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Association between vitamin B_{12}-containing supplement consumption and prevalence of biochemically defined B_{12} 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_{12} (B_{12})-containing supplement use, low B_{12} concentrations and biochemically defined B_{12} 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_{12} concentration (<148 pmol/l) and biochemical B_{12} deficiency (serum $B_{12} < 148$ 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_{12} concentrations were less prevalent among persons consuming B_{12}-containing supplements ($P = 0{\cdot}001$) with an adjusted prevalence ratio of 0·6 (95% CI 0·3, 1·0). Biochemical B_{12} deficiency showed a similar trend ($P = 0{\cdot}0002$), 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_{12} and biochemical deficiency was proportionally higher.

Conclusions: Consumption of B_{12}-containing supplements was associated with at least 50% lower prevalence of both low serum B_{12} and biochemical B_{12} deficiency in a nationally representative sample of US adults, suggesting increased consumption of B_{12} from supplements or from fortified foods may reduce the prevalence of B_{12} deficiency. Additionally, the current Recommended Daily Allowance for B_{12} of 2·4 μg may be insufficient for those aged >50 years.

Keywords
Biochemical B_{12} deficiency
Cobalamin deficiency
NHANES III
Vitamin B_{12}
Vitamin supplementation

The Institute of Medicine (IOM) extensively reviewed the available data on vitamin B_{12} (B_{12}) deficiency and requirements, and has identified biochemical B_{12} deficiency and effective methods to reduce risk of B_{12} 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_{12} in older individuals, the IOM recommended adults over the age of 50 years meet the RDA for B_{12} of 2·4 μg by consuming synthetic vitamin B_{12} in B_{12}-fortified foods or B_{12} supplements[1]. Previously, three population-based studies examined biochemical indicators of B_{12} deficiency (serum B_{12} and/or homocysteine and methylmalonic acid (MMA) concentrations) in relation to vitamin supplement status and concluded that B_{12}-containing supplements may protect against B_{12} 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_{12} supplement use and biochemically defined B_{12} deficiency in a large, nationally representative population sample of the US adult population.

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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 oversampled 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_{12} and homocysteine are available (*n* 8394). Laboratory measurements, including serum B_{12} 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_{12} 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_{12} nutriture is altered in these populations[8,9].

Analysis

There is no gold standard test or definition for B_{12} deficiency. We used a conservative serum B_{12} cut-off point of $<$148 pmol/l (200 pg/ml)[10] to define low B_{12} concentration. Subjects with serum $B_{12} < 148$ pmol/l and concomitant serum homocysteine $>$10·0 μmol/l (1·35 mg/l) were defined as biochemically defined B_{12}-deficient.

The primary exposure variable of interest, B_{12}-containing supplement use, was ascertained by calculating the total (single dose) supplemental B_{12} 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_{12} 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_{12} 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_{12} 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 $>$50 years)[1], and included as a continuous variable in the logistic regression models. Those participants who reported being current smokers of cigarettes, pipes or cigars were classified as current smokers. Study participants' average daily alcohol consumption was estimated from the NHANES survey alcohol consumption questions (number of drinks on drinking days$\times$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_{12} 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, $\alpha = 0{\cdot}05$ 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, PROCSURVEY 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_{12} 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_{12} deficiency

Applying the above criteria yielded a crude sample size of 8394 for the analysis of low B_{12} and 7404 for the analysis of biochemical B_{12} deficiency (Table 1). The overall estimated US adult population prevalence of biochemically defined B_{12} 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_{12} 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_{12}-containing supplement consumption and prevalence ratios

Supplement consumption was associated with lower prevalence of both low serum B_{12} concentration and biochemically determined B_{12} deficiency in the total population and in both age categories (18–50 and >50 years, Tables 1–3). Among the over-50 s 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_{12}-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_{12}-containing supplements, 99·8 % consumed at least 2·4 μg B_{12}/d (the RDA suggested by the IOM) and 80·7 % consumed 6–25 μg B_{12}/d (the amounts most commonly found in multivitamin supplements). Of supplement users in the second supplement

Table 1 Prevalence and unadjusted and adjusted prevalence ratio of low serum vitamin B_{12} concentration and biochemically defined vitamin B_{12} deficiency by B_{12} supplement dose for all persons aged 18 years and older in NHANES III (Third National Health and Nutrition Examination Survey; Phase 2, 1991–4)

	Low serum B_{12}*					Biochemically defined B_{12} deficiency†				
B_{12}-containing supplement use	Prevalence (%)	95 % CI	Unadj. PR	Adj. PR‡	Adj. 95 % CI	Prevalence (%)	95 % CI	Unadj. PR	Adj. PR§	95 % CI
All adults (*n* 8394‖)	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¶	0·7, 2·8	0·4	0·6	0·3, 1·0	0·4**	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††	0·0, 1·2

Unadj., unadjusted; Adj., adjusted; PR, prevalence ratio.
The PROCSURVEY 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.
*Low serum B_{12}: serum B_{12} < 148 pmol/l.
†Biochemically defined B_{12} deficiency: serum B_{12} < 148 pmol/l with serum homocysteine > 10 μmol/l.
‡Adj. PR = prevalence ratio adjusted for age (continuous), gender, race (black race or other), folate deficiency status and education (<8 years *v.* >8 years).
§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).
‖Nine hundred and seventy-two participants had missing homocysteine measurements, so estimates are based on 7404 survey participants for the biochemical B_{12} deficiency analysis and on 8394 survey participants for the low B_{12} analysis.
¶Rao–Scott *P* value = 0·001 compared with supplement non-users.
**Rao–Scott *P* value = 0·0002 compared with supplement non-users.
††PR = 0·02, rounded to 0·0.

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

	Low serum B_{12}*					Biochemically defined B_{12} deficiency†				
B_{12}-containing supplement use	Prevalence (%)	95 % CI	Unadj. PR	Adj. PR‡	Adj. 95 % CI	Prevalence (%)	95 % CI	Unadj. PR	Adj. PR§	95 % CI
Adults aged 18–50 years (*n* 4944‖)	2·6	1·4, 3·8	–	–	–	1·2	0·5, 1·8	–	–	–
Supplement non-users (*n* 3768)	2·9	1·6, 4·3	(referent)	–	–	1·5	0·7, 2·3	(referent)	–	–
Supplement users										
Any amount (*n* 1176)	1·9¶	0·5, 3·3	0·7	0·8	0·4, 1·7	0·4**	0·0, 1·1	0·3	0·5	0·1, 2·2
>0 to 6 μg (*n* 754)	1·8 (10 cases)	0·3, 3·2	0·6	0·8	0·4, 1·5	0·6 (2 cases)	0·0, 1·7	0·4	0·6	0·1, 2·9
>6 to 25 μg (*n* 261)	2·5 (3 cases)	0·0, 6·0	0·9	1·0	0·3, 3·3	0 (no cases)	n/a	n/a	<0·001	n/a
>25 μg (*n* 161)	1·6 (2 cases)	0·0, 4·3	0·6	0·7	0·1, 4·8	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 PROCSURVEY 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_{12}: serum $B_{12} < 148$ pmol/l.
†Biochemically defined B_{12} deficiency: serum $B_{12} < 148$ pmol/l with serum homocysteine > 10 μmol/l.
‡Adj. PR = prevalence ratio adjusted for age (continuous), gender, race (black race or other), folate deficiency status and education (<8 years *v.* >8 years).
§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).
‖Estimates are based on 7404 survey participants for the biochemical B_{12} deficiency analysis and on 8376 survey participants for the low B_{12} analysis.
¶Rao–Scott *P* value = 0·2 compared with supplement non-users.
**Rao–Scott *P* value = 0·06 compared with supplement non-users.

Table 3 Prevalence and unadjusted and adjusted prevalence ratio of low serum vitamin B_{12} concentration and biochemically defined vitamin B_{12} deficiency by B_{12} supplement dose for adults aged >50 years in NHANES III (Third National Health and Nutrition Examination Survey; Phase 2, 1991–4)

	Low serum B_{12}*					Biochemically defined B_{12} deficiency†				
B_{12}-containing supplement use	Prevalence (%)	95 % CI	Unadj. PR	Adj. PR‡	Adj. 95 % CI	Prevalence (%)	95 % CI	Unadj. PR	Adj. PR‡	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§	0·0, 3·2	0·3	0·3	0·1, 1·7	0·4¶	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‖	0·0‖	0·0, 0·3	0·0** (no cases)	–	0·0** (no cases)	<0·001	–

Unadj., unadjusted; Adj., adjusted; PR, prevalence ratio.
The PROCSURVEY 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_{12}: serum $B_{12} < 148$ pmol/l.
†Biochemically defined B_{12} deficiency: serum $B_{12} < 148$ pmol/l with serum homocysteine > 10 μmol/l.
‡Adj. PR = prevalence ratio adjusted for age (continuous) gender, race (black race or other) and folate deficiency status.
§Rao–Scott *P* value = 0·001 compared with supplement non-users.
‖Rao–Scott *P* value = 0·0002 compared with supplement non-users.
¶Point estimate for PR was 0·03, rounded to 0·0.
**There were no cases of biochemical B_{12} deficiency in the 149 survey participants over age 50 who were taking >25 μg of supplemental B_{12}.

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_{12} and no cases of biochemical B_{12} deficiency occurred in supplement users taking >75 μg of B_{12} 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

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significantly associated with biochemical B_{12} deficiency. Odds ratio point estimates of biochemical B_{12} 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_{12} supplement consumption was associated with reduced odds of biochemical B_{12} deficiency.

After adjusting for survey design, 17·9% of persons had elevated serum creatinine. However, the prevalence of low serum B_{12} concentration and biochemically defined B_{12} 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_{12} and $P = 0·09$ for biochemically determined B_{12} deficiency). Similarly, the prevalence of low B_{12} 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_{12} or biochemical B_{12} deficiency. Although lower educational level showed a statistically significant association with biochemical B_{12} 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_{12} was associated with reductions in the prevalence of biochemical B_{12} deficiency and low serum B_{12} 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_{12} intake (2·4 μg) because the supplemental B_{12} 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_{12} deficiency (defined as low serum B_{12} or biochemical deficiency) among persons aged >50 years who consume B_{12} supplements is half to two-thirds the prevalence in persons who do not consume B_{12} 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_{12} daily.

Although there was overlap in the confidence intervals, the trend we observed of decreasing prevalence of low serum B_{12} and biochemical B_{12} deficiency with increasing B_{12} 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_{12} increased serum B_{12} 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_{12} as low as 6 μg can shift the distribution of B_{12} concentrations upwards in the general population and reduce biochemical B_{12} deficiency prevalence.

About 2% of people aged >50 years will still have low B_{12} concentrations despite consuming 6–25 μg of synthetic B_{12}. Daily consumption of more than 75 μg B_{12} may nearly eliminate low B_{12} and biochemical B_{12} 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_{12} or biochemical B_{12} deficiency.

Given the success of a proof of concept study by Winkels *et al.*[29], our data suggest that a B_{12} fortification programme that delivered 6 to 25 μg of synthetic vitamin B_{12} to most of the population may be highly effective in decreasing the prevalence of both low B_{12} concentrations and biochemically defined B_{12} deficiency. Were such a programme implemented, there would be fewer patients with low serum B_{12} and biochemically defined B_{12} deficiency for clinicians to investigate. Several studies have reported that adults with suboptimal B_{12} 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_{12} status with fortification would result in improved functional status or clinical benefit.

The prevalence of low B_{12} 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_{12} concentrations[15] and estimates from predominantly older, white cohorts may therefore overestimate the national prevalence of B_{12} deficiency. The higher cut-off points for defining low B_{12} (<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_{12} 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_{12} deficiency exhibited clinical signs of B_{12} deficiency. Although 6 to 25 μg of supplemental

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B_{12} 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_{12} deficiency disease should be worked up and treated with an appropriate dose of vitamin B_{12}.

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_{12} deficiency. An elevated MMA concentration is typically considered a more specific confirmatory indicator of B_{12} 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_{12}- 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_{12} 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_{12} deficiency and prevalence ratio estimates in our data.

In conclusion, consumption of supplements with 6 or 25 μg of vitamin B_{12} was associated with about a 50 % reduction in the prevalence of low B_{12} concentrations and biochemically defined B_{12} deficiency. These findings suggest that approximately three million cases of low serum B_{12} and biochemical B_{12} deficiency in the US population may be prevented if all adults consumed 6–25 μg B_{12}/d from supplements or fortified foods. We also found low vitamin B_{12} concentrations and biochemically defined vitamin B_{12} deficiency in 1–2 % of persons aged >50 years who were consuming 6 to 25 μg of B_{12} 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_{12} may have problems in absorbing free B_{12}[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_{12} deficiency, particularly in older adults who have low concentration of serum B_{12} and/or biochemically defined vitamin B_{12} deficiency in spite of consuming vitamin B_{12} supplements, and manage these patients appropriately.

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