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Vitamin B12 supplementation and vitamin B12 blood serum levels: evaluation of effect modification by gender and smoking status

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Abstract

Research suggests that high intake of supplemental vitamin B12 may be associated with increased risk of cancer, with some evidence that this association may vary by gender and smoking status. This investigation evaluates if similar patterns in association are observed for data for 11,757 adults from the National Health and Nutrition Examination Survey (1999–2006). Survey-weighted multivariable-adjusted linear regression was used to evaluate the association between regular B12 supplement use and log-transformed serum B12 levels. Persons taking vitamin B12 through a multivitamin/multimineral (MVMM) had a median supplemental intake of 12 mcg/day (Q1: 6, Q3: 25), compared to 100 mcg/day (Q1: 22, Q3: 500) for persons reporting supplemental B12 intake through a MVMM-exclusive source. MVMM users had a geometric mean serum B12 26% (95% CI: 23%−30%) higher than non-users, whereas MVMM-exclusive users' geometric mean was 61% (95% CI: 53%−70%) higher than non-users (p-trend<0.001). Although a positive trend (p-trend<0.001) was observed for both men and women, the association was stronger among women (p-interaction<0.001). No interaction was observed for smoking status (p-interaction=0.45). B12 supplementation is associated with higher levels of serum B12, with significant interaction by gender but not smoking. Further work is needed to better understand the interplay of B12 and gender.

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Authors' Contributions

HEF and EDK designed the study, with additional consultation from KO, MD, SLN, and TMB. HEF and KO conducted the statistical analyses. KO managed the database. HEF, EDK, KO, MD, SLN, and TMB aided in interpreting results. HEF and EDK drafted the manuscript in consultation, with edits provided by KO, MD, SLN, and TMB.

Declaration of interest statement

Authors have no conflict of interest to report.

Keywords

vitamin B12; supplements; smoking; gender; effect modification

Introduction

Vitamin B12, also known as cobalamin, is a water-soluble vitamin that plays a key role in various bodily functions,[1] and whose deficiency has been associated with conditions ranging from anemia to memory loss.[2] Vitamin B12 is consumed through both dietary sources (poultry, meat, fish, dairy, and fortified cereals) and use of dietary supplements.[1] In the United States, supplemental vitamin B12 use declined from 1999 to 2012, driven by a decrease in use of multi-vitamin/multi-minerals (MVMM), which contain several vitamins/minerals including B12 – but often at lower dosages than individual supplements.[3] However, use of vitamin B12 supplements exclusive of MVMM, henceforth referred to as MVMM-exclusive supplements, has increased, with 8.1% of adults reporting use in 2011–2012.[3] These trends raise further questions about factors that may affect B12 absorption/metabolism and subsequent serum concentrations. To this end, smokers consistently present with lower serum B12 levels than never-smokers [4–7], and previous cross-sectional studies have found lower serum B12 concentrations and higher prevalence of B12 deficiency among men [8–11].

Additional research suggests that higher intake of B12—usually only possible through supplements— may increase cancer risk.[12–16] B vitamins (including vitamin B2, B6, B9 (henceforth referred to as folate), and B12) affect the one-carbon metabolism pathway, which affects DNA methylation.[17] Methionine synthase utilizes B12 to transform homocysteine into the essential amino acid methionine, which is later used to create S-adenosylmethionine (SAM). SAM is the universal methyl donor and can deposit methyl groups onto DNA,[18] which affects gene expression. Methylation could allow for the expression of protooncogenes or the silencing of tumor suppressor genes, allowing for tumorigenesis.[19] As B12 is instrumental in the production of these intermediates, more research is necessary to understand how supplemental intake might impact bioavailable B12.

There has been some suggestion of a positive association between high vitamin B12 levels and cancer risk,[15,16,20] and that associations between cancer and supplemental B12 use may vary by gender and smoking status.[13,14,21] However, the extent to which these findings reflect differences in B12 intake versus differences in B12 absorption/metabolism by gender and smoking status remains unclear. This is further complicated by a lack of understanding on the extent that such findings apply to intakes of B12 from dietary supplements,[22] especially as individuals can ingest substantially higher levels of B12 from supplements than from the diet. Typically, supplement doses range from 5–25 mcg/day[23] compared the recommended dietary intake for healthy adults of 2.4 mcg/day.[24]

Our objective in this study was to address unanswered questions about the association between B12 supplement use (defined as non-use, MVMM use, MVMM-exclusive use) and serum B12 levels using nationally-representative data from the National Health and

Nutrition Examination Survey (NHANES). We hypothesize that these associations may be modified by gender and smoking status.

Materials & Methods

Study population

This project was conducted using data from the National Health and Nutrition Examination Survey (NHANES), a nationally-representative study of the non-institutionalized, civilian US population that collects data through in-person interviews, medical examinations, and questionnaires.[25]

Data for this study include persons surveyed over four continuous two-year cycles (1999–2006) for which serum B12 levels were measured, with each cycle being conducted in an independent sample. Over the 1999–2006 cycles, 17,951 participants aged 20 or older participated in the dietary recall. We excluded participants from our sample if they had a prior history of cancer (exclusive of non-melanoma skin cancer; n = 1,193), and further excluded if they were pregnant at the time of the NHANES interview (n = 1,008), given concern that associations may be markedly different in this group. Exclusions were additionally made for participants missing exposure (n=26), outcome (n=808), or covariate data (n=3,159; among which 1,303 were missing data on tobacco history), leaving 11,757 participants available for study. As de-identified data are publicly available for download, the Memorial Sloan Kettering Institutional Review Board determined that this did not constitute human subjects research and thus did not require local oversight.

Exposures

While vitamins B2 (riboflavin), B6 (pyridoxine), B9 (folate), and B12 (cobalamin) all operate within the one-carbon metabolism, vitamin B12 was selected for this study due to *a priori* associations with lung cancer, and the suggestion that this association may vary by gender and/or smoking status.[13,14] Vitamin B12 supplement use was defined as follows: no regular use, regular vitamin B12 use as part of a multivitamin/multimineral (MVMM), and regular use exclusive of a MVMM supplement. Here, regular use was defined by use at least 15 days per month in order to capture consistent health behaviors. MVMM was defined as a supplement that contains 10 or more vitamins or minerals[3]; thus, MVMM-exclusive forms of B12 supplementation include B-vitamin complexes, supplements containing solely B12, as well as sources of B12 typically consumed at higher doses than when consumed through an MVMM.

Vitamin B12 supplement use was the primary exposure of interest, but the research team wanted to ensure that results were not influenced greatly by the amount of supplemental or dietary intake. Thus, in sensitivity analyses, we alternatively examined associations pertaining to i) dose of vitamin B12 supplement use (mcg/day), ii) estimated dietary vitamin B12 intake (mcg/day), and iii) total intake from combined supplement and dietary sources (mcg/day). To obtain information on supplemental dose for sensitivity analyses, interviewers asked participants if they had used any supplements in the past 30 days, and if yes, they requested to see the supplement label for ingredient and dosage information.

If the interviewer was unable to see the bottle, the participant was asked to provide information to the best of their ability, which trained nutritionists matched to the NCHS dietary supplement database that contains detailed information on supplement products and blends. NHANES documentation provides more details of this matching process and database usage.[26] Supplement information was combined with self-reported information on use (e.g. frequency of use, pills/day) to determine total dose/day of supplemental B12 (mcg/day), which was then divided into four groups: non-use and tertiles of supplement dose. Dose of supplemental vitamin B12 ranged from 0.065 mcg/day to 18,000 mcg/day, and the cut-offs for supplement dose tertiles are as follows: 6.14 and 25 mcg/day.

For estimated dietary intake, we utilized NHANES dietary recall data, the methods of which have been detailed elsewhere.[27] The NHANES survey collects dietary information from each participant through two 24-hour dietary recalls—one conducted on the day of the interview/medical examination, and for a subset of participants, another conducted over the phone 3 to 10 days later. NHANES estimates the amount of B12 in micrograms (mcg) in each food and calculates a total dietary intake for the recall period. To preserve the temporal sequence, only the first dietary recall was used to create a dietary B12 intake variable because the food would have been ingested before the blood sample was taken. The continuous dietary intake variable (mcg/day) was divided into quartiles, with cutoffs as follows: 2.04, 3.69, and 6.13 mcg/day.

A total supplemental & dietary intake variable was created using the continuous supplemental dosage data with continuous dietary intake data. After combining the dietary intake and supplement dosage, the continuous total intake variable (mcg/day) was divided into quartiles, with total supplement+diet quartile cutoffs as follows: 2.82, 6.16, 12.95 mcg/day.

Outcome

The outcome of interest was blood serum levels of vitamin B12 (pmol/L) measured using Bio-Rad Laboratories Quataphase II Folate/Vitamin B12 radioassay kits.[28] The distribution of serum B12 was comparable across NHANES cycles. However, as the B12 blood serum levels were right skewed, a natural log transformation was used to normalize the distribution.

Statistical Analysis

Linear regression was used to model the association between vitamin B12 intake and serum vitamin B12. As the outcome was log-transformed, coefficients are presented in exponentiated form, representing the ratio of geometric mean of serum vitamin B12 for MVMM or MVMM-exclusive users as compared to the non-user reference group. Minimally-adjusted and fully-adjusted linear regression models were conducted for each exposure, including the primary exposure (vitamin B12 supplement use). Minimally-adjusted models adjust for both age and gender. Fully-adjusted models additionally include the following variables: race/ethnicity, education level, poverty-to-income ratio, body mass index (BMI), smoking status, number of pack-years, alcohol use, current health status, diabetes status, heart disease history, history of anemia treatment, and history of memory loss. These covariates were selected *a priori* based on their expected association with

exposure and outcome. Details on covariate modeling are provided in tables and table footnotes. Sensitivity analyses were conducted, with exposure alternatively defined by dietary intake (mcg/day), supplemental dose (mcg/day), and total dietary+supplemental intake (mcg/day). Our research team felt these additional exposures were inherently linked to our primary exposure, but were less descriptive of long-term supplement behaviors as NHANES only collects dietary data over two days.

The multivariate model relating 3-level vitamin B12 supplement use and serum vitamin B12 was first stratified separately by gender and smoking status (current, former, or never smoker). For each, we examined linear trends within strata.

Given prior literature to suggest potential for joint interaction by gender and smoking status, we conducted a secondary analysis of interaction alternatively using six jointly-defined strata: male never smokers, male former smokers, male current smokers, female never smokers, female former smokers, and female current smokers. However, given the small number of MVMM-exclusive vitamin B12 users within each of the 6 strata, this analysis had to be alternatively conducted using the total diet+supplement B12 intake variable.

As NHANES oversamples people 60 and older, African Americans, Asians, and Hispanics, all analyses are weighted to account for oversampling and non-response. All analyses were conducted using StataSE 15 software.[29] All P values were two-sided, with $P<0.05$ considered statistically significant.

Results

In this analysis of US adults, aged ≥20 years, B12 supplement use ranged from 0.065 to 2,800 mcg/day in the MVMM group (median: 12; Q1: 6, Q3: 25) and 2 to 18,000 mcg/day in the MVMM-exclusive group (median: 100; Q1: 22, Q3:500). Distributions of serum B12 were comparable across survey years. Regular use of any supplement containing vitamin B12– defined as MVMM or MVMM-exclusive supplementation at least 15 days of the month -- was most common among those 80 years or older (46.3% reporting use), and least common among 20–29-year-olds (18.6%). This pattern generally held with MVMM-exclusive vitamin B12 supplement use: use was most common amongst those 70–79 years, of whom 9.0% regularly used MVMM-exclusive B12 supplements, as compared to 1.4% of persons 20–29 years of age. Within Non-Hispanic White participants, 5.4% regularly used MVMM-exclusive B12 supplements as compared to 2.2% of non-Hispanic Black participants, and 2.1% of Mexican Americans. MVMM-exclusive B12 use increased with poverty-to-income ratio level, with higher income individuals reporting more use than lower income individuals. Women were more likely be MVMM-exclusive B12 users (5.5%) than men (3.8%). Current smokers were less likely be MVMM-exclusive B12 supplement users (3.1%) compared to never (4.8%) and former (5.9%) smokers (Table 1).

In fully-adjusted models, persons taking vitamin B12 through an MVMM source had a 26% (95% CI: 23% - 30%) higher geometric mean than non-users; among those using B12 individual supplements (exclusive of MVMM), the geometric mean of serum B12 was 61% (95% CI: 53%−70%) higher than the non-use group. Secondary analyses for supplemental

dose, dietary B12 intake, and total dietary+supplemental intake revealed a similar pattern of association. Specifically, when examining associations for supplemental B12 intake (mcg/day), we observed that that the highest dose users (tertile 3) had a 46% (95% CI: 42% - 51%) higher geometric mean of serum vitamin B12 than B12 non-users (p-for-trend < 0.001).When examining the association between dietary B12 intake and serum B12 levels, the highest dietary intake quartile group's geometric mean was 18% (95% CI: 14% - 22%) higher than the lowest quartile (p-for-trend < 0.001). Finally, the total intake model (corresponding to dietary+supplemental intake) showed the highest dose quartile group had a geometric mean 43% (95% CI: 39% - 48%) higher than the lowest group (p-for-trend < 0.001) (Table 2)

We examined whether the association between B12 supplement use and serum B12 was modified by participants' gender and smoking status. While a significant linear trend was observed among both males and females ($p < 0.001$), the association was stronger among females (p-interaction=0.01). Among males, the MVMM-exclusive group had a geometric mean 51% (95% CI, 44%–58%) higher than the non-use group. Among females, the MVMM-exclusive group's geometric mean was 69% (95% CI, 55%−85%) higher than non-users. There was no significant interaction between smoking and use of B12 supplements (p=0.45), with significant linear trend observed for all groups (Table 3).

Secondary analyses of the association between total vitamin B12 intake and serum B12 levels in gender-smoking jointly-defined strata revealed a statistically non-significant interaction by gender/smoking status (p-interaction: 0.07). Significant trends were observed in all groups (p-trend< 0.05), with the strongest association observed for female former smokers (Q4 vs Q1 Ratio: 1.51; 95% CI: 1.34, 1.70; p-trend < 0.001) and the weakest association observed for male never-smokers (Q4 vs Q1 Ratio: 1.32; 95% CI: 1.23, 1.42; p-trend< 0.001) (Table 4)

We have also conducted a series of sensitivity analyses to evaluate the robustness of study findings. First, to address concern that supplement use and dietary exposure may not be independent, we conducted a sensitivity analysis using a mutually adjusted model, including both vitamin B12 supplement use and dietary intake. No evident change in coefficients was observed, and therefore these results are not presented. Second, recognizing that the supplemental dose ranged up to 18,000 mcg/day (mean: 82.5, median: 15) for regular users, we were concerned that the threshold of the most highly exposed group may be too low to offer a meaningful comparison. This is particularly true of the dietary+supplemental variable, where the distribution is driven down by non-users (Q4 ranges includes values from 12.95 mcg/day upward). Thus, all total diet+supplemental models were run again alternatively using deciles of total dietary+supplement intake as the exposure variable (top decile cut-point = 100 mcg/day). Results were similar to quartile models, so they are not presented here.Use of metformin, aspirin and proton pump inhibitors (PPIs) have also been associated with vitamin B12 deficiency and absorption issues. Data on metformin was available for all four cycles of NHANES included in this study. However, NHANES only collected data on aspirin use from 1999 to 2004, and during the 2003–2004 cycles, PPIs became available over-the-counter (with over-the-counter use not captured in NHANES, thus making exposure hard to accurately capture from 2003–2004 onward). For each of

the medications described, a sensitivity analysis using years with available data was run comparing a model of B12 supplement use level with B12 serum levels adjusted for all covariates including medication of interest (aspirin, metformin, or PPIs) to a similar model not including the medication. Results were unchanged and therefore these three medications were not included in the final models.

Finally, the age range of the dataset (20+) was large, and given concern associations may vary by age, we assessed whether the diet+supplement associations (overall and stratified by gender and smoking status) held when restricted to those age 50+; again, these analyses were conducted using the diet+supplement total exposure variable (as opposed to the 3-level supplement variable), given increased power for this exposure. In a sensitivity analysis of participants 50 years or older (n = 5,607), the multivariate-adjusted model comparing B12 supplementation level to B12 serum had a significant linear trend (see supplemental Table 1). MVMM users had a geometric mean 33% higher than the non-user group, and MVMM exclusive users had one 76% higher. Significant linear trends also were observed for stratified models by gender and smoking status ($p < 0.001$), with associations stronger than in the original model. Women continued to present with stronger relationships than men between B12 supplement intake group and serum B12 level. MVMM exclusive female and male users had an 85% and 66% higher geometric mean than their designated non-user groups, respectively. However, no significant interaction by gender was observed (see supplemental Table 2). Among the smoking groups, MVMM exclusive never, former, and current smokers showed 85%, 62%, and 74% higher geometric means respectively than non-user groups, again, with the interaction not significant. To provide results comparable to cohorts using 44+ as an age cut-point, so we have also provided supplemental tables for this subset of the study population (n=6,911). With the larger sample size, effect modification by gender was statistically significant (see supplemental Tables 3 & 4).

Discussion

In this large, nationally-representative study, vitamin B12 supplement use was significantly associated with higher blood serum levels than non-use, as expected, with comparable associations observed in sensitivity analyses for supplemental dose, dietary intake, and total supplemental + dietary intake. While significant associations between vitamin B12 supplement use and serum vitamin B12 were observed among both men and women, there was significant heterogeneity by gender, with stronger associations observed among women than men. No interaction was observed by smoking status, with the relationship between vitamin B12 supplement use and vitamin B12 blood levels significant for all groups (never, former, and current smokers).

Regular MVMM-exclusive B12 users presented with 61% higher geometric mean B12 serum levels than non-users (95% CI: 53% - 70%), an association stronger than that observed for lower dose as consumed through a MVMM (Ratio: 1.26; 95% CI: 1.23–1.30). Secondary analyses by dose (supplemental intake, dietary intake, or combined supplemental + dietary intake in mcg/day) revealed a significant linear trend, regardless of source, in agreement with previous studies[30–33]—including a cross-sectional analysis of B12 intake and plasma B12 from male and female Framingham Heart Study participants which showed

linear trends for combined dietary/supplemental intake of B12 and B12 blood concentrations among both supplement and non-supplement users.[34] It should be noted that while the range of MVMM and MVMM-exclusive intakes was wide (min-max: 0.065–2,800 and 2–18,000 mcg/day respectively), the thresholds in the highest dose group in our diet+supplemental intake variable (Q4 defined by intake more than 12.95 mcg/day) did not come close to that commonly found in MVMM-exclusive B vitamins. As a water-soluble vitamin, intakes in excess of what the body needs are excreted in the urine, thus no tolerable upper limit has been set for vitamin B12.

Prior analysis of NHANES III with data from 1991–1994 also showed that supplemental B12 use was associated with increased B12 serum levels. As with our findings, the association was attenuated slightly among older individuals ≥50 years, owing to reduced absorption often seen in older adults.[35] Similarly, a randomized controlled trial comparing 2000 mcg/day of MVMM-exclusive oral supplementation to intermuscular injections revealed an increase in serum B12 among the oral supplement group.[36] In another six-month randomized controlled trial of New Zealand women, significant increases in serum B12 were associated with supplementation even after women were stratified by dietary intake characteristics.[30]

Previous cross-sectional studies have found lower serum B12 concentrations and higher prevalence of B12 deficiency among men.[8–11] However, these articles did not evaluate this association in the context of long-term supplementation, so we further examined the relationship between regular supplement use and serum B12, stratified by gender, and observed that the association between supplementation category and serum B12 was significantly stronger among women than men. Females had a greater difference in geometric mean serum B12 between MVMM-exclusive users and non-users (69%; 95% CI: 58% - 98%) compared to male MVMM-exclusive users and non-users (51%; 95% CI: 44% – 58%). One might expect this result if B12 intake was higher among women to begin with, but male and female intakes among both regular user groups were comparable. Our current finding of a significant interaction between gender and serum B12 provides more evidence that females and males may metabolize vitamin B12 differently.

It is hypothesized that these gender differences could be partially explained by genetic variation. One cross-sectional study found that levels of transcobalamin, the necessary glycoprotein for vitamin B12's active form, were also higher among females than males.[37] Estrogen has been shown to increase levels of important enzymes for the one-carbon metabolism such as phosphatidylethanolamine N-methyltransferase (PEMT).[38] Female sex hormones are also associated with fluctuations in homocysteine and folate serum levels during menstrual cycles,[39–41] and based on NHANES II data from 1991–1994, pregnant women presented with significantly lower serum homocysteine levels than non-pregnant, non-oral-contraceptive-using women.[42] These studies can be extrapolated to the interaction between vitamin B12 and gender as B12 is frequently associated with homocysteine and folate levels in the blood.[8,43] These findings might partially explain the weakened interaction by gender in the subset of the study population 50 years or older as hormone levels decline rapidly in postmenopausal women.

A recent cross-sectional study found that the protective association between female sex on serum B12 cannot be explained by estrogen alone.[44] Mathematical modeling has described a difference in the amount of one-carbon metabolism enzymes in women and men, with females presenting higher levels of PEMT, choline, betaine, and S-andenosyl-homocysteine, while males present with higher levels of homocysteine and S-andenosylmethionine.[45] Differences in the enzymes that drive the one-carbon metabolism could explain gender divides in serum B12 metabolism observed in this study and others, but more research is necessary to fully understand the biological mechanisms affecting this relationship.

As with gender, significant linear trends were observed between supplement category and serum vitamin B12 in all groups, regardless of smoking status (non-, former, and current smokers), with no significant interaction by smoking status. Decreased levels of serum B12 levels among smokers compared to non-smokers have been well documented.[4–7] In one randomized controlled trial of hospitalized elderly individuals were given 100% of the Reference Nutrient Intake for B12 and other micronutrients in a micronutrient drink or a placebo drink for six weeks; smokers and ex-smokers presented with lower B12 serum levels than never smokers overall, but no significant difference between groups was observed for change in B12 status.[22] Similarly, a case-control study of Iranian men revealed no significant difference between total serum B12 in smokers and non-smokers, but further investigation revealed that the ratio of active forms of B12 to total serum B12 was lower among smokers.[46] These results would seem to suggest that future studies should evaluate the relationship between active forms of B12 and smoking status.

Understanding factors that affect B12 absorption and metabolism is important as previous studies have shown an increased risk of some cancers with higher vitamin B12 intakes. B12 is a substrate of the methionine synthase enzyme within the one-carbon metabolism. Higher levels of B12 provide more substrate for S-adenosylmethionine (SAM) production. SAM acts as the universal methyl donor and can alter expression of protooncogenes and tumor suppressor genes, which are associated with cancer.[18,19] High plasma B12 was associated with high 1-year risk of cancer (any site) among a Danish cohort within a primary care health improvement setting. Researchers hypothesized that individuals with undiagnosed cancers might metabolize B12 differently,[15] as previously observed for folic acid intake and colorectal cancer progression.[47–49] Another cohort study of hospital patients found that individuals with persistently high plasma B12 were at higher risk of solid tumor incidence within 5 years.[50] A randomized controlled trial of individuals taking 500 mcg/day of B12 as well as 400 mcg/day of folic acid for 2–3 years exhibited increased colorectal cancer risk in the experimental group.[16]

However, cohort studies appear to disagree on whether women have higher risk of cancer than men after B12 supplementation. A pooled analysis from the Lung Cancer Cohort Consortium revealed a positive association between serum B12 and lung cancer adenocarcinoma risk, but a significant dose-response association was only observed for women, current and former smokers.[13] Long-term high-dose MVMM-exclusive B12 supplementation within a prospective cohort study was associated with increased lung cancer risk among men, but not women.[14] Further research of the Women’s Health Initiative similarly revealed no association between B12 supplementation and lung cancer among

women.[21] These results are at odds with findings from our study, which imply that those taking the highest supplements have higher levels of B12 in the blood. Additionally, in our study, women have higher B12 serum levels across supplementation levels than men. Further research on B12 supplementation by sex and smoking status is necessary to understand how differences in metabolism may affect cancer risk.

One strength of this study is that NHANES collects detailed information on supplements from an in-home interview, documenting detailed information, including ingredients and dosages. Furthermore, as dietary recall data were available, we were able to consider both supplemental and dietary sources in our analyses. This large, well-powered study is nationally-representative of non-incarcerated U.S. citizens, facilitating the generalizability of these findings. There are however several important limitations to consider. Firstly, several B vitamins affect the one carbon metabolism,[17] but only B12 was analyzed here. One or more of these vitamins may have confounded results. It was also unclear if individuals had ingested their B12 supplements the day before their B12 blood serum levels were taken (even so, by defining use by regular use, we hope to have largely mitigated this issue). Additionally, dose values recorded may not match exactly what was consumed by survey participants because in some cases, the NHANES interviewer may not have seen the supplement bottle during the interview, meaning it would be recorded as a generic B12 supplement. Similarly, there may be measurement error in dietary recall as underreporting of energy intake has been seen in NHANES data collection[51] which has been linked to underreporting of micronutrients as well.[52] While we would expect such measurement error to attenuate results toward the null overall, it is possible that such measurement error could vary by gender or smoking status, which could bias effect estimates of interaction. Lastly, while extensive effort was made to control for confounding, we were not able to include all factors that might affect B12 blood serum levels, which may have resulted in the low R^2 values for the multivariate linear regression models (11%). Even so, efforts were made to adjust for all known factors, so we have some confidence in results.

In summary, this study revealed that increased regular B12 supplement use was associated with higher B12 blood serum levels, with significant effect modification by gender but not smoking status. Further research on this topic would be of merit to establish the biological mechanisms that affect absorption of B12 from supplemental sources.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1.

Population Characteristics, by Supplement Use

	Total	Supplement Use		
	N = 11,757 N (Wgt %)	**No B_{12} N = 8304 (67.9%)**	**MVMM B_{12} N = 2936 (27.4%)**	**MVMM-Exclusive B_{12} N= 517 (4.7%)**
Gender				
Male	5945 (49.7)	4372 (71.3)	1344 (24.9)	229 (3.8)
Female	5812 (50.3)	3932 (64.5)	1592 (30.0)	288 (5.5)
Age				
20–29	1928 (17.6)	1598 (81.4)	308 (17.2)	22 (1.4)
30–39	1982 (20.7)	1531 (73.8)	406 (23.6)	45 (2.6)
40–49	2240 (21.9)	1641 (68.2)	496 (26.5)	103 (5.3)
50–59	1661 (17.6)	1109 (62.5)	460 (31.2)	92 (6.3)
60–69	1903 (11.8)	1202 (55.0)	588 (38.5)	113 (6.5)
70–79	1275 (7.2)	807 (57.1)	376 (33.8)	92 (9.0)
80+	768 (3.1)	416 (53.7)	302 (39.7)	50 (6.6)
Race/ethnicity				
NH White	6023 (73.7)	3749 (63.6)	1920 (31.0)	354 (5.4)
NH African American	2343 (10.5)	1875 (82.4)	403 (15.4)	65 (2.2)
Mexican American	2518 (6.9)	2019 (81.3)	438 (16.6)	61 (2.1)
Other Hispanic	476 (4.5)	385 (79.7)	76 (17.5)	15 (2.8)
Other Race/Mixed Race	397 (4.5)	276 (71.1)	99 (23.7)	22 (5.2)
Poverty to income ratio				
<1 (lower income)	1979 (11.9)	1649 (81.3)	287 (15.7)	43 (3.0)
1–1.99	3015 (20.1)	2277 (75.2)	614 (20.3)	124 (4.6)
2–3.99	3436 (30.5)	2362 (68.4)	906 (26.9)	168 (4.7)
>= 4 (higher income)	3327 (37.5)	2016 (59.3)	1129 (35.5)	182 (5.3)
Education				
Less than high school	3470 (18.0)	2812 (79.6)	560 (17.4)	98 (3.0)
High school/GED or equivalent	2789 (25.5)	1972 (71.1)	693 (24.3)	124 (4.6)
Some college or AA degree	3181 (30.6)	2183 (66.9)	871 (27.7)	172 (5.4)
College grad or above	2317 (25.9)	1382 (57.8)	812 (37.2)	123 (5.0)
Body Mass Index (BMI)				
Underweight (<18.5)	165 (1.6)	123 (74.2)	36 (22.9)	6 (2.8)
Normal weight (18.5 – 24.9)	3466 (32.1)	2355 (65.9)	937 (29.4)	174 (4.7)
Overweight (25 – 29.9)	4176 (34.1)	2912 (67.1)	1083 (28.2)	179 (4.7)
Obese (≥ 30)	3950 (32.2)	2912 (70.4)	880 (24.9)	158 (4.7)
Smoking Status				
Never	6,592 (55.3)	4,594 (66.3)	1,715 (28.8)	283 (4.8)
Former[a]	2786 (22.1)	1814 (61.2)	815 (32.9)	157 (5.9)
1st tertile of pack-years	*938 (34.9)*	*644 (62.8)*	*254 (32.6)*	*40 (4.6)*
2nd tertile of pack-years	*925 (34.3)*	590 *(60.2)*	*280 (33.5)*	*55 (6.3)*

	Total	Supplement Use		
	N = 11,757 N (Wgt %)	**No B_{12} N = 8304 (67.9%)**	**MVMM B_{12} N = 2936 (27.4%)**	**MVMM-Exclusive B_{12} N= 517 (4.7%)**
3rd tertile of pack-years	*923 (30.8)*	580 *(60.3)*	*281 (32.7)*	*62 (7.0)*
Current[b]	2379 (22.6)	1896 (78.2)	406 (18.7)	77 (3.1)
1st tertile of pack-years	*805 (31.5)*	*679 (82.7)*	*112 (15.8)*	*14 (1.5)*
2nd tertile of pack-years	*787 (34.5)*	*626 (79.1)*	*136 (18.1)*	*25 (2.9)*
3rd tertile of pack-years	*787 (34.0)*	*591 (73.2)*	*158 (22.1)*	*38 (4.7)*
Alcohol Use				
< 1 / month	5991 (45.3)	4266 (69.1)	1463 (26.1)	262 (4.8)
≥ 1 / month to < 4 / week	3260 (30.1)	2269 (65.7)	857 (29.8)	134 (4.5)
≥ 4 / month to < 2 / day	1898 (18.5)	1323 (67.4)	479 (27.8)	96 (4.8)
≥ 2 / day	608 (6.1)	446 (70.7)	137 (25.1)	25 (4.2)
Current health status				
Poor	482 (3.0)	336 (74.7)	97 (21.7)	19 (3.6)
Fair	2077 (12.8)	1592 (72.9)	398 (22.2)	87 (5.0)
Good	3832 (30.4)	2793 (70.8)	898 (25.3)	141 (3.9)
Very Good	3186 (32.2)	2116 (65.2)	892 (29.2)	178 (5.6)
Excellent	2173 (21.6)	1432 (67.9)	649 (31.7)	92 (4.4)
Diabetes				
No	10562 (92.9)	7442 (67.9)	2669 (27.5)	451 (4.5)
Yes	1195 (7.1)	862 (67.1)	267 (26.4)	66 (6.4)
Heart disease				
No	10485 (91.4)	7438 (68.1)	2583 (27.4)	437 (4.5)
Yes	1299 (8.6)	866 (65.2)	353 (28.3)	80 (6.5)
Anemia				
No	11430 (97.8)	8101 (68.0)	2849 (27.5)	480 (4.5)
Yes	327 (2.2)	203 (61.2)	87 (26.8)	37 (12.0)
Memory Loss				
No	10855 (93.9)	7667 (67.7)	2717 (27.6)	471 (4.7)
Yes	902 (6.1)	637 (70.4)	219 (24.9)	46 (4.7)

ABBREVIATIONS: MVMM (Multi-vitamin/mineral supplement), NH (Non-Hispanic)

[a]Tertile 1: (< 5.55 pack-years), Tertile 2: (5.55 – 24.0 pack-years), Tertile 3: (> 24.0 pack-years)

[b]Tertile 1: (< 9.10 pack-years), Tertile 2: (9.10 – 26.0 pack-years), Tertile 3: (> 26.0 pack-years)

Table 2.

Association between Vitamin B_{12} Consumption and Serum Vitamin B_{12}

	Cohort N (Wgt %)	Unadjusted Geometric Mean of B_{12} serum (pmol/L)		Minimally-Adjusted[a] Ratio (95% CI)		Multivariable-Adjusted[b] Ratio (95% CI)	
	N = 11,757	Mean	95% CI	Ratio	95% CI	Ratio	95% CI
Regular Supplement B_{12}Use							
No B_{12}	8304 (67.9)	313.78	(308.51, 319.05)	1.00	Ref	1.00	Ref
MVMM B_{12}	2936 (27.4)	389.59	(381.00, 398.18)	1.24	(1.21, 1.28)	1.26	(1.23, 1.30)
MVMM-exclusive B_{12}	517 (4.7)	493.88	(469.71, 518.04)	1.58	(1.50, 1.66)	1.61	(1.53, 1.70)
				p-trend < 0.001		**p-trend < 0.001**	
Supplemental B12 Intake[c](mcg)							
Non-users	8304 (67.9)	313.78	(308.51, 319.05)	1.00	Ref	1.00	Ref
1st Tertile	1313 (12.4)	379.77	(367.76, 391.77)	1.21	(1.17, 1.25)	1.23	(1.19, 1.27)
2nd Tertile	1306 (11.8)	394.21	(382.80, 405.62)	1.26	(1.21, 1.31)	1.28	(1.23, 1.33)
3rd Tertile	834 (8.0)	458.05	(441.43, 474.67)	1.47	(1.42, 1.52)	1.49	(1.44, 1.54)
				p-trend < 0.001		**p-trend < 0.001**	
Dietary B_{12}Intake[d](mcg)							
1st Quartile	2945 (22.9)	319.04	(309.88, 328.21)	1.00	Ref	1.00	Ref
2nd Quartile	2936 (23.6)	325.94	(318.04, 333.85)	1.03	(0.99, 1.06)	1.03	(1.00, 1.07)
3rd Quartile	2937 (26.5)	347.13	(338.63, 355.62)	1.10	(1.07, 1.13)	1.11	(1.08, 1.15)
4th Quartile	2939 (27.0)	365.34	(358.00, 372.67)	1.17	(1.13, 1.20)	1.18	(1.14, 1.22)
				p-trend < 0.001		**p-trend < 0.001**	
Dietary+Supplement B_{12}Intake[e](mcg)							
1st Quartile	2943 (21.7)	291.83	(284.75, 298.91)	1.00	Ref	1.00	Ref
2nd Quartile	2941 (24.5)	310.14	(302.23, 318.05)	1.06	(1.04, 1.10)	1.08	(1.05, 1.11)
3rd Quartile	2935 (26.8)	349.96	(341.51, 358.42)	1.21	(1.17, 1.25)	1.23	(1.19, 1.27)
4th Quartile	2938 (27.0)	406.52	(399.48, 413.56)	1.40	(1.36, 1.44)	1.43	(1.39, 1.48)
				p-trend < 0.001		**p-trend < 0.001**	

ABREVIATIONS: MVMM (Multi-vitamin/mineral supplement), mcg (micrograms)

[a] Adjusted for age and gender

[b] Additionally adjusted for race, poverty to income ratio, education, body mass index, smoking status, alcohol use, current health status, diabetes status, heart disease status, anemia treatment, memory loss

[c] Non-users (0 mcg/day), Tertile 1 (0.065– 6.0 mcg/day), Tertile 2 (6.14 – 25 mcg/day), Tertile 3 (> 25.00 mcg/day)

[d] Quartile 1 (< 2.04 mcg/day), Quartile 2 (2.04 – 3.68 mcg/day), Quartile 3 (3.69 – 6.13 mcg/day), Quartile 4 (> 6.13 mcg/day)

[e] Quartile 1 (< 2.82 mcg/day), Quartile 2 (2.82 – 6.15 mcg/day), Quartile 3 (6.16 – 12.95 mcg/day), Quartile 4 (> 12.95 mcg/day)

Table 3.

Stratified Association between Vitamin B_{12} Supplement Use and Serum Vitamin B_{12}

	B_{12} Supplement Category									p-trend
	None			MVMM			MVMM exclusive			
	N (Wgt %)	Ratio[a]	95% CI	N (Wgt %)	Ratio[a]	95% CI	N (Wgt %)	Ratio[a]	95% CI	
Gender										
Male	4372 (71.3)	1.00	Ref	1344 (24.9)	1.22	(1.19, 1.26)	229 (3.8)	1.51	(1.44, 1.58)	< 0.001
Female	3932 (64.5)	1.00	Ref	1592 (30.0)	1.30	(1.25, 1.35)	288 (5.5)	1.69	(1.55, 1.85)	< 0.001
p-interaction < 0.001										
Smoking Status										
Never	4594 (66.3)	1.00	Ref	1715 (28.8)	1.25	(1.21, 1.29)	283 (4.8)	1.66	(1.54, 1.78)	< 0.001
Former	1814 (61.2)	1.00	Ref	815 (32.9)	1.30	(1.23, 1.36)	157 (5.9)	1.55	(1.42, 1.69)	< 0.001
Current	1896 (78.2)	1.00	Ref	406 (18.7)	1.25	(1.17, 1.34)	77 (3.1)	1.53	(1.37, 1.72)	< 0.001
p-interaction = 0.45										

[a]Adjusted for sex, age, race, poverty to income ratio, education, body mass index, smoking status, alcohol use, current health status, diabetes status, heart disease status, anemia treatment, memory loss

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Table 4.

Stratified Association between Vitamin B_{12} Total Intake and Serum Vitamin B_{12} by Combined Gender and Smoking Status

		Gender					
		Male			Female		
Smoking Status	**B_{12} Supplemental & Dietary Combined Intake[b] (mcg)**	**N (wgt %)**	**Ratio[a]**	**95% CI**	**N (wgt %)**	**Ratio[a]**	**95% CI**
Never Smoker	Quartile 1	568 (15.8)	1.00	Ref	1094 (26.2)	1.00	Ref
	Quartile 2	727 (26.2)	1.03	(0.96, 1.10)	878 (21.1)	1.12	(1.07, 1.17)
	Quartile 3	780 (30.3)	1.09	(1.03, 1.17)	901 (25.8)	1.30	(1.22, 1.38)
	Quartile 4	710 (27.8)	1.32	(1.23, 1.42)	934 (26.9)	1.47	(1.39, 1.55)
			p-trend <000.1			**p-trend <000.1**	
Former Smoker	Quartile 1	356 (15.1)	1.00	Ref	272 (21.0)	1.00	Ref
	Quartile 2	459 (25.8)	1.06	(0.98, 1.15)	205 (19.6)	1.04	(0.92, 1.17)
	Quartile 3	429 (27.3)	1.19	(1.10, 1.28)	231 (25.3)	1.39	(1.23, 1.56)
	Quartile 4	506 (31.8)	1.44	(1.35, 1.54)	328 (34.1)	1.51	(1.34, 1.70)
			p-trend <000.1			**p-trend <000.1**	
Current Smoker	Quartile 1	306 (19.6)	1.00	Ref	347 (34.5)	1.00	Ref
	Quartile 2	412 (290)	1.11	(1.04, 1.18)	260 (28.0)	1.02	(0.93, 1.13)
	Quartile 3	401 (28.2)	1.20	(1.10, 1.27)	193 (19.9)	1.29	(1.12, 1.48)
	Quartile 4	291 (23.3)	1.34	(1.25, 1.43)	169 (17.6)	1.50	(1.33, 1.68)
			p-trend <000.1			**p-trend <000.1**	
		p-interaction = 0.07					

[a]Adjusted for sex, age, race, poverty to income ratio, education, body mass index, smoking status, pack-years, alcohol use, current health status, diabetes status, heart disease status, anemia treatment, memory loss

[b]Quartile 1: (< 2.82 mcg/day), Quartile 2: (2.82–6.15 mcg/day), Quartile 3: (6.16– 12.95 mcg/day), Quartile 4: (≥ 12.95 mcg/day)