 between 222 and 300 pmol/L,  $p<0.001$ . No statistically significant difference was detected for incidence of abnormal folate or Hcy.

**Conclusions:** B-12 is a nutrient of a concern for vegetarian teenagers. To improve B-12 status, vegetarian adolescents should consume foods fortified with B-12, and/or take B-12 supplements.

**INTRODUCTION**

Studies with adults have shown that vegetarian diets have been associated with a lower risk of several health conditions, including ischemic heart disease, some cancers, type 2 diabetes, and obesity [1,2]. These diets are also associated with an improved profile of several risk factors for chronic health problems, such as blood lipid profile, glycemic control, blood pressure, higher intake of fruits and vegetables, and lower body mass [3,4]. Nonetheless, vegetarians, in comparison to non-vegetarians, are at a higher risk of developing inadequate status of some nutrients, including vitamin B-12 (B-12) [5]. Consequently, vegetarians may develop an elevated homocysteine (Hcy), concentration [5]. In fact, due to the risk of B-12 deficiency, the strictest forms of vegetarian diets, such as vegan diets, are not recommended in certain periods of the lifecycle, including adolescence, by the German Nutrition Society [6].

Hcy is a sulfur containing amino acid. Its status depends, mainly, on B-12 and folate, although other B-vitamins, especially

vitamin B-6, also play a role. Hyperhomocysteinemia (Hcy  $> 10 \mu$ mol/L, HHcy), is associated with endothelial dysfunction and is considered an independent risk factor for cardiovascular disease (CVD) [5,7]. In adults, HHcy also constitutes an independent risk factor for osteoporotic bone fractures [8]. HHcy increases one's risk of brain atrophy, organic mental disorders, and a variety of diabetic complications [9-13]. In adolescents, both low serum B-12 and HHcy have been associated with low bone mineral density [14,15].

Several studies have assessed Hcy concentration in adult vegetarians. Findings consistently have shown higher Hcy concentrations among vegetarians, compared to non-vegetarians, with the means above cutoffs that constitute low risk of CVD [5,16]. Considering the importance of nutrients, such as folate and B-12, in human growth and development, and their impact on Hcy concentration, it is important to evaluate their status in youth. Data on Hcy concentration in adolescents are very scarce.

This is even more true for vegetarian adolescents. Even fewer studies have compared the status of folate, B-12, and Hcy among adolescent vegetarians and non-vegetarians. This study fills a gap in the literature regarding the status of these important vitamins, along with Hcy, in adolescent vegetarians and non-vegetarians.

## GOAL/OBJECTIVES

The goal of this study included assessment of folate, B-12, and Hcy status among Australian teenagers adhering to different diet patterns. Specifically, the objectives included: 1) analysis of differences between the mean folate, B-12, and Hcy concentrations between the two respective diet groups; 2) assessment of incidence rate of abnormal folate, B-12, and Hcy within each diet group; and 3) assessment of associations between folate and B-12 with Hcy status.

## MATERIALS AND METHODS

The study protocol was approved by the Sydney Adventist Hospital Human Research Ethics Committee. The analyses included a cross-sectional assessment of plasma folate, B-12, and Hcy concentrations among 49 vegetarian and 639 non-vegetarian 14-17 year-old participants.

### Recruitment and data collection

The analysis is based on combined datasets from two previous studies: the Sydney Newcastle Adventist Schools Teen Physical Activity and Nutrition Study and the Healthy under the Skin You're In' study. Detailed description of recruitment has been described previously [17,18]. Recruitment of participants took place at both private and public secondary schools in the Sydney, Central Coast regions and Hunter regions of New South Wales, Australia. Parental consent and participants' assent was obtained.

### Blood samples collection

Following an overnight, ~12 hour, fast, 5-10 mL of blood was drawn by normal venipuncture. Serum samples for biochemical analyses were stored at -80°C until analysis. All assays were performed by the pathology department at the Sydney Adventist Hospital using routine methods.

### Definition of normal/abnormal status

Categories of folate included: < 6.8 nmol/L, 6.8 to < 13.4 nmol/L, 13.4 to < 45.3 nmol/L, and ≥ 45.3 nmol/L. Abnormally low folate was considered < 6.8 nmol/L. There were four categories of B-12 status, including < 149 pmol/L, 149 to 222 pmol/L, 222 to 300 pmol/L, and > 300 pmol/L. Hcy concentration > 10 μmol/L was considered HHcy.

### Analyses

Univariate analysis began by visually comparing the

distributions using side-by-side boxplots. Numerical summaries included median, mean, standard deviation, and geometric mean. The two-sample t-test (variances not assumed equal), was used to compare the groups on the raw scales for each variable. As there were outliers and skewness, the two-sample t-test was also used on the log-transformed data. Additionally, each of these variables was categorized into ordinal data. Distributions in the two groups were compared inferentially using Fisher's exact test. The relationship between B-12 and folate with Hcy was investigated using scatter plots both on the raw scale and on the log scale. Using the log scale resulted in approximately linear point clouds. Significance was set at  $p < 0.05$ . Data analysis was performed with the statistical software R, version 3.4.2 [19].

## RESULTS

Of a total of 688 cases, there were 49 vegetarian and 639 non-vegetarian participants. Vegetarians were defined as those individuals who did not ingest any meat, chicken, or fish. Males constituted 12.1% of the sample (14.4% of the vegetarian group and 11.6% of the non-vegetarians). Gender status for three participants was missing. Demographic characteristics of participants are found in Table 1.

### Comparison of folate, B-12, and Hcy between diet groups

The mean (range) of folate, B-12, and Hcy were: 33.4 (9.57-101) vs. 27.7 (2.7-86),  $p = 0.033$ ; 287.81 (134-702) vs. 392.22 (119-1300),  $p < 0.001$ ; and 8.82 (3.1-28.7) vs. 8.19 (2.9-30.8),  $p = 0.33$ , in vegetarians and non-vegetarians, respectively. Geometric means for folate, B-12, and Hcy were: 25.07 vs. 29.44; 265.61 vs. 361.03; and 8.18 vs. 7.85 for vegetarians and non-vegetarians, respectively. Detailed results are found in Table 2.

### Incidence of abnormal values

In comparison to non-vegetarians, vegetarians displayed a higher percentage of participants in lower serum B-12 categories, 8.2% vs. 0.9%, for <148 pmol/L,  $p = 0.003$ ; 22.4% vs. 9.4%, for B-12 between 148 and 222 pmol/L,  $p < 0.001$ ; and 36.7% vs. 22.5%, for B-12 between 222 and 300 pmol/L,  $p < 0.001$ . No statistically significant difference was detected for incidence of folate deficiency nor for elevated Hcy. Figure 1 depicts percentages of participants in the respective diet groups by different categories of folate and B-12.

### Association of folate and B-12 with Hcy

There was a negative correlation between folate and Hcy in both diet groups ( $r = -0.46$  for vegetarian and  $r = -0.42$  for non-vegetarians) when both variables were log-transformed (Figure 2). Log transformations were used because the relationship was approximately linear when using the log scale. Similarly, B-12

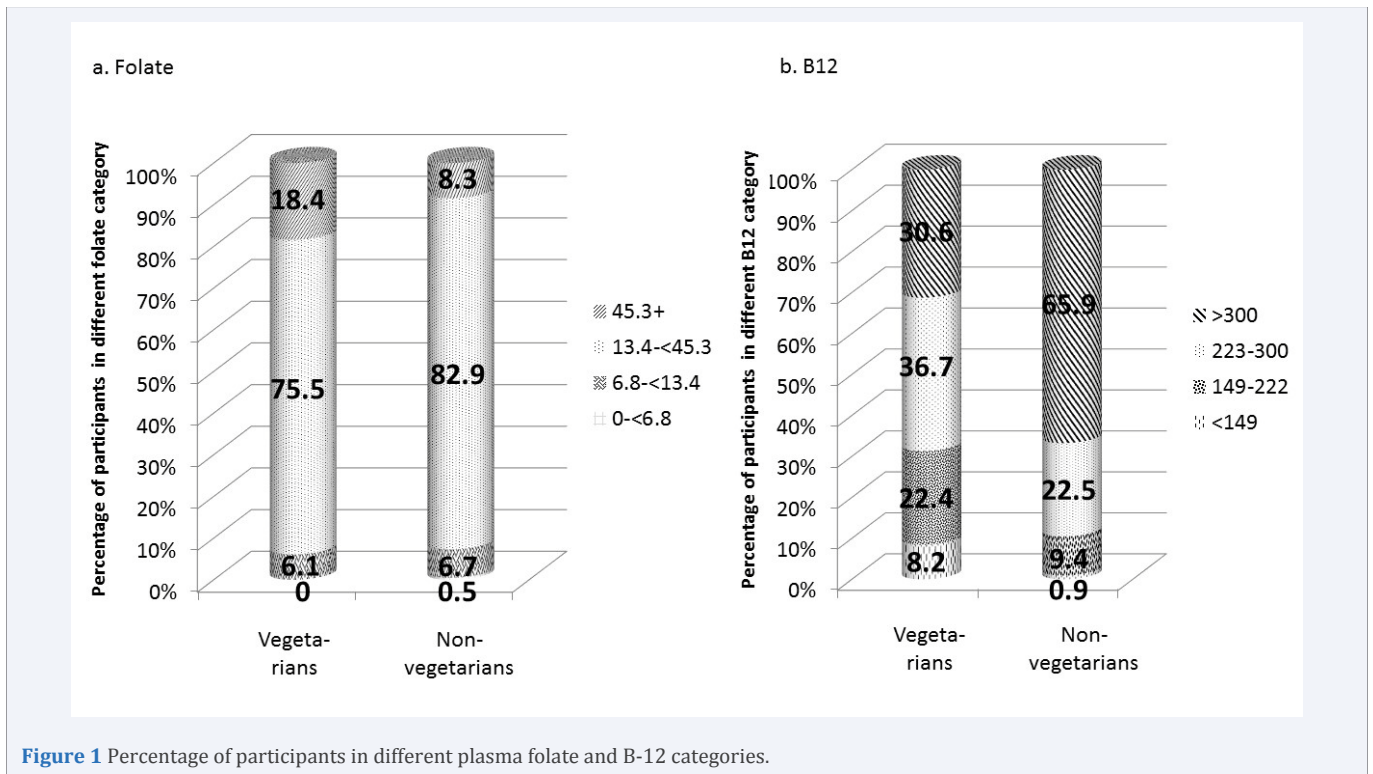
**Table 1:** Demographic and anthropometric characteristics of participants.

	Vegetarian (n = 49)		Non-vegetarian (n = 639)	
	Females n=39*	Males n=9*	Females n=563*	Males n=74*
<b>Gender</b>				
<b>Age</b> (mean ± SD)	15.6 ± 1	15.2 ± 0.5	15.6 ± 1.1	15.1 ± 0.6
<b>BMI</b> (mean ± SD)	21 ± 3.4	20 ± 2.4	21.6 ± 3.6	20.9 ± 3.4

\*- gender status was missing for one vegetarian and two non-vegetarian participants

**Table 2:** Comparison of folate, B-12, and Hcy status between vegetarians and non-vegetarians.

	Vegetarian (n = 49)	Non-vegetarian (n = 639)	Significance
<b>Folate (nmol/L)</b>			
Mean ± SD	33.4 ± 18.0	27.7 ± 12.9	p = 0.033
Median	30.8	25.0	
Geometric mean	29.4	25.1	
Range	9.6-101	2.7-86	
<b>Vitamin B-12 (pmol/L)</b>			
Mean ± SD	287.8 ± 126.7	392.2 ± 167.4	p < 0.001
Median	246.0	360.0	
Geometric mean	235.6	361.0	
Range	134-702	119-1300	
<b>Homocysteine (µmol/L)</b>			
Mean ± SD	8.8 ± 4.4	8.2 ± 2.6	p = 0.33
Median	7.9	7.8	
Geometric mean	8.17	7.9	
Range	3.1-28.7	2.9-30.8	
	25.6	27.9	



**Figure 1** Percentage of participants in different plasma folate and B-12 categories.

was inversely correlated with Hcy ( $r = -0.38$  for vegetarians and  $r = -0.43$  for non-vegetarians).

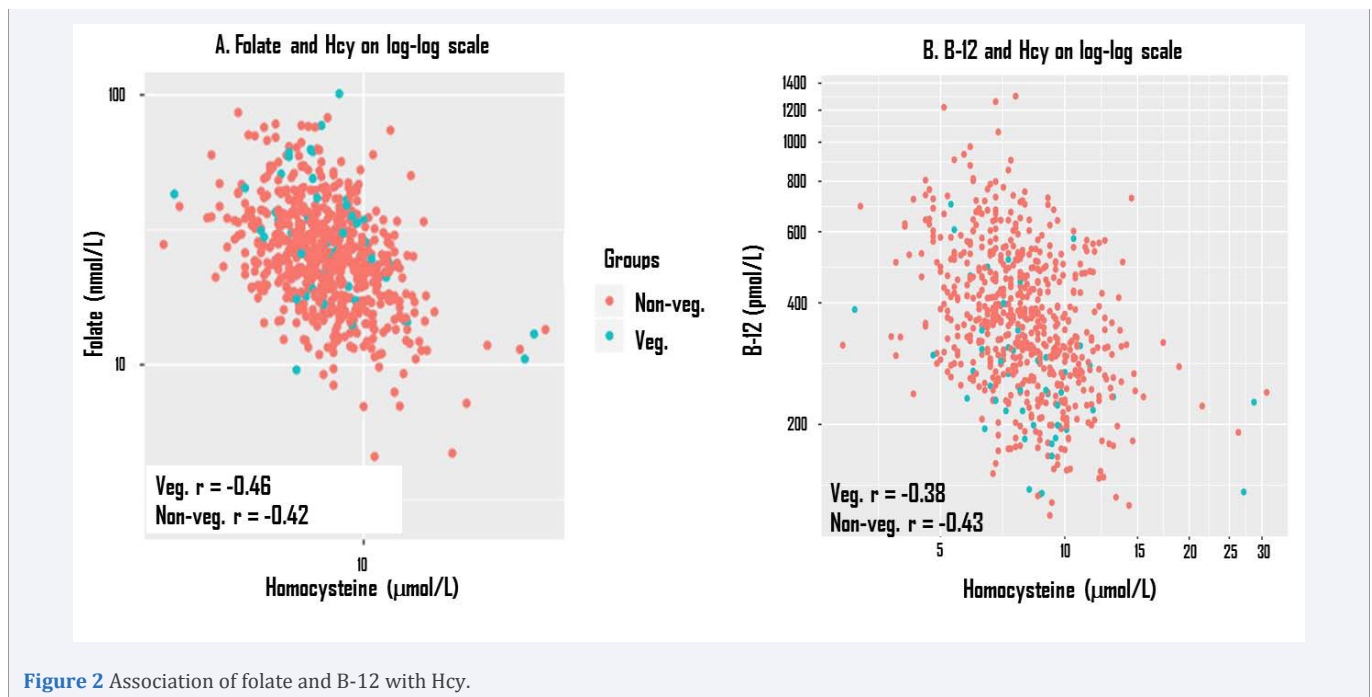
**DISCUSSION**

Although folate, B-12, and Hcy play a very important role in growth, development and disease prevention in all stages of the lifecycle, few studies to date have assessed their status among adolescents. In the current study, we have compared folate, B-12, and Hcy status among vegetarian and non-vegetarian teenagers from New South Wales, Australia. Consistent with our hypothesis, these findings have shown higher serum folate and lower B-12 among vegetarians, compared to non-vegetarians. The higher mean folate and lower B-12 among vegetarians, compared to non-vegetarians, are consistent with those reported previously

in studies with adults [5]. We found no statistically significant difference in Hcy concentration between the two diet groups, which was contrary to what we have expected. This may have reflected a partial compensation in the methylation cycle due to higher folate levels observed in vegetarian adolescents.

**Folate, B-12 and Hcy status among vegetarians and non-vegetarians**

We were only able to find one study that assessed folate, B-12, and Hcy status among 69 Australian children and teenagers (mean age  $12.8 \pm 2.3$  years) [20]. The mean serum folate among participants of that study was  $27.8 \pm 8.8$  nmol/L, compared to  $27.7 \pm 12.9$  nmol/L and  $33.4 \pm 18.0$  nmol/L, for non-vegetarians and vegetarians, respectively, in our study. B-12 levels in non-



**Figure 2** Association of folate and B-12 with Hcy.

vegetarian participants in our study was somewhat comparable in both studies ( $392.2 \pm 167.4$  pmol/L vs.  $413.3 \pm 187.5$  pmol/L), however, it was lower among vegetarians from our study ( $287.8 \pm 126.7$  pmol/L) [20]. For both, vegetarians and non-vegetarians in our study, Hcy concentration was higher than that previously reported ( $8.8 \pm 4.4$  vs.  $8.2 \pm 2.6$   $\mu\text{mol/L}$  for vegetarians and non-vegetarians, vs.  $6.2 \pm 2.1$   $\mu\text{mol/L}$ ) [20].

Two American studies have evaluated folate, B-12, and Hcy status among adolescents. Lutsey et al., reported a mean of folate, B-12, and Hcy of 18.9 and 20.4; 460 and 428; and 6.4 and 5.1, for boys and girls, respectively. Participants in that study were, on average, about 3 years older, compared to our sample (mean age: boys =  $18.3 \pm 0.5$  and girls =  $18.2 \pm 0.5$ ) [21]. Pfeiffer et al. reported Hcy concentrations among adolescents in the United States participating in the National Health and Nutrition Examination Survey (NHANES). In the analysis based on NHANES 1999-2004 (post folate fortification era), the 50<sup>th</sup> percentile of Hcy concentration among 12 to 19 year old participants was 6.37  $\mu\text{mol/L}$  for males and 5.68  $\mu\text{mol/L}$  for females. The median Hcy concentration in NHANES 2003-2004 was 6.58  $\mu\text{mol/L}$  and 5.84, for boys and girls, respectively, which was lower than the median values among both vegetarian and non-vegetarian participants in our study (see Table 2) [22].

Folate, B-12, and Hcy were also assessed in a group of adolescents from the United Kingdom. Median serum folate concentration in 11 to 14-year-old boys ( $n = 142$ ), and girls ( $n = 126$ ), were 20.2 and 19.0 nmol/L, and in 15 to 18-year-old ( $n = 120$  for boys and  $n = 132$  for girls), 16.1 and 15.6 nmol/L, respectively [23]. Serum B-12 levels were 341.0 and 332.0 pmol/L for 11 to 14-year-old, and 275.0 and 257.0 pmol/L for 15 to 18-year-old, boys and girls, respectively. Hcy was higher in the older age category, increasing from 6.3  $\mu\text{mol/L}$ , in each gender group for 11 to 14-year-old to 7.9 in boys and 7.8 in girls among the older participants [23].

The findings described above indicate that vegetarian teenagers in Australia have higher serum folate values, compared to non-vegetarians. This finding is consistent regardless of whether the findings are compared only to Australian non-vegetarian teenagers or those from other industrial countries, such as the United States or the United Kingdom. These findings are likely a result of greater intake of folate-rich foods, such as fruits, vegetables, and legumes, which may also indicate a higher diet quality, among vegetarians. This suggestion is consistent with some, though not all, previous studies with vegetarian and non-vegetarian adults. For example, Clarys et al., found vegan participants having the highest scores on the Health Eating Index (HEI) scale, with non-vegetarian having the lowest score [24]. Similarly, Conrad et al. reported meat abstinent having substantially higher HEI scores among participants of the National Health and Nutrition Examination Survey (NHANES), 2007-2012 [25].

The lower B-12 concentrations among vegetarians, compared to non-vegetarians in our study, and those from other studies, except for participants in the study from the United Kingdom, is likely reflective of lower dietary B-12 intake. Considering that, naturally, in vegetarian diets, B-12 is only found in milk, dairy, and eggs, vegetarians have few food options that constitute a good source of this nutrient. Foods that are fortified with B-12, along with the use of B-12-containing dietary supplements, can make a substantial contribution to the overall intake of B-12. Kerr et al. found an association between the use of breakfast cereal fortified with B-vitamins and the use of B-vitamin supplements and their serum level along with a decline in Hcy concentration [23].

### Incidence of inadequate folate, B-12 and Hcy status

We compared folate status among vegetarian and non-vegetarian groups based on criteria set by the World Health Organization (WHO) [26]. We found no statistically significant



difference between the two dietary groups in the percentage of folate concentration in different folate status (Figure 1a). Consistent with our assumptions, vegetarians had a higher percentage of participants in lower serum B-12 categories, compared to non-vegetarians (see figure 1b). Although the prevalence of low B-12 status, based on the traditional B-12 deficiency criteria ( $<148\text{pmol/L}$ ), was relatively low (8.2% vs. 0.9%, for vegetarian and non-vegetarian participants, respectively,  $p = 0.003$ ), Smith and Refsum have shown that a considerably higher serum B-12 concentration ( $<400\text{pmol/L}$ ), is associated with biochemical B-12 deficiency [27]. Thus, a considerable percentage of the participants from both diet groups, with a much higher percentage among vegetarians, may have had inadequate B-12 status.

It should be worrisome that, at such a young age, in a total sample, over 18% of participants had elevated Hcy concentration ( $>10\text{ }\mu\text{mol/L}$ ). Kerr et al., observed a decline in folate and B-12 concentrations and an increase in Hcy concentration in participants in older (15-18-year-old), vs. younger adolescent groups (11 to 14-year-old and children 4 to 10-year-old) [23]. These findings imply that it is likely that an even higher percentage of participants will develop HHcy as they age. HHcy is a risk factor for several health conditions, including atherosclerosis, bone fractures, brain atrophy, and dementia. Although clinical manifestations of most of these health problems are generally not seen until individuals are in their 40 or 50s, the process of developing them begins in childhood [28,29]. Thus, the findings of this study indicate that education of the public regarding the importance of keeping Hcy within normal concentration should be done at a very young age.

### Association between folate and B-12 with Hcy

Both folate and B-12 correlated with Hcy concentration ( $r = -0.46$  for vegetarians and  $r = -0.42$  for non-vegetarians for folate and  $r = -0.38$  for vegetarians and  $r = -0.43$  in non-vegetarians for B-12), on a long-transformed scale (figures 2). This finding differs from those reported in studies with adults, where in vegetarians and those with adequate folate intake, B-12 was the predominant predictor of Hcy status [5]. For example, findings reported by Waldmann et al., with 154 German vegans have shown a strong correlation of B-12 with Hcy ( $r = -0.684$ ) but not red blood cell folate ( $r = 0.033$ ) [30]. Similarly, in a study by Karabudak et al. with 26 women from Turkey, there was a strong correlation of B-12 with Hcy (regression coefficient = 0.969) but not folate (regression correlation = 0.004) [31].

### Strengths and Limitations

One of the strengths of our study is somewhat narrow age range of participants (14-17 years), in both diet groups. Nonetheless, the findings are based on a cross-sectional analysis with adolescents from Central Coast regions and Hunter regions of New South Wales, Australia. We have not asked participants for their ethnic background. However, most of them were Caucasian. Thus, the findings may not represent folate, B-12, and Hcy concentrations for adolescents from other regions of Australia and other countries or those from other ethnic backgrounds.

### CONCLUSIONS

The findings are somewhat consistent with those reported in research with adult vegetarian and non-vegetarian individuals showing a higher mean concentration of folate and a lower mean concentration of B-12. Although there was no statistically significant difference in the Hcy concentration among participants in the respective diet groups, this finding is likely a result of relatively high folate intake among vegetarians. Consistent with findings among vegetarian adults, B-12 is a nutrient of a concern among vegetarian teenagers. To improve B-12 status, vegetarian adolescents should consume foods fortified with B-12, and/or take B-12 supplements.

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### CONFLICT OF INTEREST AND FUNDING DISCLOSURES

Authors have no conflict of interest of any kind.

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