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## Association between vitamin B<sub>12</sub>-containing supplement consumption and prevalence of biochemically defined B<sub>12</sub> deficiency in adults in NHANES III (Third National Health and Nutrition Examination Survey)

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### Abstract

**Objective**—To explore the association between vitamin B<sub>12</sub> (B<sub>12</sub>)-containing supplement use, low B<sub>12</sub> concentrations and biochemically defined B<sub>12</sub> deficiency in US adults.

**Design**—A cross-sectional study with adjustment for survey design. Prevalence ratios for two age groups (18–50 and >50 years) were estimated using unconditional logistic models. Outcome measures included prevalence of low serum B<sub>12</sub> concentration (<148 pmol/l) and biochemical B<sub>12</sub> deficiency (serum B<sub>12</sub><148 pmol/l with concomitant homocysteine >10 μmol/l).

**Setting**—A population survey of health and nutritional measures.

**Subjects**—Subjects were non-institutionalized adults, aged 18 years and older, who participated in Phase 2 of NHANES III (Third National Health and Nutrition Examination Survey).

**Results**—Low B<sub>12</sub> concentrations were less prevalent among persons consuming B<sub>12</sub>-containing supplements ( $P = 0.001$ ) with an adjusted prevalence ratio of 0.6 (95% CI 0.3, 1.0). Biochemical B<sub>12</sub> deficiency showed a similar trend ( $P = 0.0002$ ), with an adjusted prevalence ratio of 0.3 (95% CI 0.1, 0.8). Prevalence ratios were similar in adults >50 years of age, although the prevalence of low B<sub>12</sub> and biochemical deficiency was proportionally higher.

**Conclusions**—Consumption of B<sub>12</sub>-containing supplements was associated with at least 50% lower prevalence of both low serum B<sub>12</sub> and biochemical B<sub>12</sub> deficiency in a nationally representative sample of US adults, suggesting increased consumption of B<sub>12</sub> from supplements or from fortified foods may reduce the prevalence of B<sub>12</sub> deficiency. Additionally, the current Recommended Daily Allowance for B<sub>12</sub> of 2.4 μg may be insufficient for those aged >50 years.

### Keywords

Biochemical B<sub>12</sub> deficiency; Cobalamin deficiency; NHANES III; Vitamin B<sub>12</sub>; Vitamin supplementation

The Institute of Medicine (IOM) extensively reviewed the available data on vitamin B<sub>12</sub> (B<sub>12</sub>) deficiency and requirements, and has identified biochemical B<sub>12</sub> deficiency and effective methods to reduce risk of B<sub>12</sub> deficiency as high priorities for research(1). The Recommended Daily Allowance (RDA) was set at 120% of the daily requirement and is expected to protect 97–98% of healthy persons from deficiency. Based on the lower bioavailability of food-bound B<sub>12</sub> in older individuals, the IOM recommended adults over the age of 50 years meet the RDA for B<sub>12</sub> of 2.4 µg by consuming synthetic vitamin B<sub>12</sub> in B<sub>12</sub>-fortified foods or B<sub>12</sub> supplements(1). Previously, three population-based studies examined biochemical indicators of B<sub>12</sub> deficiency (serum B<sub>12</sub> and/or homocysteine and methylmalonic acid (MMA) concentrations) in relation to vitamin supplement status and concluded that B<sub>12</sub>-containing supplements may protect against B<sub>12</sub> deficiency(2-4). However, all three studies used data from elderly, racially homogeneous cohorts with relatively small sample sizes, limiting the generalizability of their findings to the US adult population. Therefore, we used data from NHANES III (Third National Health and Nutrition Examination Survey) to further explore the relationship between B<sub>12</sub> supplement use and biochemically defined B<sub>12</sub> deficiency in a large, nationally representative population sample of the US adult population.

## Methods

### Subjects

NHANES III was conducted with a complex, multistage probability design(5). Persons older than 60 years as well as African-American and Mexican-Americans were over-sampled to more precisely assess US health and nutritional measures. Importantly, NHANES III was the first NHANES survey without an upper age limit, thus improving the estimates of health and nutrition in older segments of the population. The survey design was approved by the National Center for Health Statistics institutional review board (IRB) and participants gave written informed consent prior to undergoing survey procedures(5); the present analysis was thus deemed exempt from review by the Emory University IRB. The survey was divided into two phases (Phase 1, 1988–91 and Phase 2, 1991–4), designed to allow individual or combined phase analysis. Because homocysteine concentrations were measured only in Phase 2, the current analysis focuses on data from non-pregnant adults (age 18 years and older) in Phase 2 (September 1991–October 1994) for whom supplement use information and laboratory analyses of serum B<sub>12</sub> and homocysteine are available (*n* 8394). Laboratory measurements, including serum B<sub>12</sub> and total serum homocysteine concentrations, were collected and measured in accordance with NHANES III procedures(5-7).

Per NHANES protocol, persons who were institutionalized, had haemophilia or had undergone chemotherapy for cancer within the previous four weeks were excluded from phlebotomy and thus from the present analysis. Serum B<sub>12</sub> concentrations were analysed with a commercially available radioprotein binding assay kit (Quantaphase II from Bio-Rad Laboratories, Hercules, CA, USA; during NHANES III, Phase 2). EDTA-treated plasma was not available from NHANES III and serum homocysteine concentration was measured by HPLC with fluorometric detection. Further survey procedure details, including how demographic, medical history and medication and supplement intakes were collected, may be found in the NHANES III plan and operation(5) and in the data documentation on the NHANES website (<http://www.cdc.gov/nchs/nhanes.htm>). In addition, pregnant females (*n* 167) and persons who reported taking antiretroviral prescription medication for HIV (*n* 7) were excluded because B<sub>12</sub> nutriture is altered in these populations(8,9).

## Analysis

There is no gold standard test or definition for B<sub>12</sub> deficiency. We used a conservative serum B<sub>12</sub> cut-off point of <148 pmol/l (200 pg/ml)(10) to define low B<sub>12</sub> concentration. Subjects with serum B<sub>12</sub><148 pmol/l and concomitant serum homocysteine >10.0 µmol/l (1.35 µg/l) were defined as biochemically defined B<sub>12</sub>-deficient.

The primary exposure variable of interest, B<sub>12</sub>-containing supplement use, was ascertained by calculating the total (single dose) supplemental B<sub>12</sub> intake among supplement users from the NHANES III PUVITMIN and SUPLCONC data sets, which define supplement use in this cohort. Supplement users were defined as having total oral B<sub>12</sub> supplement intake >0 µg. Supplement dose categories were defined as: 0 µg (non-users), >0 to 6 µg (89% of whom ingested 6 µg), >6 to 25 µg (88% of whom ingested 9–25 µg) and >25 µg. The categories were chosen such that the lowest category of use included the dose found in many over-the-counter multivitamin supplements (6 µg). The second category of supplement users includes the 25 µg amount found in many senior daily multivitamin supplements, and the highest category included the higher doses (100, 500, 1000 µg) found in high-dose B-vitamin supplements.

Predictors of B<sub>12</sub> and/or homocysteine concentrations which could potentially confound the analysis include age, race, impaired renal function, smoking(11), heavy alcohol intake(12,13),hypothyroidism(14) and folate deficiency. Whites tend to have lower serum B<sub>12</sub> concentrations than blacks or Mexican-Americans(15) and Mexican-American Hispanics tend to have lower homocysteine concentrations than non-Hispanic whites or non-Hispanic blacks(16). Except where noted, potential confounding exposure variables were dichotomized for the univariate analysis and multivariate logistic regression modelling.

After examining the distribution of cases within quartiles of age and ensuring adequate sample size, age was classified as a dichotomous variable to match the age recommendations discussed in the IOM report (18–50 years and >50 years)(1), and included as a continuous variable in the logistic regression models. Those participants who reported being current smokers of cigarettes, pipes or cigars were classified as current smokers. Study participants' average daily alcohol consumption was estimated from the NHANES survey alcohol consumption questions (number of drinks on drinking days×number of days per year drinking/365). If the number of days per year drinking was missing, the daily average was calculated by multiplying the number of drinks on drinking days by the number days per year with 9+ drinks, and then dividing by 365 days per year. Based on the US Department of Agriculture's dietary guidelines for alcohol (no more than one to two drinks of alcohol daily) (17,18) and the definition of heavy drinking given on the Centers for Disease Control and Prevention (CDC) alcohol fact sheet, participants whose calculated average daily alcohol consumption was three or more drinks were classified as 'heavy drinkers'(17,18).

Due to possible alteration in B<sub>12</sub> and/or homocysteine status in renal impairment, serum creatinine was included as a continuous variable in the multivariate analysis. Participants with thyroid-stimulating hormone concentration greater than 5 µIU/l were considered 'hypothyroid' for the present analysis(19). Race was classified into three groups, white, black and other, based on NHANES analysis guidelines and available crude sample size(20). Folate is a major determinant of homocysteine concentrations(7,21,22), and folate deficiency was therefore defined as red blood cell (RBC) folate <232 nmol/l (102.6 ng/ml; in home-examined participants, for whom RBC folate analysis was not done, serum folate <13.3 nmol/l (5.89 ng/ml))(6,23).

In all analyses,  $\alpha = 0.05$  was considered significant and 95% confidence intervals are reported. To account for the complex survey design, SURVEY procedures in the SAS

statistical software package version 9.1 (SAS Institute Inc., Cary, NC, USA) were used to estimate the population prevalence and prevalence ratio estimates. To calculate crude prevalence ratios, PROC SURVEY FREQ outcome prevalence estimates from the exposed (supplemented participants) are divided by the prevalence estimates in the unexposed (non-supplemented participants).

Unconditional logistic regression techniques were used to evaluate the univariate frequencies of each potential exposure variable and measures of association were computed, adjusting for supplement intake. Those exposures variables which were significantly related to the outcome variable were then included in a multivariate logistic regression model. Exposure variables were then removed from the model by backwards elimination and those exposures which altered the prevalence odds ratio by more than 10% were kept in the model. The prevalence of biochemical B<sub>12</sub> deficiency in the NHANES sample meets the rare disease assumption, and adjusted prevalence ratios are approximated from the multivariate logistic regression odds ratios using the method described by Zhang and Yu(24).

## Results

### Prevalence of biochemical B<sub>12</sub> deficiency

Applying the above criteria yielded a crude sample size of 8394 for the analysis of low B<sub>12</sub> and 7404 for the analysis of biochemical B<sub>12</sub> deficiency (Table 1). The overall estimated US adult population prevalence of biochemically defined B<sub>12</sub> deficiency was 1.6%. The prevalence was 1.2% for those aged 18–50 years and 2.5% for those aged >50 years. The prevalence of low serum B<sub>12</sub> concentrations was 3.2% for all adults, 2.6% for adults aged 18–50 years and 4.4% for those aged >50 years (Tables 1, 2 and 3).

### B<sub>12</sub>-containing supplement consumption and prevalence ratios

Supplement consumption was associated with lower prevalence of both low serum B<sub>12</sub> concentration and biochemically determined B<sub>12</sub> deficiency in the total population and in both age categories (18–50 and >50 years, Tables 1-3). Among the over-50s taking >0–6 µg or >6–25 µg, supplements reduced the occurrence of both outcomes by 60–70%. The prevalence was even lower among those consuming >25 µg.

Among all B<sub>12</sub>-supplement users, the distribution of total supplemental intakes was skewed, with a mean of 31.4 µg/d and a median of 6.0 µg/d (range 0.19–2075 µg/d). Of participants who consumed B<sub>12</sub>-containing supplements, 99.8% consumed at least 2.4 µg B<sub>12</sub>/d (the RDA suggested by the IOM) and 80.7% consumed 6–25 µg B<sub>12</sub>/d (the amounts most commonly found in multivitamin supplements). Of supplement users in the second supplement dose category, >0 to 6 µg, most (89%) participants took 6 µg, the dose found in 'regular' (non-geriatric) multivitamin supplements. Only 2.1% of supplement users took large doses (250 µg or more) of supplemental B<sub>12</sub> and no cases of biochemical B<sub>12</sub> deficiency occurred in supplement users taking >75 µg of B<sub>12</sub> daily.

### Univariate analysis and multivariate logistic regression modelling

Logistic regression analysis of each potentially confounding exposure variable (with adjustment for NHANES design and supplement status) revealed that age <50 years and 8 years or less of education were both significantly associated with biochemical B<sub>12</sub> deficiency. Odds ratio point estimates of biochemical B<sub>12</sub> deficiency were above unity for male gender, non-white or African-American race, as well as hypothyroid state, but none of these associations was significant. For each demographic exposure category (old and young

age categories, gender, white and black races, and all education levels) B<sub>12</sub> supplement consumption was associated with reduced odds of biochemical B<sub>12</sub> deficiency.

After adjusting for survey design, 17.9% of persons had elevated serum creatinine. However, the prevalence of low serum B<sub>12</sub> concentration and biochemically defined B<sub>12</sub> deficiency in persons with and without elevated creatinine (>1.1 mg/dl for females, >1.4 mg/dl for males) was not significantly different ( $P = 0.5$  for low B<sub>12</sub> and  $P = 0.09$  for biochemically determined B<sub>12</sub> deficiency). Similarly, the prevalence of low B<sub>12</sub> in participants aged >50 years with normal creatinine (4.1%) was not significantly different from that in older participants with elevated creatinine (5.2%,  $P = 0.26$ ).

Multivariate logistic regression model analysis revealed that hypothyroidism, heavy alcohol use and smoking status were not significant predictors of low serum B<sub>12</sub> or biochemical B<sub>12</sub> deficiency. Although lower educational level showed a statistically significant association with biochemical B<sub>12</sub> deficiency in the univariate analysis, removing it from the model did not change the prevalence ratio estimates. Similarly, removing serum creatinine concentration from the multivariate model did not affect the point estimates or confidence intervals.

## Discussion

Consistent with our a priori hypothesis, we found that intake of supplements providing 6 or 25 µg of vitamin B<sub>12</sub> was associated with reductions in the prevalence of biochemical B<sub>12</sub> deficiency and low serum B<sub>12</sub> concentrations in US adults (aged both 18 years and older and above 50 years). The majority of supplement users met or exceeded the IOM RDA of B<sub>12</sub> intake (2.4 µg) because the supplemental B<sub>12</sub> quantities commonly found in over-the-counter multivitamin supplements range from 6 to 25 µg. Although we cannot conclude that there is a significant benefit to consuming 6 µg v. 2.4 µg supplement, or 25 µg v. 6 µg supplement, our data suggest that the prevalence of B<sub>12</sub> deficiency (defined as low serum B<sub>12</sub> or biochemical deficiency) among persons aged >50 years who consume B<sub>12</sub> supplements is half to two-thirds the prevalence in persons who do not consume B<sub>12</sub> supplements; the difference between prevalence for supplement users v. non-users in persons aged 18–50 years is about a third. The prevalence reduction was even higher in persons taking >25 µg supplemental B<sub>12</sub> daily.

Although there was overlap in the confidence intervals, the trend we observed of decreasing prevalence of low serum B<sub>12</sub> and biochemical B<sub>12</sub> deficiency with increasing B<sub>12</sub> supplement dose is consistent with results seen in vitamin supplement intervention trials in the elderly(25-28). In three of these studies, 10–500 µg of supplemental B<sub>12</sub> increased serum B<sub>12</sub> concentrations by 18.5–166 pmol/l (25–225 pg/ml)(25-27). Along with our findings, these studies indicate that daily doses of supplemental B<sub>12</sub> as low as 6 µg can shift the distribution of B<sub>12</sub> concentrations upwards in the general population and reduce biochemical B<sub>12</sub> deficiency prevalence.

About 2% of people aged >50 years will still have low B<sub>12</sub> concentrations despite consuming 6–25 µg of synthetic B<sub>12</sub>. Daily consumption of more than 75 µg B<sub>12</sub> may nearly eliminate low B<sub>12</sub> and biochemical B<sub>12</sub> deficiency, but more research is needed to determine how much supplement is needed by those individuals for whom 6 or 25 µg/d does not prevent low B<sub>12</sub> or biochemical B<sub>12</sub> deficiency.

Given the success of a proof of concept study by Winkels *et al.*(29), our data suggest that a B<sub>12</sub> fortification programme that delivered 6 to 25 µg of synthetic vitamin B<sub>12</sub> to most of the population may be highly effective in decreasing the prevalence of both low B<sub>12</sub> concentrations and biochemically defined B<sub>12</sub> deficiency. Were such a programme

implemented, there would be fewer patients with low serum B<sub>12</sub> and biochemically defined B<sub>12</sub> deficiency for clinicians to investigate. Several studies have reported that adults with suboptimal B<sub>12</sub> status have poorer cognitive functioning or could improve with supplementation(30-32). However, results from other B-vitamin supplement trials have failed to yield clinically measurable benefit(33,34). Further investigations are required to determine whether improving B<sub>12</sub> status with fortification would result in improved functional status or clinical benefit.

The prevalence of low B<sub>12</sub> in NHANES III (3.2%) was similar to that previously observed in NHANES III (3%)(7), but was lower than that observed in other cohort studies in the USA (5.3–14.5%)(2,3,35). However, those studies focused on racially homogeneous, elderly cohorts rather than the entire adult population, and they had relatively small sample sizes. Increased age and white race have both been associated with lower B<sub>12</sub> concentrations(15) and estimates from predominantly older, white cohorts may therefore overestimate the national prevalence of B<sub>12</sub> deficiency. The higher cut-off points for defining low B<sub>12</sub> (<221 pmol/l or 300 pg/ml) in three non-institutionalized adult populations(2,3,35) and two outpatient source populations(35,36) may also explain the lower prevalence of B<sub>12</sub> deficiency in our study population. Overall, our study supports the results of previous studies, but widens their generalizability for the US population.

We did not examine the limited physical examination data in NHANES III addressing whether persons with biochemically determined B<sub>12</sub> deficiency exhibited clinical signs of B<sub>12</sub> deficiency. Although 6 to 25 µg of supplemental B<sub>12</sub> appears to prevent most preclinical deficiency indicators in those aged 18 years and older, higher doses may be warranted in persons over 50 years old or with clinical signs such as anaemia and/or neuropathy(25). We remind clinicians that patients with clinical symptoms consistent with clinical B<sub>12</sub> deficiency disease should be worked up and treated with an appropriate dose of vitamin B<sub>12</sub>.

The primary strength of our analysis is that it examines a nationally representative population sample of ambulatory persons in the USA. With the largest sample size reported to date (over 8000 participants), we were able to adjust prevalence ratio estimates for the most common potential confounding exposures, including age, race, educational level, renal insufficiency and folate status.

A potential weakness of our study is the absence of MMA data to use as a criterion for biochemical determination of B<sub>12</sub> deficiency. An elevated MMA concentration is typically considered a more specific confirmatory indicator of B<sub>12</sub> deficiency than elevated homocysteine. Because NHANES III MMA data are not publicly available, we chose a homocysteine cut-off that is within the range expected in B<sub>12</sub>- and folate-replete individuals(23,37). Another potential weakness of our study is the incomplete exclusion of HIV-positive individuals(38). Given that low B<sub>12</sub> concentrations are reportedly less likely in HIV after antiretroviral therapy is started(8), those excluded were least likely to be cases. HIV patients use supplements at a higher rate (over 70%) than the general population and the participants not excluded would likely increase the number of exposed controls, potentially resulting in bias towards the null. However, at the estimated prevalence of HIV of 0.34%(38), unidentified HIV-positive individuals would have virtually no impact on the prevalence of B<sub>12</sub> deficiency and prevalence ratio estimates in our data.

In conclusion, consumption of supplements with 6 or 25 µg of vitamin B<sub>12</sub> was associated with about a 50% reduction in the prevalence of low B<sub>12</sub> concentrations and biochemically defined B<sub>12</sub> deficiency. These findings suggest that approximately three million cases of low serum B<sub>12</sub> and biochemical B<sub>12</sub> deficiency in the US population may be prevented if all adults consumed 6–25 µg B<sub>12</sub>/d from supplements or fortified foods. We also found low

vitamin B<sub>12</sub> concentrations and biochemically defined vitamin B<sub>12</sub> deficiency in 1–2% of persons aged >50 years who were consuming 6 to 25 µg of B<sub>12</sub> from supplements. This relatively high prevalence in older supplement users suggests that the current RDA of 2.4 µg/d may be too low for this age group. As Berry *et al.* noted, people who have low serum concentrations of B<sub>12</sub> may have problems in absorbing free B<sub>12</sub>(39) and have the preclinical pernicious anaemia that Carmel *et al.* have identified among those over 50 years of age(10). Clinicians should be vigilant for subtle signs and symptoms of pernicious anaemia and clinical vitamin B<sub>12</sub> deficiency, particularly in older adults who have low concentration of serum B<sub>12</sub> and/or biochemically defined vitamin B<sub>12</sub> deficiency in spite of consuming vitamin B<sub>12</sub> supplements, and manage these patients appropriately.

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Prevalence and unadjusted and adjusted prevalence ratio of low serum vitamin B<sub>12</sub> concentration and biochemically defined vitamin B<sub>12</sub> deficiency by B<sub>12</sub> supplement dose for all persons aged 18 years and older in NHANES III (Third National Health and Nutrition Examination Survey; Phase 2, 1991–4)

**Table 1**

B <sub>12</sub> -containing supplement use	Low serum B <sub>12</sub> <sup>*</sup>				Biochemically defined B <sub>12</sub> deficiency <sup>†</sup>			
	Prevalence (%)	95% CI	Unadj. PR	Adj. PR <sup>‡</sup> 95% CI	Prevalence (%)	95% CI	Unadj. PR	Adj. PR <sup>§</sup> 95% CI
All adults (n 8394) <sup>¶</sup>	3.2	2.2, 4.2	–	–	1.6	1.1, 2.1	–	–
Supplement non-users (n 6192)	3.9	2.8, 5.0	(referent)	–	2.2	1.6, 2.7	(referent)	–
Supplement users								
Any amount (n 2202)	1.7 <sup>¶¶</sup>	0.7, 2.8	0.4	0.6 0.3, 1.0	0.4 <sup>**</sup>	0.0, 0.9	0.2	0.3 0.1, 0.8
>0 to 6 µg (n 1377)	1.8	0.6, 3.0	0.5	0.6 0.3, 1.1	0.5	0.0, 1.2	0.2	0.3 0.1, 1.3
>6 to 25 µg (n 515)	2.0	0.0, 4.7	0.5	0.6 0.2, 2.0	0.5	0.0, 1.5	0.2	0.3 0.1, 1.6
>25 µg (n 310)	1.1	0.0, 3.0	0.3	0.4 0.1, 2.0	0.2	0.0, 0.6	0.1	0.0 <sup>††</sup> 0.0, 1.2

Unadj., unadjusted; Adj., adjusted; PR, prevalence ratio.

The PROCURVEY FREQ procedure in SAS version 9.1 (SAS Institute Inc., Cary, NC, USA) and the NHANES III, Phase 2 data sets were used to obtain these US population prevalence proportion estimates in adults aged 18 years and older.

<sup>\*</sup> Low serum B<sub>12</sub>: serum B<sub>12</sub> < 148 pmol/l.

<sup>†</sup> Biochemically defined B<sub>12</sub> deficiency: serum B<sub>12</sub> < 148 pmol/l with serum homocysteine > 10 µmol/l.

<sup>‡</sup> Adj. PR=prevalence ratio adjusted for age (continuous), gender, race (black race or other), folate deficiency status and education (<8 years v. >8 years).

<sup>§</sup> Adj. PR=prevalence ratio adjusted for age (continuous), gender, race (black race or other), folate deficiency status, serum creatinine and education (<8 years v. >8 years).

<sup>¶</sup> Nine hundred and seventy-two participants had missing homocysteine measurements, so estimates are based on 7404 survey participants for the bio-chemical B<sub>12</sub> deficiency analysis and on 8394 survey participants for the low B<sub>12</sub> analysis.

<sup>¶¶</sup> Rao–Scott *P* value=0.001 compared with supplement non-users.

<sup>\*\*</sup> Rao–Scott *P* value=0.0002 compared with supplement non-users.

<sup>††</sup> PR=0.02, rounded to 0.0.

Prevalence and unadjusted and adjusted prevalence ratio of low serum vitamin B<sub>12</sub> concentration and biochemically defined vitamin B<sub>12</sub> deficiency by B<sub>12</sub> supplement dose for adults aged 18–50 years in NHANES III (Third National Health and Nutrition Examination Survey; Phase 2, 1991–4)

Table 2

B <sub>12</sub> -containing supplement use	Low serum B <sub>12</sub> <sup>*</sup>				Biochemically defined B <sub>12</sub> deficiency <sup>†</sup>				
	Prevalence (%)	95% CI	Unadj. PR	Adj. PR <sup>‡</sup>	Prevalence (%)	95% CI	Unadj. PR	Adj. PR <sup>§</sup>	95% CI
Adults aged 18–50 years ( <i>n</i> 4944) <sup>¶</sup>	2.6	1.4, 3.8	–	–	1.2	0.5, 1.8	–	–	–
Supplement non-users ( <i>n</i> 3768)	2.9	1.6, 4.3	(referent)	–	1.5	0.7, 2.3	(referent)	–	–
Supplement users									
Any amount ( <i>n</i> 1176)	1.9 <sup>¶¶</sup>	0.5, 3.3	0.7	0.8	0.4 <sup>**</sup>	0.0, 1.1	0.3	0.5	0.1, 2.2
>0 to 6 µg ( <i>n</i> 754)	1.8 (10 cases)	0.3, 3.2	0.6	0.8	0.6 (2 cases)	0.0, 1.7	0.4	0.6	0.1, 2.9
>6 to 25 µg ( <i>n</i> 261)	2.5 (3 cases)	0.0, 6.0	0.9	1.0	0 (no cases)	n/a	n/a	<0.001	n/a
>25 µg ( <i>n</i> 161)	1.6 (2 cases)	0.0, 4.3	0.6	0.7	0.3 (1 case)	0.0, 0.9	0.2	0.3	0.0, 3.5

Unadj., unadjusted; Adj., adjusted; PR, prevalence ratio; n/a, not applicable.

The PROC SURVEY FREQ procedure in SAS version 9.1 (SAS Institute Inc., Cary, NC, USA) and the NHANES III, Phase 2 data sets were used to obtain these US population prevalence proportion estimates in adults aged 18–50 years.

\* Low serum B<sub>12</sub>: serum B<sub>12</sub> < 148 pmol/l.

<sup>†</sup> Biochemically defined B<sub>12</sub> deficiency: serum B<sub>12</sub> < 148 pmol/l with serum homocysteine > 10 µmol/l.

<sup>‡</sup> Adj. PR = prevalence ratio adjusted for age (continuous), gender, race (black race or other), folate deficiency status and education (< 8 years v. > 8 years).

<sup>§</sup> Adj. PR = prevalence ratio adjusted for age (continuous), gender, race (black race or other), folate deficiency status, serum creatinine and education (< 8 years v. > 8 years).

<sup>¶</sup> Estimates are based on 7404 survey participants for the biochemical B<sub>12</sub> deficiency analysis and on 8376 survey participants for the low B<sub>12</sub> analysis.

<sup>¶¶</sup> Rao–Scott *P* value = 0.2 compared with supplement non-users.

\*\* Rao–Scott *P* value = 0.06 compared with supplement non-users.

Prevalence and unadjusted and adjusted prevalence ratio of low serum vitamin B<sub>12</sub> concentration and biochemically defined vitamin B<sub>12</sub> deficiency by B<sub>12</sub> supplement dose for adults aged >50 years in NHANES III (Third National Health and Nutrition Examination Survey; Phase 2, 1991–4)

Table 3

B <sub>12</sub> -containing supplement use	Low serum B <sub>12</sub> <sup>*</sup>				Biochemically defined B <sub>12</sub> deficiency <sup>†</sup>			
	Prevalence (%)	95% CI	Unadj. PR	Adj. PR <sup>‡</sup> 95% CI	Prevalence (%)	95% CI	Unadj. PR	Adj. PR <sup>‡</sup> 95% CI
Adults aged >50 years (n 3450)	4.4	3.1, 5.7	—	—	2.5	1.7, 3.4	—	—
Supplement non-users (n 2424)	5.9	4.1, 7.7	(referent)	—	3.7	2.5, 4.8	(referent)	—
Supplement users								
Any amount (n 1026)	1.5 <sup>§</sup>	0.0, 3.2	0.3	0.3 0.1, 1.7	0.4 <sup>¶</sup>	0, 1.1	0.3	0.4 0.1, 1.7
>0 to 6 µg (n 623)	1.9 (5 cases)	0.0, 4.7	0.3	0.4 0.1, 1.6	0.1 (2 cases)	0.0, 0.3	0.2	0.5 0.1, 3.0
>6 to 25 µg (n 254)	1.2 (3 cases)	0.0, 3.4	0.2	0.3 0.0, 1.3	1.3 (2 cases)	0.0, 3.9	0.2	0.4 0.1, 2.4
>25 µg (n 149)	0.2 (1 case)	0.0, 0.5	0.0 <sup>¶¶</sup>	0.0 <sup>¶¶</sup> 0.0, 0.3	0.0 <sup>**</sup> (no cases)	—	0.0 <sup>**</sup> (no cases)	<0.001 —

Unadj., unadjusted; Adj., adjusted; PR, prevalence ratio.

The PROC SURVEY FREQ procedure in SAS version 9.1 (SAS Institute Inc., Cary, NC, USA) and the NHANES III, Phase 2 data sets were used to obtain these US population prevalence proportion estimates in adults aged >50 years.

\* Low serum B<sub>12</sub>: serum B<sub>12</sub> <148 pmol/l.

<sup>†</sup> Biochemically defined B<sub>12</sub> deficiency: serum B<sub>12</sub> <148 pmol/l with serum homocysteine >10 µmol/l.

<sup>‡</sup> Adj. PR=prevalence ratio adjusted for age (continuous) gender, race (black race or other) and folate deficiency status.

<sup>§</sup> Rao–Scott *P* value=0.001 compared with supplement non-users.

<sup>¶</sup> Rao–Scott *P* value=0.0002 compared with supplement non-users.

<sup>¶¶</sup> Point estimate for PR was 0.03, rounded to 0.0.

\*\* There were no cases of biochemical B<sub>12</sub> deficiency in the 149 survey participants over age 50 who were taking >25 µg of supplemental B<sub>12</sub>.