Association of Biochemical B_{12} Deficiency With Metformin Therapy and Vitamin B_{12} Supplements

The National Health and Nutrition Examination Survey, 1999–2006

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OBJECTIVE—To describe the prevalence of biochemical B_{12} deficiency in adults with type 2 diabetes taking metformin compared with those not taking metformin and those without diabetes, and explore whether this relationship is modified by vitamin B_{12} supplements.

RESEARCH DESIGN AND METHODS—Analysis of data on U.S. adults ≥50 years of age with (n = 1,621) or without type 2 diabetes (n = 6,867) from the National Health and Nutrition Examination Survey (NHANES), 1999–2006. Type 2 diabetes was defined as clinical diagnosis after age 30 without initiation of insulin therapy within 1 year. Those with diabetes were classified according to their current metformin use. Biochemical B_{12} deficiency was defined as serum B_{12} concentrations ≤148 pmol/L and borderline deficiency was defined as >148 to ≤221 pmol/L.

RESULTS—Biochemical B_{12} deficiency was present in 5.8% of those with diabetes using metformin compared with 2.4% of those not using metformin (P = 0.0026) and 3.3% of those without diabetes (P = 0.0002). Among those with diabetes, metformin use was associated with biochemical B_{12} deficiency (adjusted odds ratio 2.92; 95% CI 1.26–6.78). Consumption of any supplement containing B_{12} was not associated with a reduction in the prevalence of biochemical B_{12} deficiency among those with diabetes, whereas consumption of any supplement containing B_{12} was associated with a two-thirds reduction among those without diabetes.

CONCLUSIONS—Metformin therapy is associated with a higher prevalence of biochemical B_{12} deficiency. The amount of B_{12} recommended by the Institute of Medicine (IOM) (2.4 μg/day) and the amount available in general multivitamins (6 μg) may not be enough to correct this deficiency among those with diabetes.

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It is well known that the risks of both type 2 diabetes and B_{12} deficiency increase with age (1,2). Recent national data estimate a 21.2% prevalence of diagnosed diabetes among adults ≥65 years of age and a 6 and 20% prevalence of biochemical B_{12} deficiency (serum B_{12} <148 pmol/L) and borderline deficiency (serum B_{12} ≥148–221 pmol/L) among adults ≥60 years of age (3,4).

The diabetes drug metformin has been reported to cause a decrease in serum B_{12} concentrations. In the first efficacy trial, DeFronzo and Goodman (5) demonstrated that although metformin offers superior control of glycosylated hemoglobin levels and fasting plasma glucose levels compared with glyburide, serum B_{12} concentrations were lowered by 22% compared with placebo, and 29% compared with glyburide therapy after 29 weeks of treatment. A recent, randomized control trial designed to examine the temporal relationship between metformin and serum B_{12} found a 19% reduction in serum B_{12} levels compared with placebo after 4 years (6). Several other randomized control trials and cross-sectional surveys reported reductions in B_{12} ranging from 9 to 52% (7–16). Although classical B_{12} deficiency presents with clinical symptoms such as anemia, peripheral neuropathy, depression, and cognitive impairment, these symptoms are usually absent in those with biochemical B_{12} deficiency (17).

Several researchers have made recommendations to screen those with type 2 diabetes on metformin for serum B_{12} levels (6,7,14–16,18–21). However, no formal recommendations have been provided by the medical community or the U.S. Prevention Services Task Force. High-dose B_{12} injection therapy has been successfully used to correct the metformin-induced decline in serum B_{12} (15,21,22). The use of B_{12} supplements among those with type 2 diabetes on metformin in a nationally representative sample and their potentially protective effect against biochemical B_{12} deficiency has not been reported. It is therefore the aim of the current study to use the nationally representative National Health and Nutrition Examination Survey (NHANES) population to determine the prevalence of biochemical B_{12} deficiency among those with type 2 diabetes ≥50 years of age taking metformin compared with those with type 2 diabetes not taking metformin and those without diabetes, and to explore how these relationships are modified by B_{12} supplement consumption.

RESEARCH DESIGN AND METHODS

Design overview

NHANES is a nationally representative sample of the noninstitutionalized U.S. population with targeted oversampling of U.S. adults ≥60 years of age, African Americans, and Hispanics. Details of these surveys have been described elsewhere (23). All participants gave written informed consent, and the survey protocol was approved by a human subjects review board.

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Setting and participants

Our study included adults ≥50 years of age from NHANES 1999–2006. Participants with positive HIV antibody test results, high creatinine levels (>1.7 mg/dL for men and >1.5 mg/dL for women), and prescription B_{12} injections were excluded from the analysis. Participants who reported having prediabetes or borderline diabetes (n = 226) were removed because they could not be definitively grouped as having or not having type 2 diabetes. We also excluded pregnant women, those with type 1 diabetes, and those without diabetes taking metformin. Based on clinical aspects described by the American Diabetes Association and previous work in NHANES, those who were diagnosed before the age of 30 and began insulin therapy within 1 year of diagnosis were classified as having type 1 diabetes (24,25). Type 2 diabetes status in adults was dichotomized as yes/no. Participants who reported receiving a physician's diagnosis after age 30 (excluding gestational diabetes) and did not initiate insulin therapy within 1 year of diagnosis were classified as having type 2 diabetes.

Outcomes and follow-up

The primary outcome was biochemical B_{12} deficiency determined by serum B_{12} concentrations. Serum B_{12} levels were quantified using the Quantaphase II folate/vitamin B_{12} radioassay kit from Bio-Rad Laboratories (Hercules, CA). We defined biochemical B_{12} deficiency as serum levels ≤148 pmol/L, borderline deficiency as serum B_{12} >148 to ≤221 pmol/L, and normal as >221 pmol/L (26).

The main exposure of interest was metformin use. Using data collected in the prescription medicine questionnaire, those with type 2 diabetes were classified as currently using metformin therapy (alone or in combination therapy) versus those not currently using metformin. Length of metformin therapy was used to assess the relationship between duration of metformin therapy and biochemical B_{12} deficiency. In the final analysis, two control groups were used to allow the comparison of those with type 2 diabetes taking metformin with those with type 2 diabetes not taking metformin and those without diabetes.

To determine whether the association between metformin and biochemical B_{12} deficiency is modified by supplemental B_{12} intake, data from the dietary supplement questionnaire were used. Information regarding the dose and frequency was used to calculate average daily supplemental B_{12} intake. We categorized supplemental B_{12} intake as 0 μg (no B_{12} containing supplement), >0–6 μg, >6–25 μg, and >25 μg. The lower intake group, >0–6 μg, includes 6 μg, the amount of vitamin B_{12} typically found in over-the-counter multivitamins, and 2.4 μg, the daily amount the IOM recommends for all adults ≥50 years of age to consume through supplements or fortified food (1). The next group, >6–25 μg, includes 25 μg, the amount available in many multivitamins marketed toward senior adults. The highest group contains the amount found in high-dose B-vitamin supplements.

Table 1—*Demographic and biological characteristics of U.S. adults ≥50 years of age: NHANES 1999–2006*

	Type 2 diabetes				
	Metformin users N = 575	Nonmetformin users N = 1,046	P value*	Without diabetes N = 6,867	P value*
Age, mean (SE)	63.4 (0.5)	66.4 (0.5)	<0.0001	63.4 (0.2)	0.9446
Male, n (%)	293 (50.3%)	507 (43.7%)	0.0848	3,382 (45.5%)	0.0961
Race, n (%)					
Non-Hispanic white	226 (66.7%)	415 (65.9%)	0.1820	4,177 (81.3%)	<0.0001
Non-Hispanic black	135 (14.6%)	272 (15.4%)		1,110 (7.9%)	
Hispanic	188 (10.9%)	323 (13.3%)		1,387 (7.1%)	
Other	26 (7.8%)	36 (5.5%)		193 (3.7%)	
BMI (kg/m^2), n (%)					
Normal (<25)	87 (15.0%)	178 (18.3%)	0.0987	2,059 (31.5%)	<0.0001
Overweight (25 to <30)	196 (29.5%)	361 (33.6%)		2,546 (37.8%)	
Obese (>30)	269 (55.5%)	441 (48.1%)		1,994 (30.7%)	
Years with diabetes, mean (SE)	12.0 (0.8)	14.1 (0.6)	0.0207		
Insulin use, n (%)	90 (15.4%)	318 (32.3%)	<0.0001		
Take folic acid, n (%)	201 (39.6%)	336 (38.1%)	0.6359	2,873 (47.3%)	0.0069
Take vitamin B_{12}, n (%)	209 (40.7%)	360 (40.1%)	0.8640	2,912 (47.5%)	0.0180
Take calcium, n (%)	220 (44.0%)	382 (44.4%)	0.8974	3,314 (54.4%)	0.0002
Serum folate (nmol/L), mean (SE)	36.8 (1.4)	42.2 (1.7)	0.1210	38.6 (0.6)	0.2398
Homocysteine (μmol/L), mean (SE)	9.8 (0.2)	10.4 (0.3)	0.1051	9.7 (0.1)	0.3702
A1C (%), mean (SE)	7.3 (0.1)	7.3 (0.1)	0.5222	5.5 (0.0)	<0.0001
Hemoglobin (g/dL), mean (SE)	13.9 (0.1)	14.1 (0.1)	0.1210	14.4 (0.0)	<0.0001
Serum B_{12} (pmol/L), mean† (SE)	317.5 (9.6)	386.7 (7.8)	0.0116	350.8 (2.9)	0.0011
Macrocytosis‡, n (%)	12 (1.9%)	35 (2.7%)	0.4577	346 (4.5%)	0.0017
Anemia§, n (%)	104 (16.2%)	222 (17.1%)	0.6594	731 (8.0%)	<0.0001
Antacid use, n (%)	45 (9.9%)	52 (6.6%)	0.0784	553 (9.6%)	0.7986
Proton pump inhibitor use, n (%)	60 (11.7%)	113 (12.3%)	0.7819	690 (10.5%)	0.5315
H_2 blocker use, n (%)	24 (4.0%)	64 (6.2%)	0.1356	221 (3.2%)	0.5278

*Versus metformin users. †Geometric mean. ‡Mean cell volume >99 fL. §Hemoglobin <13 g/dL for men, <12 g/dL for women (WHO guidelines) (36).

Statistical analysis

Statistical analysis was performed using SAS version 9.2 (SAS Institute, Cary, NC) and SUDAAN version 10.1 (Research Triangle Park, Durham, NC). Sample weights and variances were applied throughout the analysis to provide a representative sample of the U.S. population ≥50 years of age.

SAS survey procedures and SUDAAN "proc descript" were used to estimate means and proportions. SUDAAN "proc crosstab" was used to estimate the weighted prevalence adjusted for age, race, and sex. Tests of significance were performed using *t* tests for continuous variables and χ^2 test for categorical variables. Population-attributable risk for metformin use on biochemical B_{12} deficiency was calculated from the cross-sectional data using the Fleiss equation (27).

Polytomous logistic regression was performed in SUDAAN using "proc multilog" with a trilevel B_{12}-status outcome (vitamin B_{12} deficiency, borderline deficiency, and normal) to assess the association of previously identified risk factors with biochemical B_{12} deficiency and borderline deficiency in our study population. Risk factors previously identified for biochemical B_{12} deficiency were assessed as exposure variables along with metformin therapy in a full model and included age, race/ethnicity, sex, BMI (calculated as weight in kilograms divided by height in meters squared), and the use of proton pump inhibitors, H_2 blockers, antacids, B_{12} supplements, alcohol, and tobacco (12,28). The final polytomous logistic model adjusted for age, BMI, insulin, and B_{12} supplement use. Alcohol use and smoking could not be included in the model as >60% of responses were missing.

RESULTS—In the final analysis, there were 575 U.S. adults ≥50 years of age with type 2 diabetes using metformin, 1,046 with type 2 diabetes not using metformin, and 6,867 without diabetes. The demographic and biological characteristics of the groups are shown in Table 1. Among metformin users, mean age was 63.4 ± 0.5 years, 50.3% were male, 66.7% were non-Hispanic white, and 40.7% used a supplement containing B_{12}. The median duration of metformin use was 5 years. Compared with those with type 2 diabetes not taking metformin, metformin users were younger ($P < 0.0001$), reported a lower prevalence of insulin use ($P < 0.001$), and had a shorter duration of diabetes ($P = 0.0207$). Compared with those without diabetes, metformin users had a higher proportion of nonwhite racial groups ($P < 0.0001$), a higher proportion of obesity ($P < 0.0001$), a lower prevalence of macrocytosis ($P = 0.0017$), a lower prevalence of supplemental folic acid use ($P = 0.0069$), a lower prevalence of supplemental vitamin B_{12} use ($P = 0.0180$), and a lower prevalence of calcium supplement use ($P = 0.0002$). There was a twofold difference in the prevalence of anemia among those with type 2 diabetes versus those without, and no difference between the groups with diabetes.

The geometric mean serum B_{12} concentration among those with type 2 diabetes taking metformin was 317.5 pmol/L. This was significantly lower than the geometric mean concentration in those with type 2 diabetes not taking metformin (386.7 pmol/L; $P = 0.0116$) and those without diabetes (350.8 pmol/L; $P = 0.0011$). As seen in Fig. 1, the weighted prevalence of biochemical B_{12} deficiency adjusted for age, race, and sex was 5.8% for those with type 2 diabetes taking metformin, 2.2% for those with type 2 diabetes not taking metformin ($P = 0.0002$), and 3.3% for those without diabetes ($P = 0.0026$). Among the three aforementioned groups, borderline deficiency was present in 16.2, 5.5, and 8.8%, respectively ($P < 0.0001$). Applying the Fleiss formula for calculating attributable risk from cross-sectional data (27), among all of the cases of biochemical B_{12} deficiency, 3.5% of the cases were attributable to metformin use; and among those with diabetes, 41% of the deficient cases were attributable to metformin use. When the prevalence of biochemical B_{12} deficiency among those with diabetes taking metformin was analyzed by duration of metformin therapy, there was no notable increase in the prevalence of biochemical B_{12} deficiency as the duration of metformin use increased. The prevalence of biochemical B_{12} deficiency was 4.1% among those taking metformin <1 year, 6.3% among those taking metformin ≥1–3 years, 4.1% among those taking metformin >3–10 years, and 8.1% among those taking metformin >10 years ($P = 0.3219$ for <1 year vs. >10 years). Similarly, there was no clear increase in the prevalence of borderline deficiency as the duration of metformin use increased (15.9% among those taking metformin >10 years vs. 11.4% among those taking metformin <1 year; $P = 0.4365$).

Table 2 presents a stratified analysis of the weighted prevalence of biochemical

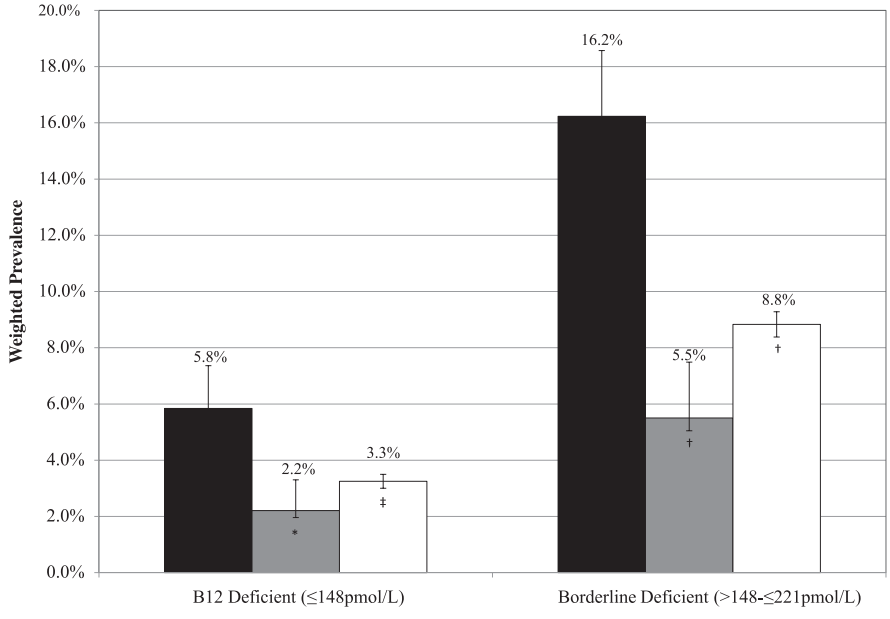

Figure 1—*Weighted prevalence of biochemical B_{12} deficiency and borderline deficiency adjusted for age, race, and sex in U.S. adults ≥50 years of age: NHANES 1999–2006. Black bars are those with type 2 diabetes on metformin, gray bars are those with type 2 diabetes not on metformin, and the white bars are those without diabetes.* *P = *0.0002 vs. type 2 diabetes on metformin.* †P < *0.0001 vs. type 2 diabetes on metformin.* ‡P = *0.0026 vs. type 2 diabetes on metformin.*

B_{12} deficiency and borderline deficiency by B_{12} supplement use. For those without diabetes, B_{12} supplement use was associated with an ~66.7% lower prevalence of both biochemical B_{12} deficiency (4.8 vs. 1.6%; $P < 0.0001$) and borderline deficiency (16.6 vs. 5.5%; $P < 0.0001$). A decrease in the prevalence of biochemical B_{12} deficiency was seen at all levels of supplemental B_{12} intake compared with nonusers of supplements. Among those with type 2 diabetes taking metformin, supplement use was not associated with a decrease in the prevalence of either biochemical B_{12} deficiency (5.6 vs. 5.3%; $P = 0.9137$) or borderline deficiency (15.5 vs. 8.8%; $P = 0.0826$). Among the metformin users who also used supplements, those who consumed >0–6 μg of B_{12} had a prevalence of biochemical B_{12} deficiency of 14.1%. However, consumption of a supplement containing >6 μg of B_{12} was associated with a prevalence of biochemical B_{12} deficiency of 1.8% ($P = 0.0273$ for linear trend). Similar trends were seen in the association of supplemental B_{12} intake and the prevalence of borderline deficiency. For those with type 2 diabetes not taking metformin, supplement use was also not associated with a decrease in the prevalence of biochemical B_{12} deficiency (2.1 vs. 2.0%; $P = 0.9568$) but was associated with a 54% reduction in the prevalence of borderline deficiency (7.8 vs. 3.4%; $P = 0.0057$ for linear trend).

Table 3 demonstrates the association of various risk factors with biochemical B_{12} deficiency. Metformin therapy was associated with biochemical B_{12} deficiency (odds ratio [OR] 2.89; 95% CI 1.33–6.28) and borderline deficiency (OR 2.32; 95% CI 1.31–4.12) in a crude model (results not shown). After adjusting for age, BMI, and insulin and supplement use, metformin maintained a significant association with biochemical B_{12} deficiency (OR 2.92; 95% CI 1.28–6.66) and borderline deficiency (OR 2.16; 95% CI 1.22–3.85). Similar to Table 2, B_{12} supplements were protective against borderline (OR 0.43; 95% CI 0.23–0.81), but not biochemical, B_{12} deficiency (OR 0.76; 95% CI 0.34–1.70) among those with type 2 diabetes. Among those without diabetes, B_{12} supplement use was ~70% protective against biochemical B_{12} deficiency (OR 0.26; 95% CI 0.17–0.38) and borderline deficiency (OR 0.27; 95% CI 0.21–0.35).

Table 2—*Comparison of average daily B_{12} supplement intake by weighted prevalence of biochemical B_{12} deficiency (serum B_{12} ≤148 pmol/L) and borderline deficiency (serum B_{12} >148 to ≤221 pmol/L) among U.S. adults ≥50 years of age: NHANES 1999–2006*

	Type 2 diabetes, taking metformin			Type 2 diabetes, not taking metformin			Without diabetes		
B_{12} supplement intake	Total *N**	Deficient, % (95% CI)†	Borderline, % (95% CI)†	Total *N**	Deficient, % (95% CI)†	Borderline, % (95% CI)†	Total *N**	Deficient, % (95% CI)†	Borderline, % (95% CI)†
Overall	575	5.6 (2.5–8.6)	13.1 (8.9–17.2)	1,046‡	2.0 (0.9–3.2)	6.2 (3.9–8.5)	6,867§	3.3 (2.8–3.8)	11.4 (10.4–12.3)
No supplement	366	5.6 (2.0–9.2)	15.5 (10.5–20.5)	683	2.1 (0.2–3.9)	7.8 (5.2–10.4)	3,939	4.8 (4.0–5.6)	16.6 (15.2–17.9)
Any supplement	209‖	5.3 (0.8–9.8)¶	8.8 (2.4–15.2)#	360**	2.0 (0.0–4.0)††	3.4 (0.6–6.3)‡‡	2,912§§	1.6 (1.0–2.2)‡‡	5.5 (4.3–6.6)‡‡
>0–6 μg	68	14.1 (0.0–28.3)	20.1 (1.8–38.4)	108	0.0 (0.0–0.0)	4.0 (0.0–8.0)	1,054	1.8 (1.0–2.6)	7.2 (5.2–9.2)
>6–25 μg							1,083	2.3 (1.1–3.6)	5.4 (3.6–7.2)
>25 μg	127	1.8 (0.0–4.6)‖‖	4.3 (0.0–8.7)‖‖	219	2.9 (0.0–6.0)‖‖	3.5 (0.0–7.6%)‖‖	672	0.3 (0.0–0.9)	3.1 (1.5–4.6)

*Crude *N* for each intake category. †Weighted to represent noninstitutionalized U.S. population. ‡*n* = 3 missing for age. §*n* = 16 missing data for supplement use. ‖*n* = 14 missing data for daily intake. ¶*P* = 0.0273 for linear trend. #*P* = 0.0016 for linear trend. **n = 33 missing data for daily intake. ††*P* = 0.0015 for linear trend. ‡‡*P* < 0.0001 for linear trend. §§*n* = 103 missing data for daily intake. ‖‖>6–25 μg and >25 μg were combined to ensure reliable estimates.

CONCLUSIONS—The IOM has highlighted the detection and diagnosis of B_{12}

Table 3—*Polytomous logistic regression for potential risk factors of biochemical B_{12} deficiency and borderline deficiency among U.S. adults ≥50 years of age: NHANES 1999–2006, OR (95% CI)*

		Type 2 diabetes		Without diabetes	
		B_{12} def vs. normal	Border vs. normal	B_{12} def vs. normal	Border vs. normal
Metformin use, model 1*	Yes	**2.92 (1.28–6.66)**	**2.16 (1.22–3.85)**		
	No	1.00 (REF)	1.00 (REF)		
Metformin use, model 2†	Yes	**2.92 (1.26–6.78)**	**2.15 (1.20–3.86)**		
	No	1.00 (REF)	1.00 (REF)		
Age	Per year	**1.03 (1.00–1.06)**	1.02 (0.98–1.05)	**1.02 (1.00–1.03)**	1.00 (0.99–1.01)
Sex	Male	1.49 (0.76–2.94)	1.32 (0.78–2.22)	0.79 (0.57–1.10)	0.81 (0.67–0.99)
	Female	1.00 (REF)	1.00 (REF)	1.00 (REF)	1.00 (REF)
Race/ethnicity	Non-Hispanic black	0.39 (0.12–1.25)	0.79 (0.46–1.37)	0.23 (0.12–0.45)	**0.53 (0.42–0.67)**
	Hispanic	0.49 (0.18–1.34)	0.67 (0.28–1.63)	0.71 (0.36–1.39)	0.71 (0.51–1.01)
	Other	0.37 (0.05–2.78)	0.15 (0.02–1.21)	1.36 (0.55–3.32)	0.54 (0.27–1.06)
	Non-Hispanic white	1.00 (REF)	1.00 (REF)	1.00 (REF)	1.00 (REF)
BMI (kg/m^2)	Obese >30	0.74 (0.38–1.42)	1.08 (0.56–2.10)	1.31 (0.75–2.29)	**1.48 (1.16–1.88)**
	Overweight 25–30	**0.37 (0.16–0.88)**	0.72 (0.34–1.52)	0.94 (0.59–1.51)	1.24 (0.98–1.57)
	Normal <25	1.00 (REF)	1.00 (REF)	1.00 (REF)	1.00 (REF)
B_{12}-lowering med use‡	Yes	1.39 (0.49–3.95)	1.61 (0.95–2.74)	0.60 (0.34–1.03)	**0.79 (0.65–0.97)**
	No	1.00 (REF)	1.00 (REF)	1.00 (REF)	1.00 (REF)
Insulin use	Yes	0.51 (0.17–1.54)	**0.57 (0.33–1.00)**		
	No	1.00 (REF)	1.00 (REF)		
B_{12} supplement use	Yes	0.76 (0.34–1.70)	**0.43 (0.23–0.81)**	**0.26 (0.17–0.38)**	**0.27 (0.21–0.35)**
	No	1.00 (REF)	1.00 (REF)	1.00 (REF)	1.00 (REF)

Border, borderline; def, deficiency; med, medication; REF, reference. *Adjusted for age, BMI, insulin use, and B_{12} supplement use. †Includes all exposure variables; ORs are individual associations within the full model. ‡Medications include H_2 blockers, proton pump inhibitors, and antacids. Boldface estimates are significant at a 5% significance level.

deficiency as a high-priority topic for research (1). Our results suggest several findings that add to the complexity and importance of B_{12} research and its relation to diabetes, and offer new insight into the benefits of B_{12} supplements. Our data confirm the relationship between metformin and reduced serum B_{12} levels beyond the background prevalence of biochemical B_{12} deficiency. Our data demonstrate that an intake of >0–6 μg of B_{12}, which includes the dose most commonly found in over-the-counter multivitamins, was associated with a two-thirds reduction of biochemical B_{12} deficiency and borderline deficiency among adults without diabetes. This relationship has been previously reported with NHANES and Framingham population data (4,29). In contrast, we did not find that >0–6 μg of B_{12} was associated with a decrease in the prevalence of biochemical B_{12} deficiency or borderline deficiency among adults with type 2 diabetes taking metformin. This observation suggests that metformin reduces serum B_{12} by a mechanism that is additive to or different from the mechanism in older adults. It is also possible that metformin may exacerbate the deficiency among older adults with low serum B_{12}. Our sample size was too small to determine which amount >6 μg was associated with maximum protection, but we did find a dose-response trend.

We were surprised to find that those with type 2 diabetes not using metformin had the lowest prevalence of biochemical B_{12} deficiency. It is possible that these individuals may seek medical care more frequently than the general population and therefore are being treated for their biochemical B_{12} deficiency. Or perhaps, because this population had a longer duration of diabetes and a higher proportion of insulin users compared with metformin users, they have been switched from metformin to other diabetic treatments due to low serum B_{12} concentrations or uncontrolled glucose levels and these new treatments may increase serum B_{12} concentrations. Despite the observed effects of metformin on serum B_{12} levels, it remains unclear whether or not this reduction is a public health concern. With lifetime risks of diabetes estimated to be one in three and with metformin being a first-line intervention, it is important to increase our understanding of the effects of oral vitamin B_{12} on metformin-associated biochemical deficiency (20,21).

The strengths of this study include its nationally representative, population-based sample, its detailed information on supplement usage, and its relevant biochemical markers. This is the first study to use a nationally representative sample to examine the association between serum B_{12} concentration, diabetes status, and metformin use as well as examine how this relationship may be modified by vitamin B_{12} supplementation. The data available regarding supplement usage provided specific information regarding dose and frequency. This aspect of NHANES allowed us to observe the dose-response relationship in Table 2 and to compare it within our three study groups.

This study is also subject to limitations. First, NHANES is a cross-sectional survey and it cannot assess time as a factor, and therefore the results are associations and not causal relationships. A second limitation arises in our definition of biochemical B_{12} deficiency. There is no general consensus on how to define normal versus low serum B_{12} levels. Some researchers include the functional biomarker methylmalonic acid (MMA) in the definition, but this has yet to be agreed upon (30–34). Recently, an NHANES roundtable discussion suggested that definitions of biochemical B_{12} deficiency should incorporate one biomarker (serum B_{12} or holotranscobalamin) and

one functional biomarker (MMA or total homocysteine) to address problems with sensitivity and specificity of the individual biomarkers. However, they also cited a need for more research on how the biomarkers are related in the general population to prevent misclassification (34). MMA was only measured for six of our survey years; one-third of participants in our final analysis were missing serum MMA levels. Moreover, it has recently been reported that MMA values are significantly greater among the elderly with diabetes as compared with the elderly without diabetes even when controlling for serum B_{12} concentrations and age, suggesting that having diabetes may independently increase the levels of MMA (35). This unique property of MMA in elderly adults with diabetes makes it unsuitable as part of a definition of biochemical B_{12} deficiency in our specific population groups. Our study may also be subject to misclassification bias. NHANES does not differentiate between diabetes types 1 and 2 in the surveys; our definition may not capture adults with type 2 diabetes exclusively. Additionally, we used responses to the question "Have you received a physician's diagnosis of diabetes" to categorize participants as having or not having diabetes. Therefore, we failed to capture undiagnosed diabetes. Finally, we could only assess current metformin use. We cannot determine if nonmetformin users have ever used metformin or if they were not using it at the time of the survey.

Our data demonstrate several important conclusions. First, there is a clear association between metformin and biochemical B_{12} deficiency among adults with type 2 diabetes. This analysis shows that 6 μg of B_{12} offered in most multivitamins is associated with two-thirds reduction in biochemical B_{12} deficiency in the general population, and that this same dose is not associated with protection against biochemical B_{12} deficiency among those with type 2 diabetes taking metformin. Our results have public health and clinical implications by suggesting that neither 2.4 μg, the current IOM recommendation for daily B_{12} intake, nor 6 μg, the amount found in most multivitamins, is sufficient for those with type 2 diabetes taking metformin.

This analysis suggests a need for further research. One research design would be to identify those with biochemical B_{12} deficiency and randomize them to receive various doses of supplemental B_{12} chronically and then evaluate any improvement in serum B_{12} concentrations and/or clinical outcomes. Another design would use existing cohorts to determine clinical outcomes associated with biochemical B_{12} deficiency and how they are affected by B_{12} supplements at various doses. Given that a significant proportion of the population ≥50 years of age have biochemical B_{12} deficiency and that those with diabetes taking metformin have an even higher proportion of biochemical B_{12} deficiency, we suggest that support for further research is a reasonable priority.

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