Folate and Vitamin B_{12} Status of a Multiethnic Adult Population

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Background: Folic acid and vitamin B_{12} are of particular interest for their diverse biological functions and preventive roles in many prevalent chronic diseases. However, ethnic differences on the status of these vitamins have not been investigated among multiethnic adult college students.

Methods: A cross-sectional study (n=177) was conducted to determine the dietary intakes and levels of serum concentrations of folate and vitamin B_{12} among triethnic college students—non-Hispanic white, Hispanic and non-Hispanic black. Dietary intake was assessed using a validated food frequency questionnaire, and serum was analyzed for folate and vitamin B_{12} using standardized methods.

Results: Mean intakes of both vitamins without supplementation was higher ($P<0.05$) among non-Hispanic white males than females, and non-Hispanic white and non-Hispanic black males and females. Non-Hispanic white females had a significantly lower mean dietary intake of vitamin B_{12} than the females of other ethnic groups ($P<0.01$). There was a positive correlation between B_{12} intake and serum concentrations. More than 52% of the females did not meet the required folate intake of 400 µg/day.

Conclusions: The data suggest that there was no difference in overall mean intake of folate and vitamin B_{12} or serum concentrations in regard to gender or ethnicity. One-fourth of the female subjects failed to meet the recommended folate intake when supplement was excluded.

Key words: folate ■ vitamin B_{12} ■ minorities ■ adults

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INTRODUCTION

Folic acid and vitamin B_{12}, two water-soluble vitamins, are of special interest for their diverse biological functions and roles in many chronic diseases.[1,2] These vitamins are required in the remethylation of homocysteine to generate methionine and numerous other methylation reactions involving DNA synthesis and repair. An elevated level of plasma total homocysteine (tHcy) is considered an independent risk factor for cardiovascular diseases (CVDs).[3-7] In a meta-analysis, Boushey et al. reported an increased risk of coronary artery disease associated with increased plasma tHcy levels.[6] In a cohort of 1,153 young males and females, elevated plasma tHcy was an independent risk factor for coronary artery disease.[7] In addition, meta-analysis of randomized trials has shown that supplementation with folic acid and vitamin B_{12} may effectively lower tHcy concentrations.[8-10]

Folate deficiency may play a role in the alterations in gene expression and increased DNA damage.[11] The interrelationship between folate and vitamin B_{12} may provide insight into the carcinogenesis since both vitamins are involved in the synthesis, repair and methylation of DNA. Studies have documented an inverse relationship between optimal intakes of folate and vitamin B_{12} with several types of cancers.[12,13] Hyperhomocysteinemia due to folate and vitamin-B_{12} deficiency may also cause depression,[14] dementia and Alzheimer's disease[15,16] by impairing neuronal plasticity and promoting neuronal degeneration.[17]

In the United States, among adults aged ≥25 years, 58% of all deaths result from CVD (35.2%) and cancer (22.8%).[18] Because of the possible health benefits of folate and vitamin B_{12} in preventing these diseases,[19] it is important to determine the dietary intakes and serum levels of these vitamins in young adults. Differences in the status of these vitamins have not been reported among multiethnic college students. Since both vitamins are involved in several common metabolic pathways and vitamin

B_{12} interacts with folate, the nutritional status of these two vitamins needs to be assessed together. Therefore, we studied the dietary intakes and serum levels of folate and vitamin B_{12} of a triethnic college student (non-Hispanic white, Hispanic and non-Hispanic black) population. The relationship between dietary intakes with or without supplements and serum levels of these vitamins were also studied. Additionally, we report supplemental uses of these two vitamins because of the special interest expressed in the literature.[20]

METHODS

Subjects were part of a large cross-sectional study conducted in one of the southeast's largest public universities with an ethnically diverse student population. The study protocol was approved by the institutional review board (IRB) at Florida International University.

Study objectives were briefly explained and study flyers were distributed to the interested students. In addition, flyers were posted in two of the university campuses where students usually congregate. Volunteer students reported to the Human Nutrition Laboratory at University Park campus and signed an informed consent form agreeing to participate in the study. The inclusion criteria were: age 18–40 years, any major except nutrition (to prevent selection bias), not pregnant and not having any chronic major medical conditions. A demographic questionnaire was administered to collect information about gender, age, race/ethnic background, academic major and class level.

Dietary and supplement intakes of folic acid and vitamin B_{12} were obtained using a semiquantitative food frequency questionnaire (FFQ). The validity and reliability of the FFQ have been described previously.[21,22] The past or current use of folic acid and multivitamin supplement preparations, dosage and brand names were also documented. The FFQs were analyzed by the Channing Laboratory (Boston, MA). Analysis of FFQs also estimated the folate intake from fortified grains and grain products.

Following an overnight fast, 15 ml of blood was collected into serum-separator Vacutainer® tubes from the antecubital vein of each subject. Blood was allowed to clot for 30 min at room temperature protected from light. Samples were then centrifuged in an Eppendorf Centrifuge 5403 at 100 RCF for 15 min. Serum was placed in plastic tubes protected from light and frozen at -70° C until analyzed for folate and vitamin B_{12}. Automated chemiluminescence method was used (ACS:180, Bayer Corp. Diagnostic Division, Norwood, MA) for folate and vitamin-B_{12} analysis. Blood analysis was done by Oracle Diagnostic Laboratories (Davie, FL).

All data were analyzed by gender and ethnicity using SPSS for Windows® version

Table 1. General characteristics of subjects

Characteristics	Total	Males	Females
Gender (N, %)	177 (100)	89 (50.3)	88 (49.7)
Age (year)	21.3 ± 4.1	21.3 ± 3.7	21.3 ± 4.5
Ethnicity			
Non-Hispanic white	59 (33.3)	30 (33.7)	29 (33.0)
Hispanic	60 (33.9)	30 (33.7)	30 (34.1)
Non-Hispanic black	58 (32.8)	29 (32.6)	29 (33.0)

Data are mean ± SD or N (%)

Table 2. Mean intakes and serum levels of folate and vitamin B_{12} of subjects by gender and ethnicity

	Folate, µg with Supplement	B_{12} µg with Supplement	Folate, µg without Supplement	B_{12} µg without Supplement	Folate, ng/ml Serum Concentrations	B_{12} pg/ml Serum Concentrations
All subjects (n=177)	437.4 ± 258.6	9.3 ± 9.3	338.3 ± 177.5	7.1 ± 6.1	11.7 ± 6.3	446.8 ± 189.6
Male (n=89)	453.5 ± 283.0	9.8 ± 7.5	365.0 ± 205.2*	8.7 ± 6.8**	11.8 ± 6.0	472.2 ± 183.3
Female (n=88)	421.1 ± 231.8	8.8 ± 10.9	311.3 ± 140.4	5.5 ± 4.9	11.6 ± 6.6	421.1 ± 193.4
Males						
NHW (n=30)	535.3 ± 368.5	11.8 ± 8.6	442.0 ± 289.8^{a*}	10.4 ± 7.6	11.1 ± 5.3	469.4 ± 194.4
Hispanic (n=30)	399.0 ± 226.6	9.2 ± 6.4	303.7 ± 129.3^{b}	7.9 ± 6.0	12.1 ± 6.4	485.2 ± 175.9
NHB (n=29)	425.5 ± 216.0	8.5 ± 7.3	348.6 ± 131.6^{ab}	7.6 ± 6.6	12.3 ± 6.5	461.8 ± 184.6
Females						
NHW (n=29)	429.3 ± 250.2	6.3 ± 3.9^{a*}	295.9 ± 105.4	4.1 ± 2.67^{a**}	11.8 ± 6.1	420.7 ± 190.7
Hispanic (n=30)	409.7 ± 224.5	8.9 ± 7.6^{b}	321.3 ± 151.01	7.7 ± 7.21^{b}	10.8 ± 6.7	417.3 ± 214.1
NHB (n=29)	424.7 ± 227.9	11.2 ± 16.7^{ab}	316.4 ± 161.79	4.7 ± 2.53^{ab}	12.3 ± 7.0	425.4 ± 180.0

Data are means ± SD; NHW: non-Hispanic white; NHB: non-Hispanic black; * P<0.05, ** P<0.01; Means with different superscripts are significantly different using Bonferroni's post hoc test, P<0.05

10.0 (Chicago, IL) software program. One-way Analyses of Variance (ANOVA) was used to determine the mean differences in serum folate and vitamin-B_{12} concentrations within gender and ethnicity. Post hoc pairwise comparisons for all pairs of group comparisons were performed with Bonferroni's test. Pearson's correlations were used to examine the correlations between dietary intakes and serum folate and vitamin-B_{12} concentrations. Differences were considered significant at $P<0.05$.

RESULTS

One-hundred-seventy-seven subjects—89 males and 88 females—participated in the study. The mean age was 21.3 ± 3.7 and 21.3 ± 4.5 year for males and females, respectively. Other demographic characteristics of the subjects are presented in Table 1. The mean folate and vitamin-B_{12} intakes (with supplementation) were 437.4 ± 258.6 μg/day and 9.3 ± 9.3 μg/day, respectively (Table 2). Although males consumed more folate and vitamin B_{12} than females, only the intakes excluding supplementation were significantly higher ($P <0.05$) for males compared to females. Mean intakes of both vitamins excluding supplementation was higher ($P <0.05$) among non-Hispanic white males than females, and non-Hispanic white and non-Hispanic black males and females (Table 2). Non-Hispanic white females had a significantly lower mean dietary intake of vitamin B_{12} than the females of other ethnic groups ($P <0.01$) (Table 2). No correlation was found between folate intake and serum folate concentrations (Table 3). There was a positive correlation between vitamin-B_{12} intake and serum concentrations with ($r=0.16$, $P <0.05$) or without ($r=0.15$, $P<0.05$) supplement use in all subjects; however, when comparing gender and ethnic groups, the positive correlation between vitamin-B_{12} intake and serum levels was significant ($r=0.23$, $P<0.05$) only in males (Table 3).

Optimal intake of folate among females of childbearing age is of great concern because of its association with neural tube defects. Therefore, we examined the folate intake from food and supplemental sources for the females. More than 52% of the females did not meet their required folate intake of 400 μg/day even with including supplementation. More than three-fourths of the females did not have required folate intake when supplement use was excluded (Table 4). A significant percent (86.2%) of non-Hispanic white females did not meet the Dietary Reference Intakes (DRIs) for folate without supplementation.

DISCUSSION

Folate and vitamin B_{12} are involved in myriad biochemical reactions and may prevent many chronic diseases.[1,2] Little is known about the concurrent folate and vitamin-B_{12} intakes with and without supplement use and serum levels of several ethnic groups, especially at younger age groups. Ford et al.[23] reported the dietary intake of folate in U.S. adults ≥40 years of age. Others reported either dietary intake or serum levels of one or both folate and vitamin B_{12}.[24-27] An earlier study[28] reported folate and vitamin-B_{12} status of only non-Hispanic black female adolescents. Wright et al.[24] reported only serum values of folate and vitamin B_{12} of non-Hispanic whites, non-Hispanic blacks and Mexican Americans. Our study presents data for folate and vitamin-B_{12} status of young adults in a multiethnic population and provides much needed information.

Overall mean folate and vitamin-B_{12} intakes were not different in gender and ethnic groups. Mean intakes of the folate and vitamin B_{12} were within normal limits (400 μg/day and 2.4 μg/day, respectively). Our results are in agreement with the values reported by Alaimo et al.[29] for the same age groups from the NHANES III-Phase 1. In our study, non-Hispanic white males consumed higher folate than any other male or female ethnic groups, and Hispanic males had the lowest mean total folate intake. In contrast, both NHANES III[23] and NHANES III-Phase 1[29] reported the lowest mean

Table 3. Pearson's correlation between dietary folate and vitamin-B_{12} intakes with serum folate and vitamin-B_{12} concentrations

	Folate	B_{12}	Folate	B_{12}
	with Supplement		without Supplement	
	r		r	
All subjects (n=177)	0.08	0.16*	0.04	0.15*
Male (n=89)	-0.01	0.23*	0.04	0.23*
Female (n=88)	0.08	0.08	0.03	0.05
Non-Hispanic white (n=59)	0.16	0.06	0.10	0.12
Hispanic (n=60)	0 .08	0.17	0.09	0.12
Non-Hispanic black (n=58)	-0.004	0.24	-0.05	0.27*

* P<0.05

folate intake among non-Hispanic black females. Overall, the mean folate intake among females in our study population was lower than the reported values from the NHANES III[30] and the Continuing Survey of Food Intake by Individuals (CSFII).[20] Non-Hispanic white females in our study had the lowest and non-Hispanic white males had the highest mean folate consumption without supplementation. Overall mean vitamin-B_{12} intake was highest in non-Hispanic white males and non-Hispanic black females and lowest among non-Hispanic white females. Results from the NHANES III are comparable to our results—non-Hispanic white females (age 20–29 years) had the lowest mean intake of vitamin B_{12}, and non-Hispanic white males in the 30–39-year age group had the highest mean intake.[29] Mean intake of vitamin B_{12} without supplementation was lowest in non-Hispanic white females and highest in males of the same ethnicity. Studies are not available to compare the supplement intakes of folate or vitamin B_{12}, particularly among multiethnic young adults.[31]

We found no differences in mean serum folate or vitamin-B_{12} concentrations between genders or among ethnic groups, and all values were within the normal range.[32] In contrast to the reported values from the NHANES III,[33] males in our study had higher serum folate and vitamin-B_{12} levels compared to females. Ford et al.[33] found that non-Hispanic whites had higher serum folate than other ethnic groups. However, we found higher serum folate levels in both non-Hispanic black males and females compared to non-Hispanic white or Hispanic counterparts. NHANES III[33] also reported that among three ethnic groups, Mexican Americans had the highest serum vitamin B_{12}. In our study, Hispanic males and non-Hispanic black females had the highest serum levels of vitamin B_{12} compared to other ethnic/gender groups. We found a positive correlation between serum vitamin B_{12} and vitamin-B_{12} intakes with and without supplementation (Table 3). One study[34] found no correlation between dietary intake and serum concentrations of vitamin B_{12}.

Although serum folate is considered a sensitive indicator of dietary folate intake,[34,35] our analysis did not indicate any positive correlations between dietary intake and serum concentrations. One possible explanation would be the dietary assessment tool used in our study to determine folate intake. Folate intake by FFQ would correlate better with erythrocyte folate because it represents folate intake over several months.[36] On the other hand, a diet recall or diet history would be a better representation of serum folate.[37] Another reason would be that fasting serum concentrations may be lower than the nonfasting state.[29]

Periconceptual intake of folate at optimal amounts may significantly reduce the risk of neural tube defects among females of childbearing age.[38] More than half of the females in our study did not meet the recommended folate intake (≥400 µg/day) despite the inclusion of folate supplementation. When folate intake from supplementation was excluded, only less than one-fourth of the females met their folate requirements. Balluz et al.[39] also reported that about 67% of females of reproductive age did not take folate supplementation. The percentages of females who did not meet daily folate requirements were lower in our study than reported from the NHANES III[33] and the CSFII.[20] Higher educational status of our cohort would be the likely reason. Many studies reported low folate intake and low serum values among non-Hispanic black females.[22,24,25] In contrast, we found that non-Hispanic black females had the highest intake of folate, and 52% of non-Hispanic black females met the DRI of folate intake, the highest among all three ethnic groups. However, Hispanic females had the highest percent (30%) of folate intake without any folate supplementation, meeting the DRI for folate followed by non-Hispanic black and non-Hispanic white females.

Table 4. Subjects (N=177) who had Intakes of folate <400 µg/day

	Intake of Folate <400 µg/day with Supplement	Intake of Folate <400 µg/day without Supplement
	N (%)	
All subjects	92 (52.0)	127 (71.8)
Male	46 (51.7)	60 (67.4)
Female	46 (52.3)	67 (76.1)
Males		
Non-Hispanic white	14 (46.7)	17 (56.7)
Hispanic	18 (60.0)	25 (83.3)
Non-Hispanic black	14 (48.3)	18 (62.1)
Females		
Non-Hispanic white	16 (55.2)	25 (86.2)
Hispanic	16 (53.3)	21 (70.0)
Non-Hispanic black	14 (48.3)	21 (72.4)

The cardiovascular health of adults is determined, in part, by environmental factors during young adulthood.[40] Atherosclerosis, the key pathological feature of CVD, commences in childhood and progresses over the decades,

often leading to a heart attack.[41,42] The inverse relationship between folate and vitamin B_{12}, and CVD has been established by several studies.[43,44] A low dietary folate intake, observed in our female population, may increase the risk of CVD, neural tube defects and certain types of cancers if this trend continues. Targeting females of childbearing age and incorporating nutrition knowledge in their course materials and university health and wellness centers may prevent costly health outcomes related to these vitamins.

The study participants were a convenient sample of college students in the southeast. The results may not be generalized to all college-aged people in other geographic regions. Ethnic differences in intakes of these vitamins may be due to dietary preferences that we cannot rule out. During analyses, several confounding variables (i.e., smoking, exercise, polymorphism of methylenetetrahydrofolate reductase gene, etc.) that may affect folate and vitamin-B_{12} status were not adjusted or controlled. Although we compared results of our study with others, caution must be taken in any comparison due to some methodological differences (assessing dietary intake, analyzing FFQ, etc.). Another limitation of this study was that we used serum folate over erythrocyte folate to assess folate status. Large-scale, population-based, randomized studies are needed to confirm our findings.

In conclusion, the present study reports folate and vitamin-B_{12} status in a multiethnic young population. The study found no difference in overall mean intake of folate and vitamin B_{12} or serum concentrations in regard to gender or ethnicity. We found no correlations between dietary folate intake and serum folate status. However, it is important to note that three-fourths of the female subjects failed to meet the DRI for folate when folate intake from supplement was excluded. Females are at high risk for the development of CVD and neural tube defects if the dietary intake of folate is not increased by increasing consumption of folate-rich foods and or taking folate supplements. Present study indicates that supplementation is an important source of folate for college students. Additional efforts are warranted to increase the folate intake for females of reproductive age either from fresh fruits and vegetables or in combination with supplements.

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