

# Association of Biochemical B<sub>12</sub> Deficiency With Metformin Therapy and Vitamin B<sub>12</sub> Supplements

The National Health and Nutrition Examination Survey, 1999–2006

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**OBJECTIVE**—To describe the prevalence of biochemical B<sub>12</sub> 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<sub>12</sub> supplements.

**RESEARCH DESIGN AND METHODS**—Analysis of data on U.S. adults  $\geq 50$  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<sub>12</sub> deficiency was defined as serum B<sub>12</sub> concentrations  $\leq 148$  pmol/L and borderline deficiency was defined as  $>148$  to  $\leq 221$  pmol/L.

**RESULTS**—Biochemical B<sub>12</sub> 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<sub>12</sub> deficiency (adjusted odds ratio 2.92; 95% CI 1.26–6.78). Consumption of any supplement containing B<sub>12</sub> was not associated with a reduction in the prevalence of biochemical B<sub>12</sub> deficiency among those with diabetes, whereas consumption of any supplement containing B<sub>12</sub> was associated with a two-thirds reduction among those without diabetes.

**CONCLUSIONS**—Metformin therapy is associated with a higher prevalence of biochemical B<sub>12</sub> deficiency. The amount of B<sub>12</sub> recommended by the Institute of Medicine (IOM) (2.4  $\mu\text{g}/\text{day}$ ) and the amount available in general multivitamins (6  $\mu\text{g}$ ) 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<sub>12</sub> deficiency increase with age (1,2). Recent national data estimate a 21.2% prevalence of diagnosed diabetes among adults  $\geq 65$  years of age and a 6 and 20% prevalence of biochemical B<sub>12</sub> deficiency (serum B<sub>12</sub>  $<148$  pmol/L) and borderline deficiency (serum B<sub>12</sub>  $\geq 148$ – $221$  pmol/L) among adults  $\geq 60$  years of age (3,4).

The diabetes drug metformin has been reported to cause a decrease in serum B<sub>12</sub> 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<sub>12</sub> 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<sub>12</sub> found a 19% reduction in serum B<sub>12</sub> levels compared with placebo after 4 years

(6). Several other randomized control trials and cross-sectional surveys reported reductions in B<sub>12</sub> ranging from 9 to 52% (7–16). Although classical B<sub>12</sub> deficiency presents with clinical symptoms such as anemia, peripheral neuropathy, depression, and cognitive impairment, these symptoms are usually absent in those with biochemical B<sub>12</sub> deficiency (17).

Several researchers have made recommendations to screen those with type 2 diabetes on metformin for serum B<sub>12</sub> 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<sub>12</sub> injection therapy has been successfully used to correct the metformin-induced decline in serum B<sub>12</sub> (15,21,22). The use of B<sub>12</sub> supplements among those with type 2 diabetes on metformin in a nationally representative sample and their potentially protective effect against biochemical B<sub>12</sub> 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<sub>12</sub> deficiency among those with type 2 diabetes  $\geq 50$  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<sub>12</sub> 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  $\geq 60$  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<sub>12</sub> 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<sub>12</sub> deficiency determined by serum B<sub>12</sub> concentrations. Serum B<sub>12</sub> levels were quantified using the Quantaphase II folate/vitamin B<sub>12</sub> radioassay kit from Bio-Rad Laboratories (Hercules, CA). We defined biochemical B<sub>12</sub> deficiency as serum levels ≤148 pmol/L, borderline deficiency as serum B<sub>12</sub> >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<sub>12</sub> 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<sub>12</sub> deficiency is modified by supplemental B<sub>12</sub> intake, data from the dietary supplement questionnaire were used. Information regarding the dose and frequency was used to calculate average daily supplemental B<sub>12</sub> intake. We categorized supplemental B<sub>12</sub> intake as 0 μg (no B<sub>12</sub> 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<sub>12</sub> 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		P value*	Without diabetes N = 6,867	P value*
	Metformin users N = 575	Nonmetformin users N = 1,046			
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 <sup>2</sup> ), 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 <sub>12</sub> , 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 <sub>12</sub> (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 <sub>2</sub> 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  $\geq 50$  years of age.

SAS survey procedures and SUDAAN "proc describe" 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  $\chi^2$  test for categorical variables. Population-attributable risk for metformin use on biochemical B<sub>12</sub> 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<sub>12</sub>-status outcome (vitamin B<sub>12</sub> deficiency, borderline deficiency, and normal) to assess the association of previously identified risk factors with biochemical B<sub>12</sub> deficiency and borderline deficiency in our study population. Risk factors previously identified for biochemical B<sub>12</sub> 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<sub>2</sub> blockers, antacids, B<sub>12</sub> supplements, alcohol, and tobacco (12,28). The final polytomous logistic model adjusted for age, BMI, insulin, and B<sub>12</sub> supplement use. Alcohol use and smoking could not be included in the model as  $>60\%$  of responses were missing.

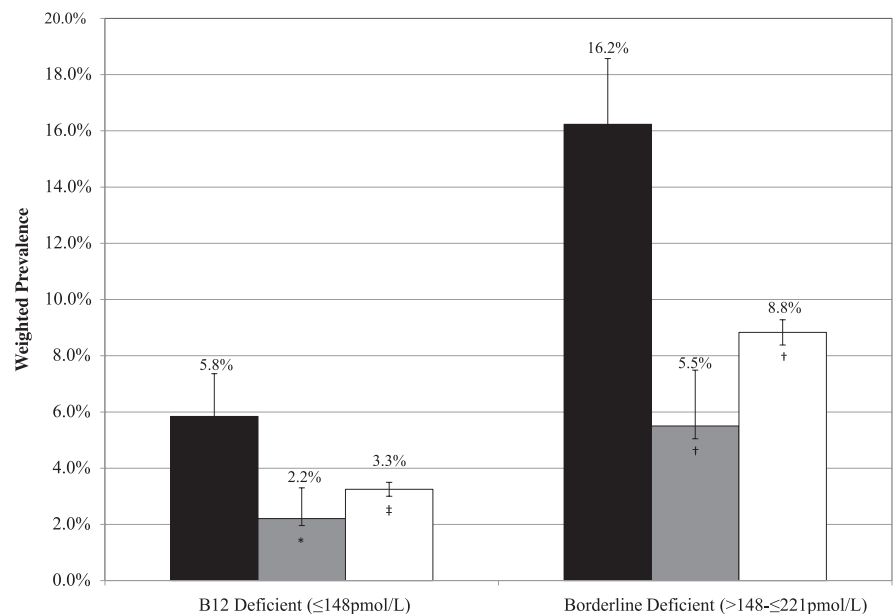
**RESULTS**—In the final analysis, there were 575 U.S. adults  $\geq 50$  years of age with type 2 diabetes using metformin, 1,046 with type 2 diabetes not using metformin, and 6,867 without diabetes. The demographic and biological characteristics of the groups are shown in Table 1. Among metformin users, mean age was  $63.4 \pm 0.5$  years, 50.3% were male, 66.7% were non-Hispanic white, and 40.7% used a supplement containing B<sub>12</sub>. 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<sub>12</sub> 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<sub>12</sub> 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<sub>12</sub> 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<sub>12</sub> 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<sub>12</sub> 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<sub>12</sub> deficiency as the duration of metformin use increased. The prevalence of biochemical B<sub>12</sub> deficiency was 4.1% among those taking metformin  $< 1$  year, 6.3% among those taking metformin  $\geq 1$ –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<sub>12</sub> deficiency and borderline deficiency adjusted for age, race, and sex in U.S. adults  $\geq 50$  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<sub>12</sub> deficiency and borderline deficiency by B<sub>12</sub> supplement use. For those without diabetes, B<sub>12</sub> supplement use was associated with an ~66.7% lower prevalence of both biochemical B<sub>12</sub> 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<sub>12</sub> deficiency was seen at all levels of supplemental B<sub>12</sub> 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<sub>12</sub> 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<sub>12</sub> had a prevalence of biochemical B<sub>12</sub> deficiency of 14.1%. However, consumption of a supplement containing >6 μg of B<sub>12</sub> was associated with a prevalence of biochemical B<sub>12</sub> deficiency of 1.8% (*P* = 0.0273 for linear trend). Similar trends were seen in the association of supplemental B<sub>12</sub> 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<sub>12</sub> 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<sub>12</sub> deficiency. Metformin therapy was associated with biochemical B<sub>12</sub> 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<sub>12</sub> 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<sub>12</sub> supplements were protective against borderline (OR 0.43; 95% CI 0.23–0.81), but not biochemical, B<sub>12</sub> deficiency (OR 0.76; 95% CI 0.34–1.70) among those with type 2 diabetes. Among those without diabetes, B<sub>12</sub> supplement use was ~70% protective against biochemical B<sub>12</sub> deficiency (OR 0.26; 95% CI 0.17–0.38) and borderline deficiency (OR 0.27; 95% CI 0.21–0.35).

**CONCLUSIONS**—The IOM has highlighted the detection and diagnosis of B<sub>12</sub>

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

B <sub>12</sub> supplement intake	Type 2 diabetes, taking metformin			Type 2 diabetes, not taking metformin			Without diabetes		
	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	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)	1,083	2.3 (1.1–3.6)	5.4 (3.6–7.2)
>25 μg							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 ≥50 years of age. ‡n = 3 missing for supplement use. §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.

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

		Type 2 diabetes		Without diabetes	
		B <sub>12</sub> def vs. normal	Border vs. normal	B <sub>12</sub> def vs. normal	Border vs. normal
Metformin use, model 1*	Yes	<b>2.92 (1.28–6.66)</b>	<b>2.16 (1.22–3.85)</b>		
	No	1.00 (REF)	1.00 (REF)		
Metformin use, model 2†	Yes	<b>2.92 (1.26–6.78)</b>	<b>2.15 (1.20–3.86)</b>		
	No	1.00 (REF)	1.00 (REF)		
Age	Per year	<b>1.03 (1.00–1.06)</b>	1.02 (0.98–1.05)	<b>1.02 (1.00–1.03)</b>	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)	<b>0.53 (0.42–0.67)</b>
	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 <sup>2</sup> )	Obese >30	0.74 (0.38–1.42)	1.08 (0.56–2.10)	1.31 (0.75–2.29)	<b>1.48 (1.16–1.88)</b>
	Overweight 25–30	<b>0.37 (0.16–0.88)</b>	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 <sub>12</sub> -lowering med use‡	Yes	1.39 (0.49–3.95)	1.61 (0.95–2.74)	0.60 (0.34–1.03)	<b>0.79 (0.65–0.97)</b>
	No	1.00 (REF)	1.00 (REF)	1.00 (REF)	1.00 (REF)
Insulin use	Yes	0.51 (0.17–1.54)	<b>0.57 (0.33–1.00)</b>		
	No	1.00 (REF)	1.00 (REF)		
B <sub>12</sub> supplement use	Yes	0.76 (0.34–1.70)	<b>0.43 (0.23–0.81)</b>	<b>0.26 (0.17–0.38)</b>	<b>0.27 (0.21–0.35)</b>
	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<sub>12</sub> supplement use. †Includes all exposure variables; ORs are individual associations within the full model. ‡Medications include H<sub>2</sub> 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<sub>12</sub> research and its relation to diabetes, and offer new insight into the benefits of B<sub>12</sub> supplements. Our data confirm the relationship between metformin and reduced serum B<sub>12</sub> levels beyond the background prevalence of biochemical B<sub>12</sub> deficiency. Our data demonstrate that an intake of >0–6 μg of B<sub>12</sub>, which includes the dose most commonly found in over-the-counter multivitamins, was associated with a two-thirds reduction of biochemical B<sub>12</sub> 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<sub>12</sub> was associated with a decrease in the prevalence of biochemical B<sub>12</sub> deficiency or borderline deficiency among adults with type 2 diabetes taking metformin. This observation suggests that metformin reduces serum B<sub>12</sub> 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<sub>12</sub>. 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<sub>12</sub> 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<sub>12</sub> 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<sub>12</sub> concentrations or uncontrolled glucose levels and these new treatments may increase serum B<sub>12</sub> concentrations. Despite the observed effects of metformin on serum B<sub>12</sub> 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<sub>12</sub> 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<sub>12</sub> concentration, diabetes status, and metformin use as well as examine how this relationship may be modified by vitamin B<sub>12</sub> 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<sub>12</sub> deficiency. There is no general consensus on how to define normal versus low serum B<sub>12</sub> 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<sub>12</sub> deficiency should incorporate one biomarker (serum B<sub>12</sub> 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<sub>12</sub> 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<sub>12</sub> 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<sub>12</sub> deficiency among adults with type 2 diabetes. This analysis shows that 6 μg of B<sub>12</sub> offered in most multivitamins is associated with two-thirds reduction in biochemical B<sub>12</sub> deficiency in the general population, and that this same dose is not associated with protection against biochemical B<sub>12</sub> 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<sub>12</sub> 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<sub>12</sub> deficiency and randomize them to receive various doses of supplemental B<sub>12</sub> chronically and then evaluate any

improvement in serum B<sub>12</sub> concentrations and/or clinical outcomes. Another design would use existing cohorts to determine clinical outcomes associated with biochemical B<sub>12</sub> deficiency and how they are affected by B<sub>12</sub> supplements at various doses. Given that a significant proportion of the population ≥50 years of age have biochemical B<sub>12</sub> deficiency and that those with diabetes taking metformin have an even higher proportion of biochemical B<sub>12</sub> deficiency, we suggest that support for further research is a reasonable priority.

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## References

1. Institute of Medicine. *Food and Nutrition Board. Dietary Reference Intakes: Thiamin, Riboflavin, Niacin, Vitamin B6, Folate, Vitamin B12, Pantothenic Acid, Biotin, and Choline*. Washington, DC, National Academy Press, 1998
2. American Diabetes Association. Standards of medical care in diabetes—2010. *Diabetes Care* 2010;33(Suppl. 1):S11–S61
3. Centers for Disease Control and Prevention. *National Diabetes Fact Sheet: National Estimates and General Information on Diabetes and Prediabetes in the United States*. Atlanta, GA, Department of Health and Human Services, 2011.
4. Evatt ML, Terry PD, Ziegler TR, Oakley GP. Association between vitamin B12-containing supplement consumption and prevalence of biochemically defined B12 deficiency in adults in NHANES III (third national health and nutrition examination survey). *Public Health Nutr* 2010;13:25–31
5. DeFronzo RA, Goodman AM; The Multicenter Metformin Study Group.

Efficacy of metformin in patients with non-insulin-dependent diabetes mellitus. *N Engl J Med* 1995;333:541–549

6. de Jager J, Kooy A, Lehert P, et al. Long term treatment with metformin in patients with type 2 diabetes and risk of vitamin B-12 deficiency: randomised placebo controlled trial. *BMJ* 2010;340:c2181
7. Bauman WA, Shaw S, Jayatilleke E, Spungen AM, Herbert V. Increased intake of calcium reverses vitamin B12 malabsorption induced by metformin. *Diabetes Care* 2000;23:1227–1231
8. Carlsen SM, Følling I, Grill V, Bjerve KS, Schneede J, Refsum H. Metformin increases total serum homocysteine levels in non-diabetic male patients with coronary heart disease. *Scand J Clin Lab Invest* 1997;57:521–527
9. Leung S, Mattman A, Snyder F, Kassam R, Meneilly G, Nexo E. Metformin induces reductions in plasma cobalamin and haptocorrin bound cobalamin levels in elderly diabetic patients. *Clin Biochem* 2010;43:759–760
10. Sahin M, Tutuncu NB, Ertugrul D, Tanaci N, Guvener ND. Effects of metformin or rosiglitazone on serum concentrations of homocysteine, folate, and vitamin B12 in patients with type 2 diabetes mellitus. *J Diabetes Complications* 2007;21:118–123
11. Wulffélé MG, Kooy A, Lehert P, et al. Effects of short-term treatment with metformin on serum concentrations of homocysteine, folate and vitamin B12 in type 2 diabetes mellitus: a randomized, placebo-controlled trial. *J Intern Med* 2003;254:455–463
12. Pflipsen MC, Oh RC, Saguil A, Seehusen DA, Seaquist D, Topolski R. The prevalence of vitamin B(12) deficiency in patients with type 2 diabetes: a cross-sectional study. *J Am Board Fam Med* 2009;22:528–534
13. Pongchaidecha M, Srikusalanukul V, Chattananon A, Tanjariyaporn S. Effect of metformin on plasma homocysteine, vitamin B12 and folic acid: a cross-sectional study in patients with type 2 diabetes mellitus. *J Med Assoc Thai* 2004;87:780–787
14. Sparre Hermann L, Nilsson B, Wettre S. Vitamin B12 status of patients treated with metformin: a cross-sectional cohort study. *British Journal of Diabetes and Vascular Disease*. 2004;4:401–406
15. Tomkin GH, Hadden DR, Weaver JA, Montgomery DA. Vitamin-B12 status of patients on long-term metformin therapy. *BMJ* 1971;2:685–687
16. Wile DJ, Toth C. Association of metformin, elevated homocysteine, and methylmalonic acid levels and clinically worsened diabetic peripheral neuropathy. *Diabetes Care* 2010;33:156–161
17. Carmel R. Mandatory fortification of the food supply with cobalamin: an idea whose

- time has not yet come. *J Inher Metab Dis* 2011;34:67–73
18. Bell DS. Metformin-induced vitamin B12 deficiency presenting as a peripheral neuropathy. *South Med J* 2010;103:265–267
  19. Callaghan TS, Hadden DR, Tomkin GH. Megaloblastic anaemia due to vitamin B12 malabsorption associated with long-term metformin treatment. *BMJ* 1980;280:1214–1215
  20. Carpentier JL, Bury J, Luyckx A, Lefebvre P. Vitamin B12 and folic acid serum levels in diabetics under various therapeutic regimens. *Diabete Metab* 1976;2:187–190
  21. Filioussi K, Bonovas S, Katsaros T. Should we screen diabetic patients using biguanides for megaloblastic anaemia? *Aust Fam Physician* 2003;32:383–384
  22. Andrès E, Noel E, Goichot B. Metformin-associated vitamin B12 deficiency. *Arch Intern Med* 2002;162:2251–2252
  23. Centers for Disease Control and Prevention. *National Health and Nutrition Examination Survey Questionnaire (or Examination Protocol, or Laboratory Protocol)*. Atlanta, GA, National Center for Health Statistics, 2010
  24. American Diabetes Association. Diagnosis and classification of diabetes mellitus. *Diabetes Care* 2010;33(Suppl. 1):S62–S69
  25. Dodd AH, Colby MS, Boye KS, Fahlman C, Kim S, Briefel RR. Treatment approach and HbA1c control among US adults with type 2 diabetes: NHANES 1999–2004. *Curr Med Res Opin* 2009;25:1605–1613
  26. Carmel R, Green R, Rosenblatt DS, Watkins D. Update on cobalamin, folate, and homocysteine. *Hematology Am Soc Hematol Educ Program* 2003;62–81
  27. Fleiss JL. Inference about population attributable risk from cross-sectional studies. *Am J Epidemiol* 1979;110:103–104
  28. Ting RZ-W, Szeto CC, Chan MH-M, Ma KK, Chow KM. Risk factors of vitamin B(12) deficiency in patients receiving metformin. *Arch Intern Med* 2006;166:1975–1979
  29. Lindenbaum J, Rosenberg IH, Wilson PW, Stabler SP, Allen RH. Prevalence of cobalamin deficiency in the Framingham elderly population. *Am J Clin Nutr* 1994;60:2–11
  30. Morris MS, Jacques PF, Rosenberg IH, Selhub J. Folate and vitamin B-12 status in relation to anemia, macrocytosis, and cognitive impairment in older Americans in the age of folic acid fortification. *Am J Clin Nutr* 2007;85:193–200
  31. Selhub J, Jacques PF, Dallal G, Choumenkovitch S, Rogers G. The use of blood concentrations of vitamins and their respective functional indicators to define folate and vitamin B12 status. *Food Nutr Bull* 2008;29(Suppl.):S67–S73
  32. Selhub J, Jacques PF, Bostom AG, et al. Association between plasma homocysteine concentrations and extracranial carotid-artery stenosis. *N Engl J Med* 1995;332:286–291
  33. Solomon LR. Disorders of cobalamin (vitamin B12) metabolism: emerging concepts in pathophysiology, diagnosis and treatment. *Blood Rev* 2007;21:113–130
  34. Yetley EA, Pfeiffer CM, Phinney KW, et al. Biomarkers of vitamin B-12 status in NHANES: a roundtable summary. *Am J Clin Nutr* 2011;94:313S–321S
  35. Solomon LR. Diabetes as a cause of clinically significant functional cobalamin deficiency. *Diabetes Care* 2011;34:1077–1080
  36. WHO/UNICEF/UNU. *Iron Deficiency Anemia: Assessment, Prevention, and Control*. Geneva, World Health Organization, 2001