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Dietary supplement use and smoking are important correlates of biomarkers of water-soluble vitamin status after adjusting for sociodemographic and lifestyle variables in a representative sample of US adults[1,,2,,3]

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Abstract

Biochemical indicators of water-soluble vitamin (WSV) status have been measured in a nationally representative sample of the US population in NHANES 2003–2006. To examine whether demographic differentials in nutritional status were related to and confounded by certain variables, we assessed the association of sociodemographic (age, sex, race-ethnicity, education, income) and lifestyle variables (dietary supplement use, smoking, alcohol consumption, BMI, physical activity) with biomarkers of WSV status in adults (≥20 y): serum and RBC folate, serum pyridoxal-5′-phosphate (PLP), serum 4-pyridoxic acid, serum total cobalamin (B-12), plasma total homocysteine (tHcy), plasma methylmalonic acid (MMA), and serum ascorbic acid. Age (except for PLP) and smoking (except for MMA) were generally the strongest significant correlates of these biomarkers ($|r| \leq 0.43$) and together with supplement use explained more of the variability as compared to the other covariates in bivariate analysis. In multiple regression models, sociodemographic and lifestyle variables together explained from 7% (B-12) to 29% (tHcy) of the biomarker variability. We observed significant associations for most biomarkers (≥6 out of 8) with age, sex, race-ethnicity, supplement use, smoking, and BMI; and for some biomarkers with PIR (5/8), education (1/8), alcohol consumption (4/8), and physical activity (5/8). We noted large estimated percent changes in biomarker concentrations between race-ethnic groups (from −24% to 20%), between supplement users and nonusers (from −12% to 104%), and between smokers and nonsmokers (from −28% to 8%). In summary, age, sex, and race-ethnic differentials in biomarker concentrations remained significant after adjusting for sociodemographic and lifestyle variables. Supplement use and smoking were important correlates of biomarkers of WSV status.

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[3]Supplemental Tables 1–4 and Supplemental Figures 1–4 are 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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[4]Abbreviations used: 4PA, 4-pyridoxic acid; B-12, total cobalamin; MA, Mexican American; MET, metabolic equivalent task; MMA, methylmalonic acid; NCHS, National Center for Health Statistics; NHB, non-Hispanic black; NHW, non-Hispanic white; PIR, poverty income ratio; PLP, pyridoxal-5′-phosphate; RBC-FOL, RBC folate; S-FOL, serum folate; tHcy, total homocysteine; VIC, ascorbic acid.

INTRODUCTION

The water-soluble B vitamins folate, B-6 and B-12 are important cofactors in one-carbon metabolism, participating in methylation reactions and DNA synthesis (1). Vitamin C functions as a water-soluble antioxidant due to its high reducing power. Its best characterized function is in the synthesis of collagen connective tissue protein through the hydroxylation of proline and lysine residues of procollagen (2). While clinical deficiencies are rare for these vitamins, interest in these nutrients persists; “suboptimal” folate status is known to increase the risk of neural tube defects (3), vitamin C in combination with other supplements (vitamin E, zinc, and *beta*-carotene) has been shown to slow the progression of age-related macular degeneration (4), and “suboptimal” vitamin B or C status may modulate chronic diseases such as cardiovascular disease, cancer, and/or cognitive function (5,6). Furthermore, in the era of post-folic acid fortification, public health concerns are no longer limited to low folic acid intakes, but extend to the safety of high intakes (7), which are largely driven by supplement use (8).

Different biochemical indicators have been measured in the US population as part of the NHANES 2003–2006 to assess the status of water-soluble vitamins. The CDC’s *Second National Report on Biochemical Indicators of Diet and Nutrition in the US Population* (*Second Nutrition Report*) used these data to provide a descriptive analysis of the nutritional status of Americans by age, sex, and race-ethnicity. These analyses however, provide only limited interpretation of relative differences in nutritional status by demographic subgroup (9).

The relationship between diet and health is of great public health interest. Nutritional biomarkers are considered mediators of this relationship, avoiding reliance on biased self-reports of diet (10,11). Aside from diet, various genetic, biological, and lifestyle variables influence biomarkers. However, the associations between these variables and biomarkers are understudied. Data from national nutrition surveys in the United States, United Kingdom, Canada, and Mexico have shown that socioeconomic status and/or lifestyle variables are related to nutritional biomarkers (Table 1). Most of these studies were limited in scope, investigating the influence of 1 or few variables on 1 or 2 biomarkers (12–30). Few studies have assessed the relationship of multiple biomarkers with socioeconomic variables (31,32), smoking (33), alcohol consumption (34), or BMI (35,36). To our knowledge, no studies have examined the combined association of sociodemographic and lifestyle variables with biomarkers of water-soluble vitamin status.

To fill this knowledge gap and in order to examine whether demographic differentials in nutritional status found in the *Second Nutrition Report* were confounded by certain variables, we applied a systematic modeling approach to questionnaire and laboratory data from the adult US population participating in NHANES 2003–2006 to assess the association of 10 preselected sociodemographic (age, gender, race-ethnicity, education, and income) and lifestyle (supplement use, smoking, alcohol consumption, BMI, and physical activity) variables with 8 biomarkers of water-soluble vitamin status. These results will provide a foundation to researchers who develop predictive regression models addressing specific

hypotheses. Companion publications in this journal supplement address the same questions for other biomarker classes featured in the *Second Nutrition Report*.

SUBJECTS AND METHODS

The NHANES, designed and carried out by the National Center for Health Statistics (NCHS) [5] at CDC, collects cross-sectional data on the health and nutritional status of the civilian non-institutionalized US population (37). Since 1999, it has been conducted as a continuous survey with data released in 2-y cycles. The 2003–2006 survey cycles obtained a stratified, multistage, probability sample designed to represent the American population on the basis of age, sex, and race/ethnicity. Data collection consisted of a screening visit, during which sample persons were identified; an interview during which a wide battery of health related questions were asked; and an examination consisting of direct standardized physical examinations, including body measurements and blood and urine collection, carried out in a mobile examination center. All respondents gave their informed consent, and the NHANES protocol was reviewed and approved by the NCHS Research Ethics Review Board. Interview and examination response rates for each survey period are publically available (38).

Laboratory methods

The following biomarkers were analyzed by the CDC laboratory during all or part of NHANES 2003–2006: serum (S-FOL; short-term indicator) and RBC folate (RBC-FOL; long-term indicator), and plasma total homocysteine (tHcy; functional indicator of "suboptimal" folate, riboflavin, B-6, or B-12 status); serum pyridoxal-5′-phosphate (PLP; biologically active coenzyme form and best single indicator of vitamin B-6 status; 2005–2006 only) and serum 4-pyridoxic acid (4PA; end product of vitamin B-6 catabolism and indicator of recent intake; 2005–2006 only); serum total cobalamin (B-12) and plasma methylmalonic acid (MMA; functional indicator of "suboptimal" vitamin B-12 status; 2003–2004 only); and serum ascorbic acid (VIC; indicator of tissue stores). Information for each biomarker on the specimen matrix, the NHANES survey period assessed, and the laboratory method used is presented in Supplemental Table 1. Laboratory method details are provided elsewhere (39,40). Westgard-type QC multi-rules were used to judge assay performance (41).

Study variables

Data for all sociodemographic variables (age, sex, race-ethnicity, income, and education) and several lifestyle variables (alcohol consumption, physical activity level and supplement use) used in our analysis were self-reported. For bivariate analyses, we categorized the variables as follows: age (20–39 y, 40–59 y, and ≥60 y); sex (men and women); race-ethnicity (Mexican American [MA], non-Hispanic black [NHB], and non-Hispanic white [NHW]); education (<high school, high school, and >high school); family poverty income ratio (PIR: 0–1.85 [low], >1.85–3.5 [medium], and >3.5 [high]) (42); smoking (serum cotinine ≤10 μg/L [nonsmoker], >10 μg/L [smoker]) (43); alcohol consumption (average daily number of "standard" drinks [1 drink ≈ 15 g ethanol]: no drinks, <1 (not 0), 1–<2, and ≥2 drinks/d); BMI (kg/m^2: <18.5 [underweight], 18.5–<25 [normal], 25–<30 [overweight], and ≥30 [obese]) (44); physical activity (total metabolic equivalent task [MET]-min/wk from

leisure time physical activity; none reported, 0–<500, 500–<1000, and ≥1000 MET-min/wk) (45); supplement use (reported taking a dietary supplement within the past 30 d: yes [user], no [non-user]).

Analytic sample

All participants examined in the mobile examination center aged 20 y and older in the NHANES 2003–2004 and 2005–2006 with at least 1 biomarker of interest were eligible for inclusion in the analysis. Depending on whether the biomarker was analyzed in both survey periods or just in 1 survey period, data were available for between ~4300 and nearly 9000 adult NHANES participants (Supplemental Table 2). We did not exclude participants because our intent was to assess how these variables impact the general US population. Furthermore, considering all the potentially relevant exclusions in an analysis with such broad scope of biomarkers would have been impractical. However, we verified that excluding participants who reported to have used antibiotics in the last 30 d (~0.4% of participants) did not substantially alter the geometric mean of the two biomarkers of vitamin B-6 status PLP and 4PA compared to not excluding them.

Statistical methods

As we used the same statistical methods for the series of papers presented in this supplement, the reader is referred to Sternberg *et al.* (46) for a detailed description of the methods and for a discussion of compromises taken in developing the multiple regression model due to the limited degrees of freedom, such as the number of covariates considered, the chosen form of continuous covariates, and the consideration of interactions between covariates. In short, we explored bivariate associations between each biomarker and selected study variables by calculating Spearman correlations (for continuous variables) and by presenting the geometric means (arithmetic mean for VIC as its distribution was reasonably symmetric) and 95% CI across the variable categories.

We used multiple linear regression to assess the impact of confounding and determine whether statistical significance persists after adjusting for differences in key variables. We arranged the independent variables into 2 sets or “chunks”: 1) sociodemographic variables (age, sex, race-ethnicity, education level, and PIR) and 2) lifestyle variables (dietary supplement use, smoking, alcohol consumption, BMI, and physical activity level). We tested each chunk simultaneously to determine whether the independent variables (as a group) were related to the dependent variable; followed by a test for each individual variable while controlling for the other variables. We present the results of 3 regression models for each biomarker: simple linear regression (model 1), multiple linear regression model with the sociodemographic chunk (model 2), and multiple linear regression model with both the sociodemographic and lifestyle chunk (model 3). This allows for the comparison of results across all biomarkers. For each model we present the estimated percent change (absolute unit change for VIC) in biomarker concentrations with change in each covariate holding all other remaining covariates constant. Two-sided *P*-values were flagged as statistically significant if <0.05.

RESULTS

A description of the civilian non-institutionalized US population by the variables studied using NHANES 2003–2006 can be found in Supplemental Table 3. Most of the continuous variables (age, PIR, smoking, alcohol consumption, BMI, and exercise duration) were at best moderately significantly correlated ($|r| \leq 0.43$) with the biomarkers of water-soluble vitamin status; MMA showed a moderate significant correlation with age only ($r = 0.33$) (Table 2). Based on the magnitude of the statistically significant Spearman correlations, age and smoking were generally the strongest correlates of biomarker concentrations, with levels increasing with increasing age and decreasing with increasing exposure to cigarette smoking (except for tHcy which was positively correlated to smoking).

Bivariate methods (model 1) were used to test for significant differences among variable categories. Of the demographic variables, age (except for PLP), sex (except for 4PA and B-12) and race-ethnicity (except for VIC) were significantly associated with most biomarkers, with age and race-ethnicity, separately, accounting for the largest variability in most biomarkers (Table 3). The socioeconomic variables education (except for B-12 and MMA) and PIR (except for B-12, tHcy and MMA) were also significantly associated with most biomarkers, but other than for PLP, they did not account for much of the variability in biomarker concentration. All 5 lifestyle variables were significantly associated with all biomarker concentrations, except for MMA, which was only significantly associated with alcohol consumption and physical activity (Table 4). Supplement use and smoking, separately, accounted for the largest variability in biomarker concentrations, while the other 3 variables explained only little of the biomarker variability.

In multiple regression models, the chunk of sociodemographic variables (model 2) explained up to 27% of the variability in biomarker concentrations: 2% (B-12), 6% (PLP), 8% (VIC), 13% (4PA and MMA), 14% (S-FOL), 15% (RBC-FOL), and 27% (tHcy) (Supplemental Table 4). Together, the chunks of sociodemographic and lifestyle variables (model 3) explained up to 29% of the variability: 7% (B-12), 15% (MMA), 22% (VIC), 23% (PLP), 25% (4PA), 26% (S-FOL and RBC-FOL), and 29% (tHcy). Adjusting for sociodemographic variables generally led to a mild attenuation of *beta* coefficients, while additionally adjusting for lifestyle variables more acutely diminished the association with sociodemographic variables, suggesting that sociodemographic variables may capture some unmeasured association that was shared with lifestyle variables.

Because the log transformations may obscure the interpretation of the *beta* coefficients, we estimated the percent change in biomarker concentrations (change in μmol/L for VIC which was not log transformed) associated with each covariable (Table 5). As noted with the *beta* coefficients, the estimated effect of most of these variables changed between models 1 and 3, suggesting that at least some of the association measured in the unadjusted model may be a result of confounding with variables not included in the model. For example, the estimated percent change for S-FOL concentrations for persons who were older by 10 y fell from 9.9% in model 1 to 6.8% in model 3, for women vs. men from 13.8% to 5.8%, and for NHB vs. NHW from −23.6% to −13.0%. Based on the full regression model 3, we observed significant associations for most biomarkers with age (8/8), sex (8/8), race-ethnicity (6/8 for

NHB vs. NHW and 7/8 for MA vs. NHW), supplement use (8/8), smoking (7/8), and BMI (6/8); and for some biomarkers with PIR (5/8), education (1/8), alcohol consumption (4/8), and physical activity (5/8) (for a graphic representation, see also Supplemental Fig. 1–4). Age (being 10 y older) showed the strongest association with 4PA (15%), tHcy (10%), and MMA (9%); sex (being a women) with PLP (−21%), tHcy (−15%), and VIC (5.7 μmol/L); and race-ethnicity (being MA or NHB vs. NHW) with 4PA (−24% and −13%, respectively), MMA (−22% and −22%, respectively), and B-12 (20% and 15%, respectively). Supplement use and smoking both showed the strongest association with biomarkers of vitamin B-6 status (4PA 104% and −18%, PLP 79% and −28%, respectively), biomarkers of folate status (S-FOL 38% and −15%, RBC-FOL 24% and −12%, respectively), and VIC (16 and −11 μmol/L, respectively). Estimated vitamin concentrations in supplement users were up to twice as high compared to nonusers. As expected, the inversely correlated metabolites Hcy and MMA showed lower estimated concentrations in supplement users. Estimated vitamin concentrations in smokers were up to ~30% lower and tHcy concentrations were nearly 10% higher.

DISCUSSION

Using cross-sectional data for biomarkers of water-soluble vitamin status from a nationally representative sample of American adults participating in NHANES 2003–2006, we found that 1) age, sex, and race-ethnic differentials in biomarker concentrations remained significant, though the magnitude of the differentials was generally diminished after adjusting for key sociodemographic and lifestyle variables; and 2) of the variables studied, supplement use, smoking, and race-ethnicity were important correlates of biomarkers of water-soluble vitamin status, independent of the other sociodemographic and lifestyle variables in the model.

We used 3 different approaches to study the association between biomarkers and variables—correlations, bivariate regression, and multiple regression models and found good consistency across these approaches. Age and smoking emerged as the strongest individual correlates of the biomarkers. Using bivariate methods, age, race-ethnicity, supplement use, and smoking accounted for the largest portions of the variability in biomarker concentrations. Finally, using multiple regression models, age, sex, race-ethnicity, supplement use, and smoking continued to be significantly associated with nearly all biomarkers.

Our modeling estimated 79% higher PLP concentrations in supplement users compared to nonusers; this large difference can also be observed when comparing the prevalence of low PLP (<20 nmol/L) in supplement users (7.8%) compared to nonusers (19%) (data not shown). Morris *et al.* found similar prevalence estimates (11% in supplement users and 24% in nonusers) in NHANES 2003–2004 after adjusting for a similar list of variables plus self-reported diabetes status and intakes of protein and energy (19). Our analysis estimated S-FOL and RBC-FOL concentrations to be 39% and 24% higher, respectively in supplement users, similar to a recent NHANES 1999–2010 report (41% and 33% higher, respectively) (13). The larger proportional increase in S-FOL compared to RBC-FOL as a result of a folate dose—also noted after the introduction of folic acid fortification (13) and in response

to long-term folic acid supplementation (47)—is likely a result of the much higher RBC folate concentration compared to serum. We estimated 21% higher B-12 concentrations in supplement users compared to nonusers, which is consistent with a prevalence of low B-12 (<200 ng/L) of 2.0% in supplement users compared to 3.1% in non-users (data not shown). Evatt *et al.* found a slightly bigger difference in low B-12 prevalence between users (1.7%) and nonusers (3.9%) for persons 18 y and older in NHANES III, however they assessed specifically B-12-containing supplement consumption (20). Our analysis estimated VIC concentrations to be 16.4 μmol/L higher in supplement users. Schleicher *et al.* found age-adjusted VIC concentrations to be 25 μmol/L higher in adults who consumed vitamin C-containing supplements as part of NHANES 2003–2004 (23).

Smoking was also significantly related in our analysis with most biomarkers, with smokers having lower vitamin concentrations compared to nonsmokers: VIC (~30%), PLP (29%), 4PA (18%), S-FOL (16%), and RBC-FOL (13%). Schleicher *et al.* reported 30% (men) and 33% (women) lower age-adjusted VIC concentrations for smokers vs. nonsmokers (23). Morris *et al.* showed that adjusted PLP concentrations of current smokers compared to those who never smoked were 25% and 22% lower in supplement users and nonusers, respectively (19). Using data from NHANES III, Mannino *et al.* found adjusted (sociodemographic variables and folate intake) RBC-FOL concentrations to be 16% lower in smokers compared to nonsmokers with low exposure to passive smoking (16). As expected due to the inverse relationship of tHcy with folate, vitamin B-6 and B-12, we found higher estimated tHcy concentrations (8%) in smokers. Similar observations were made in previous analyses of the US population (25,26,28,29). The 1994/1995 British National Diet and Nutrition Survey (NDNS) of people aged 65 y and over found an inverse relationship between smoking status and nutrient intake (VIC, B-6, and folate) and between smoking status and micronutrient indices (VIC, PLP, S-FOL, and RBC-FOL) after adjusting for intake and other covariates (33). Smoking itself may predispose to lower water-soluble vitamin status. In a recent analysis from two large Norwegian B vitamin intervention trials (NORVIT and WENBIT), Ulvik *et al.* showed that smoking status was directly associated with tHcy and inversely with S-FOL and PLP in a dose-response relationship (48). More interestingly, smokers with low serum cotinine (abstained from smoking for ≥3 d) had higher S-FOL and PLP concentrations compared to smokers with high serum cotinine. The authors suggested that the short-term effects may be related to acute smoking-induced oxidative stress; long-term effects among ex-smokers may reflect changes in diet and/or restoration of vitamin concentrations in tissue after smoking cessation.

Our analysis also showed a significant relation between race-ethnicity and most biomarkers. Compared to NHW, MA and NHB had lower S-FOL, RBC-FOL, 4PA, and MMA, but higher B-12 and VIC. Similar race-ethnic differentials were found in previous descriptive analyses of the US population (13,19,49), but also in an analysis by Kant *et al.* after they adjusted for socioeconomic status (and additionally for nutrient-specific intake) (32). The authors found lower folate intake and status in NHB, both pre- and post-fortification and suggested that ethnic-specific nutrition interventions would be needed to target at-risk ethnic groups and promote dietary changes. However, given the different frequencies of the *MTHFR C677T* genotype among the three major race-ethnic groups (50) and the fact that the BioRad radioassay—used in NHANES 1988–1994 and 1999–2006—responded

differently to blood samples with the *T/T* genotype compared with either *C/C* or *C/T* genotypes (13), the association between RBC-FOL and race-ethnicity needs to be interpreted with caution. While we generally observed attenuation of the effect of race-ethnicity on biomarker concentrations with increasing adjustment, this was not the case for VIC. After adjusting for lifestyle variables, NHB had significantly higher VIC concentrations compared to NHW, whereas prior to adjustment the opposite was true, which may suggest confounding with at least 1 of the lifestyle variables, assuming the model is not misspecified.

Other sociodemographic or lifestyle variables assessed in this analysis had generally weaker and in some cases nonsignificant associations with biomarkers. After adjusting for sociodemographic and lifestyle variables, age was positively correlated with most biomarkers (negatively correlated with PLP) and women had better folate and vitamin C status, but lower vitamin B-6 and B-12 status, compared to men. Similar age and sex differences were reported in previous NHANES analyses (9,13,19,23,29,49,51). We confirmed previous findings of lower S-FOL (17,18,35,36) and higher RBC-FOL (18) concentrations with higher BMI. However, Tinker *et al.* only found BMI inversely associated with S-FOL among women who did not use folic acid-containing supplements, hypothesizing that cellular uptake and tissue distribution of folate may be altered by BMI which may be compensated by folic acid supplement use (18). Similar to findings by *Walmsley et al.* from the British NDNS (34), we also noted higher estimated PLP concentrations with higher alcohol consumption and no (RBC-FOL, 4PA, MMA, and VIC) or minimal (slightly lower S-FOL and B-12 and slightly higher tHcy) changes in all other biomarkers. This is expected based on results from a randomized intervention study of moderate alcohol consumption in postmenopausal women, which showed no (S-FOL, MMA) or small (B-12, tHcy) effects of 1 or 2 drinks/d over an 8-wk period (52).

To our knowledge, this is the first study that examined the association of demographic, socioeconomic, and lifestyle variables with all biomarkers available in the more recent continuous NHANES to interpret the status of 4 water-soluble vitamins: folate, vitamins B-6, B-12, and C. By applying a systematic modeling approach and limiting data driven decisions in the model building process we preserved the statistical properties of *P*-values and coefficients (46). Additionally, the hierarchical chunk regression modeling provided a natural way to systematically assess the magnitude of an estimated change in biomarker concentration with a change in a single covariate, holding all other variables constant, across biomarkers. Moreover, we applied the same approach to other classes of nutritional and dietary biomarkers allowing comparisons over a wide range of indicators (see other papers in this journal supplement; a summary table is presented in [46]). The large sample size in NHANES in combination with the use of 2 survey cycles that maximized the number of available biomarkers (i.e., tHcy, MMA, and VIC data are not available after 2006) allowed us to assess associations with a fair number of covariates in the same model.

Our analysis has limitations. The cross-sectional nature of NHANES prevented us from drawing any causal relationships between the biomarkers and variables in our study. Our results could be confounded by unmeasured biological and genetic factors. We did not test for interactions between variables due to limitations in degrees of freedom, nor did we

maximize the predictive power of our descriptive model. Investigating nutrient-nutrient interactions (e.g., vitamin B-6 and protein intake, vitamin C and iron), studying how various health conditions or health risk factors are associated with nutritional biomarkers, or how dietary intake or intake of *specific* dietary supplements are associated with nutritional biomarkers or interact with variables included in our analysis was outside the scope of this study. Nutrient intake is known to be a major determinant of biomarker concentrations and both intake and biomarkers are indicators of nutritional status. We chose to describe how biomarkers were associated with certain variables after adjusting for sociodemographic and lifestyle variables and within that scheme dietary intake was more naturally an outcome variable than a covariate. A few studies have shown that associations of biomarkers with different variables remained unchanged after addition of the relevant nutrient intake to regression models (16,17,32,33). Regardless, our descriptive analysis cannot answer the question whether the associations we found are explained by intake or not. In summary, we conclude that supplement use, smoking, and race-ethnicity were associated with notable changes in concentrations of most biomarkers of water-soluble vitamin status, after adjusting for preselected sociodemographic and lifestyle variables. This analysis provides a foundation for future data analyses that set out to build predictive models to address specific hypotheses between nutritional status and health.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Table 1

National nutrition surveys that assessed the association between socioeconomic and lifestyle variables and biochemical indicators or water-soluble vitamin status in adult populations

Biochemical indicator(s)[1] (specimen matrix)[2]	Variable(s) (socioeconomic and/or lifestyle)	National nutrition survey[3]	Reference
FOL (S, RBC)	Education	NHANES III	12
FOL (S, RBC)	Income, supplement use	NHANES III and 1999–2010	13
FOL (S)	Supplement use	NHANES 2001–2004	14
FOL (RBC)	Income, education, supplement use	Canadian HMS	15
FOL (S, RBC)	Smoking	NHANES III	16
FOL (S)	BMI	NHANES III and 1999–2000	17
FOL (S, RBC)	BMI, supplement use	NHANES 2003–2008	18
PLP (P)	Supplement use, smoking, alcohol consumption, BMI	NHANES 2003–2004	19
B-12 (S)	Supplement use	NHANES III	20
B-12 (S)	Income and BMI	Canadian HMS	21
VIC (S)	Smoking	NHANES III	22
VIC (S)	Income, supplement use, smoking, BMI	NHANES 2003–2004	23
tHcy (P)	Income, education	NHANES 1999–2002	24
tHcy (P)	Smoking (passive)	NHANES III	25
tHcy (P)	Smoking (passive)	NHANES 1999–2002	26
tHcy (P)	Smoking, BMI	British NDNS	27
tHcy (P)	Supplement use, smoking, alcohol consumption, BMI	NHANES III	28
tHcy (P)	Supplement use, smoking, BMI	NHANES 1999–2004	29
MMA (P)	Income, social class, education, smoking, physical activity	British NDNS	30
FOL (RBC), VIC (S), other MN	Income	Mexican National Survey	31
FOL (S, RBC), VIC (S), other MN	Income, education	NHANES III and 1999–2002	32
FOL (S, RBC), PLP (P), B-12 (S), VIC (P), other MN	Smoking	British NDNS	33
FOL (S, RBC), PLP (P), VIC (P), other MN	Alcohol consumption	British NDNS	34
FOL (S, RBC), VIC (S), other MN	BMI	NHANES III	35
FOL (S, RBC), B-12 (S), VIC (S), other MN	BMI	NHANES III	36

[1] 4PA, 4-pyridoxic acid; B-12, total cobalamin; FOL, folate; MMA, methylmalonic acid; MN, micronutrients; PLP, pyridoxal-5′-phosphate; tHcy, total homocysteine; VIC, ascorbic acid

[2] S, serum; P, plasma

[3] US National Health and Nutrition Examination Survey; Canadian Health Measures Survey; British National Diet and Nutrition Survey

Table 2

Spearman correlation coefficients describing bivariate associations between each water-soluble vitamin biomarker and selected continuous sociodemographic and lifestyle variables for adults ≥20 y, NHANES 2003–2006[1,2,3]

Variable	S-FOL	RBC-FOL	PLP	4PA	B-12	tHcy	MMA	VIC
Age	0.29*	0.29*	−0.01	0.32*	0.06*	0.43*	0.33*	0.13*
PIR[3]	0.14*	0.15*	0.19*	0.18*	0.02	−0.01	0.03	0.14*
Smoking	−0.31*	−0.29*	−0.19*	−0.22*	−0.13*	0.14*	−0	−0.31*
Alcohol consumption[4]	−0.08*	−0.04	0.18*	0.08*	−0.05*	0.14*	−0.02	−0.04
BMI	−0.08*	0.08*	−0.17*	−0.06*	−0.09*	0.06*	0	−0.18*
Physical activity[5]	−0	−0.03	0.13*	0.06*	0.03	−0	−0.04	0.08*

[1] 4PA, 4-pyridoxic acid; B-12, total cobalamin; MMA, methylmalonic acid; PIR, family poverty income ratio; PLP, pyridoxal-5′-phosphate; RBC-FOL, RBC folate; S-FOL, serum folate; tHcy, total homocysteine; VIC, ascorbic acid

[2] MMA data only available for NHANES 2003–2004; PLP and 4PA data only available for NHANES 2005–2006

[3] Sample sizes for each biomarker by variable can be found in Supplemental Table 2

[4] Alcohol consumption: calculated as average daily number of “standard” drinks [(quantity × frequency)/365.25]; 1 drink ≈ 15 g ethanol

[5] Physical activity: calculated as total metabolic equivalent task (MET)-min/wk from self-reported leisure time physical activities

* Significant correlation; $P < 0.05$

Table 3

Unadjusted biomarker concentrations of water-soluble vitamin status by sociodemographic variable categories for adults ≥20 y, NHANES 2003–2006[1,2,3]

Variable	S-FOL *μg/L*	RBC-FOL *μg/L*	PLP *nmol/L*	4PA *nmol/L*	B-12 *ng/L*	tHcy *μmol/L*	MMA *nmol/L*	VIC *μmol/L*
Age, *y*								
20–39	10.4 (10.1 – 10.7)	244 (238 – 250)	51.0 (47.7 – 54.6)	26.8 (24.3 – 29.5)	454 (443 – 465)	7.14 (7.04 – 7.24)	122 (116 – 127)	51.0 (48.8 – 53.2)
40–59	11.6 (11.2 – 12.0)	270 (264 – 276)	49.0 (45.1 – 53.3)	34.7 (31.9 – 37.7)	466 (451 – 482)	8.33 (8.17 – 8.50)	137 (129 – 144)	51.6 (49.7 – 53.4)
≥60	15.6 (15.0 – 16.1)	324 (317 – 332)	50.4 (46.7 – 54.5)	58.6 (54.7 – 62.9)	482 (468 – 496)	10.1 (9.85 – 10.4)	177 (169 – 186)	63.0 (61.5 – 64.6)
P-value[4]	*<0.0001*	*<0.0001*	*0.53*	*<0.0001*	*0.0008*	*<0.0001*	*<0.0001*	*<0.0001*
I^2, %[5]	*8*	*8*	*0*	*8*	*<1*	*15*	*9*	*3*
Sex								
Men	11.1 (10.8 – 11.5)	261 (255 – 267)	54.9 (51.7 – 58.3)	35.3 (32.5 – 38.4)	462 (452 – 472)	9.00 (8.83 – 9.18)	141 (135 – 148)	49.4 (47.7 – 51.0)
Women	12.7 (12.3 – 13.1)	280 (274 – 287)	46.0 (43.0 – 49.2)	35.9 (33.8 – 38.1)	468 (454 – 482)	7.55 (7.36 – 7.74)	136 (128 – 144)	58.3 (56.5 – 60.0)
P-value	*<0.0001*	*<0.0001*	*<0.0001*	*0.53*	*0.29*	*<0.0001*	*0.0283*	*<0.0001*
I^2, %	*2*	*1*	*1*	*0*	*0*	*7*	*<1*	*2*
Race-ethnicity[6]								
MA	10.1 (9.73 – 10.5)	246 (240 – 252)	46.8 (44.3 – 49.4)	24.5 (22.7 – 26.4)	499 (480 – 519)	7.09 (6.95 – 7.23)	114 (109 – 119)	51.3 (48.9 – 53.8)
NHB	9.67 (9.32 – 10.0)	215 (211 – 220)	38.5 (34.8 – 42.5)	23.8 (21.1 – 26.7)	514 (499 – 530)	8.22 (8.03 – 8.42)	115 (109 – 122)	50.3 (48.3 – 52.2)
NHW	12.7 (12.2 – 13.1)	287 (280 – 294)	52.5 (48.9 – 56.3)	40.9 (38.0 – 44.0)	454 (441 – 468)	8.39 (8.22 – 8.57)	146 (139 – 154)	54.9 (52.9 – 56.9)
P-value	*<0.0001*	*<0.0001*	*<0.0001*	*<0.0001*	*<0.0001*	*<0.0001*	*<0.0001*	*0.18*
I^2, %	*4*	*7*	*1*	*4*	*1*	*2*	*4*	*<1*
Education								
<High school	10.7 (10.3 – 11.1)	252 (246 – 258)	39.6 (36.8 – 42.7)	29.0 (26.6 – 31.7)	471 (455 – 488)	8.50 (8.17 – 8.84)	145 (133 – 157)	48.6 (46.5 – 50.7)
High school	11.7 (11.2 – 12.1)	267 (260 – 275)	45.2 (42.4 – 48.3)	33.6 (30.1 – 37.5)	457 (443 – 472)	8.49 (8.30 – 8.68)	139 (130 – 148)	50.5 (48.9 – 52.1)
>High school	12.4 (12.0 – 12.9)	279 (273 – 285)	56.3 (52.6 – 60.4)	38.9 (35.9 – 42.2)	467 (455 – 479)	8.00 (7.87 – 8.13)	137 (131 – 143)	57.3 (55.6 – 59.0)

Variable	S-FOL *μg/L*	RBC-FOL *μg/L*	PLP *nmol/L*	4PA *nmol/L*	B-12 *ng/L*	tHcy *μmol/L*	MMA *nmol/L*	VIC *μmol/L*
P-value	*<0.0001*	*<0.0001*	*<0.0001*	*<0.0001*	*0.24*	*<0.0001*	*0.19*	*<0.0001*
I^2, %	*1*	*1*	*3*	*1*	*0*	*1*	*<1*	*2*
PIR[7]								
Low	10.8 (10.6 – 11.1)	254 (249 – 259)	40.2 (38.1 – 42.4)	28.8 (26.8 – 31.0)	461 (451 – 472)	8.22 (8.01 – 8.43)	139 (130 – 148)	49.2 (47.4 – 51.1)
Medium	12.0 (11.5 – 12.6)	273 (266 – 281)	48.6 (44.3 – 53.2)	33.4 (30.1 – 37.2)	468 (450 – 486)	8.29 (8.11 – 8.48)	140 (132 – 149)	53.6 (51.6 – 55.6)
High	12.6 (12.2 – 13.0)	283 (276 – 289)	58.5 (55.4 – 61.9)	41.7 (38.7 – 44.9)	464 (450 – 478)	8.13 (7.98 – 8.29)	139 (132 – 146)	57.6 (55.9 – 59.3)
P-value	*<0.0001*	*<0.0001*	*<0.0001*	*<0.0001*	*0.73*	*0.16*	*0.91*	*<0.0001*
I^2, %	*1*	*1*	*3*	*2*	*0*	*0*	*0*	*1*

[1]Biomarker concentrations represent geometric means (arithmetic mean for vitamin C) and 95% CI; 4PA, 4-pyridoxic acid; B-12, total cobalamin; MMA, methylmalonic acid; PLP, pyridoxal-5′-phosphate; RBC-FOL, RBC folate; S-FOL, serum folate; tHcy, total homocysteine; VIC, ascorbic acid. SI conversion factors are as follows: FOL, ×2.266 (nmol/L) and B-12, ×0.738 (pmol/L)

[2]MMA data only available for NHANES 2003–2004; PLP and 4PA data only available for NHANES 2005–2006

[3]Sample sizes for each biomarker by variable can be found in Supplemental Table 2

[4]*P*-value based on Wald F test, which tests whether at least one of the means across the categories is significantly different

[5]I^2 based on model 1, simple linear regression, using categories as shown

[6]MA, Mexican American; NHB, non-Hispanic black; NHW, non-Hispanic white

[7]PIR, family poverty income ratio: 0–1.85 (low); >1.85–3.5 (medium); >3.5 (high)

Table 4

Unadjusted biomarker concentrations of water-soluble vitamin status by lifestyle variable categories for adults ≥20 y, NHANES 2003–2006[1,2,3]

Variable	S-FOL *μg/L*	RBC-FOL *μg/L*	PLP *nmol/L*	4PA *nmol/L*	B-12 *ng/L*	tHcy *μmol/L*	MMA *nmol/L*	VIC *μmol/L*
Supplement use[4]								
No	9.37 (9.13 – 9.62)	231 (226 – 235)	36.0 (34.2 – 37.9)	22.2 (21.0 – 23.5)	421 (410 – 431)	8.43 (8.27 – 8.59)	141 (134 – 148)	42.6 (40.7 – 44.5)
Yes	14.5 (14.1 – 15.0)	310 (304 – 317)	66.2 (62.9 – 69.7)	53.0 (48.9 – 57.5)	505 (491 – 520)	8.04 (7.86 – 8.23)	137 (129 – 144)	63.5 (62.2 – 64.9)
P-value[5]	*<0.0001*	*<0.0001*	*<0.0001*	*<0.0001*	*<0.0001*	*<0.0001*	*0.11*	*<0.0001*
I^2, %[6]	*17*	*15*	*12*	*17*	*4*	*<1*	*<1*	*13*
Smoking[7]								
No	12.9 (12.5 – 13.3)	287 (282 – 293)	55.0 (52.2 – 57.9)	39.5 (36.4 – 42.8)	478 (466 – 490)	8.02 (7.87 – 8.16)	139 (133 – 145)	58.8 (57.5 – 60.0)
Yes	9.77 (9.49 – 10.1)	234 (229 – 239)	39.5 (36.5 – 42.8)	27.3 (24.8 – 30.0)	433 (421 – 445)	8.73 (8.49 – 8.97)	137 (128 – 147)	42.1 (40.2 – 44.1)
P-value	*<0.0001*	*<0.0001*	*<0.0001*	*<0.0001*	*<0.0001*	*<0.0001*	*0.65*	*<0.0001*
I^2, %	*6*	*6*	*3*	*2*	*1*	*1*	*0*	*7*
Alcohol consumption[8]								
No drinks	12.7 (12.2 – 13.3)	280 (272 – 288)	46.6 (43.2 – 50.2)	38.6 (35.0 – 42.6)	477 (460 – 495)	8.51 (8.22 – 8.82)	149 (138 – 160)	54.8 (52.8 – 56.8)
<1 (not 0)	11.8 (11.5 – 12.2)	271 (265 – 276)	50.0 (47.1 – 53.0)	34.9 (32.6 – 37.4)	463 (451 – 475)	7.91 (7.77 – 8.04)	134 (129 – 140)	55.1 (53.4 – 56.8)
1–<2	11.4 (10.6 – 12.1)	263 (253 – 274)	62.8 (54.9 – 71.9)	38.4 (32.3 – 45.7)	438 (424 – 452)	8.66 (8.40 – 8.93)	135 (127 – 143)	51.0 (47.1 – 54.9)
≥2	10.2 (9.58 – 10.9)	256 (243 – 269)	59.5 (52.1 – 67.9)	32.8 (28.3 – 37.9)	446 (423 – 470)	9.35 (8.89 – 9.83)	131 (120 – 143)	45.7 (41.7 – 49.7)
P-value	*<0.0001*	*0.0111*	*<0.0001*	*0.0361*	*0.0009*	*<0.0001*	*0.0045*	*0.0001*
I^2, %	*1*	*1*	*1*	*<1*	*<1*	*2*	*1*	*1*
BMI[9]								
Underweight	11.7 (10.6 – 12.9)	234 (212 – 258)	46.4 (36.5 – 58.9)	27.9 (21.4 – 36.5)	520 (474 – 572)	7.88 (7.32 – 8.50)	131 (116 – 148)	57.1 (51.6 – 62.5)
Normal weight	12.3 (11.9 – 12.7)	262 (255 – 269)	56.7 (52.3 – 61.5)	37.4 (33.4 – 41.8)	481 (468 – 495)	7.94 (7.76 – 8.12)	140 (131 – 149)	59.6 (57.6 – 61.7)

Variable	S-FOL *μg/L*	RBC-FOL *μg/L*	PLP *nmol/L*	4PA *nmol/L*	B-12 *ng/L*	tHcy *μmol/L*	MMA *nmol/L*	VIC *μmol/L*
Overweight	12.3 (11.9 – 12.6)	274 (269 – 280)	57.7 (55.3 – 60.2)	40.1 (37.0 – 43.5)	473 (460 – 487)	8.43 (8.25 – 8.62)	138 (132 – 146)	55.4 (53.7 – 57.2)
Obese	11.2 (10.8 – 11.7)	279 (273 – 284)	40.2 (37.1 – 43.5)	30.9 (28.6 – 33.5)	441 (426 – 456)	8.22 (8.04 – 8.41)	137 (130 – 144)	47.4 (45.7 – 49.0)
P-value	*0.0001*	*<0.0001*	*<0.0001*	*<0.0001*	*<0.0001*	*<0.0001*	*0.60*	*<0.0001*
I^2, %	*1*	*1*	*4*	*1*	*1*	*1*	*0*	*3*
Physical activity[10]								
None reported	11.2 (10.8 – 11.5)	261 (255 – 268)	39.3 (36.7 – 42.1)	31.9 (29.7 – 34.4)	453 (440 – 466)	8.71 (8.50 – 8.93)	148 (138 – 158)	48.0 (46.3 – 49.7)
0–<500	12.1 (11.7 – 12.5)	275 (269 – 282)	50.0 (47.8 – 52.4)	34.6 (32.3 – 37.1)	463 (450 – 475)	8.04 (7.83 – 8.24)	137 (130 – 144)	53.5 (51.9 – 55.0)
500–<1000	12.9 (12.3 – 13.5)	285 (276 – 294)	53.4 (44.9 – 63.5)	39.9 (32.0 – 49.7)	466 (449 – 484)	7.96 (7.70 – 8.22)	135 (124 – 147)	57.9 (55.7 – 60.0)
≥1000	12.1 (11.6 – 12.6)	271 (265 – 278)	62.4 (58.2 – 66.9)	38.5 (34.7 – 42.7)	479 (463 – 496)	7.94 (7.80 – 8.08)	131 (127 – 135)	59.0 (56.8 – 61.2)
P-value	*<0.0001*	*<0.0001*	*<0.0001*	*0.0025*	*0.0026*	*<0.0001*	*<0.0001*	*<0.0001*
I^2, %	*1*	*1*	*4*	*1*	*<1*	*1*	*1*	*2*

[1] Biomarker concentrations represent geometric means (arithmetic mean for vitamin C) and 95% CI; 4PA, 4-pyridoxic acid; B-12, total cobalamin; MMA, methylmalonic acid; PLP, pyridoxal-5′-phosphate; RBC-FOL, RBC folate; S-FOL, serum folate; tHcy, total homocysteine; VIC, ascorbic acid. SI conversion factors are as follows: FOL, ×2.266 (nmol/L) and B-12, ×0.738 (pmol/L)

[2] MMA data only available for NHANES 2003–2004; PLP and 4PA data only available for NHANES 2005–2006

[3] Sample sizes for each biomarker by variable can be found in Supplemental Table 2

[4] "Supplement user" defined as participant who reported taking a dietary supplement within the past 30 d

[5] *P*-value based on Wald F test, which tests whether at least 1 of the means across the categories is significantly different

[6] I^2 based on model 1, simple linear regression, using categories as shown

[7] "Smoker" defined by serum cotinine concentration >10 μg/L

[8] Alcohol consumption: calculated as average daily number of "standard" drinks [(quantity × frequency)/365.25]; 1 drink ≈ 15 g ethanol

[9] BMI (kg/m^2) definitions: <18.5 (underweight); 18.5–>25 (normal weight); 25–<30 (overweight); and ≥30 (obese)

[10] Physical activity: calculated as total metabolic equivalent task (MET)-min/wk from self-reported leisure time physical activities

Table 5

Estimated change in biomarker concentration of water-soluble vitamin status after adjusting for sociodemographic and lifestyle variables through chunk-wise modeling using data for adults ≥20 y, NHANES 2003–2006[1,2,3]

Variable	S-FOL	RBC-FOL	PLP	4PA	B-12	tHcy	MMA	VIC
Age: every 10 y increase								
Model 1	9.9*	6.9*	−0.1	20.8*	1.6*	8.7*	9.6*	2.7*
Model 2	9.4*	6.5*	0.5	19.6*	2.4*	8.7*	9.0*	2.8*
Model 3	6.8*	4.5*	−2.1*	14.6*	1.0*	9.6*	9.2*	1.9*
Sex: women vs. men								
Model 1	13.8*	7.4*	−16.1*	1.6	1.3	−16.2*	−3.8*	8.9*
Model 2	13.1*	6.9*	−15.7*	0.3	0.7	−17.5*	−5.4*	8.9*
Model 3	5.8*	3.6*	−21.2*	−8.6*	−3.7*	−15.0*	−5.2*	5.7*
Race-ethnicity[4]: NHB vs. NHW								
Model 1	−23.6*	−25.0*	−26.7*	−41.9*	13.1*	−2.0	−21.5*	−4.6*
Model 2	−18.1*	−21.2*	−18.7*	−32.5*	15.9*	0.6	−20.1*	−0.9
Model 3	−13.0*	−19.7*	−7.7	−23.5*	20.2*	0.3	−22.4*	3.4*
Race-ethnicity[4]: MA vs. NHW								
Model 1	−20.3*	−14.3*	−10.8*	−40.0*	9.9*	−15.5*	−22.4*	−3.6*
Model 2	−6.9*	−4.7*	6.4	−16.7*	14.7*	−11.6*	−18.7*	4.4*
Model 3	−5.8*	−4.7*	8.1	−13.1*	15.4*	−10.6*	−21.6*	5.3*
PIR[5]: every 2 unit decrease								
Model 1	−8.3*	−6.5*	−19.0*	−19.1*	−0.8	1.3*	0.0	−5.0*
Model 2	−4.8*	−3.3*	−14.6*	−12.0*	−2.2*	3.2*	3.9*	−4.0*
Model 3	−1.3	−0.6	−8.3*	−6.3*	−0.9	1.9*	2.7*	−1.7*
Education: ≤high school vs. >high school								
Model 1	−9.1*	−6.6*	−24.0*	−18.8*	−0.8	6.1*	3.3	−7.6*
Model 2	−7.9*	−5.5*	−16.9*	−14.3*	−1.9	2.2	0.7	−6.4*
Model 3	−0.1	−0.7	−2.3	−0.6	3.1	−0.4	−1.8	−1.8*

Variable	S-FOL	RBC-FOL	PLP	4PA	B-12	tHcy	MMA	VIC
Supplement use[6]: yes vs. no								
Model 1	55.2*	34.5*	83.8*	139*	20.2*	−4.6*	−3.0	20.9*
Model 3	38.4*	24.1*	78.7*	104*	20.8*	−8.4*	−12.1*	16.2*
Smoking[7]: yes vs. no								
Model 1	−24.2*	−18.7*	−28.1*	−30.9*	−9.4*	8.9*	−1.1	−16.6*
Model 3	−14.9*	−12.2*	−27.6*	−18.1*	−6.2*	7.8*	−0.9	−11.0*
Alcohol consumption[8]: 1 vs. 0 drinks/d								
Model 1	−7.9*	−3.3*	16.0*	−1.4	−3.8*	5.6*	−4.1*	−3.5*
Model 3	−2.4*	1.6	10.6*	−0.3	−3.3*	3.2*	−1.9	−0.8
BMI[9]: 25% increase								
Model 1	−4.0*	3.3*	−14.5*	−7.0*	−4.0*	1.1*	−0.4	−5.3*
Model 3	−4.1*	3.8*	−12.6*	−7.4*	−4.3*	−0.1	−1.7	−5.3*
Physical activity[10]: 750 vs. 150 MET-min/wk								
Model 1	2.0*	1.0*	9.3*	4.0*	1.0*	−1.9*	−2.3*	2.2*
Model 3	1.4*	0.6*	3.1*	1.3	0.6	−0.4	−1.4*	1.4*

[1]Change represents percent change (%) in geometric mean for all biomarkers except for vitamin C where change in arithmetic mean represents concentration units (μmol/L); 4PA, 4-pyridoxic acid; B-12, total cobalamin; MMA, methylmalonic acid; PLP, pyridoxal-5′-phosphate; RBC-FOL, RBC folate; S-FOL, serum folate; tHcy, total homocysteine; VIC, ascorbic acid

[2]Model 1, simple linear regression; model 2, multiple linear regression by adjusting for sociodemographic variables; model 3, multiple linear regression by adjusting for sociodemographic and lifestyle variables; change in covariate was carried out while holding any other variables in the model constant

[3]Sample sizes for each biomarker by variable can be found in Supplemental Table 2 (model 1) and Supplemental Table 4 (models 2–4)

[4]NHB, non-Hispanic black; NHW, non-Hispanic white

[5]PIR, family poverty income ratio

[6]"Supplement user" defined as participant who reported taking a dietary supplement within the past 30 d

[7]"Smoker" defined by serum cotinine concentration >10 μg/L

[8]Alcohol consumption: calculated as average daily number of "standard" drinks [(quantity × frequency)/365.25]; 1 drink ≈ 15 g ethanol

[9]A 25% increase in BMI is comparable to a change from being normal weight to overweight

[10] Physical activity: calculated as total metabolic equivalent task (MET)-min/wk from self-reported leisure time physical activities

* Change is significantly different from zero; $P < 0.05$