ORIGINAL ARTICLE

Long-term Metformin Use and Vitamin B12 Deficiency in the Diabetes Prevention Program Outcomes Study

Vanita R. Aroda, Sharon L. Edelstein, Ronald B. Goldberg, William C. Knowler, Santica M. Marcovina, Trevor J. Orchard, George A. Bray, David S. Schade, Marinella G. Temprosa, Neil H. White, Jill P. Crandall, and the Diabetes Prevention Program Research Group

MedStar Health Research Institute (V.R.A.), Hyattsville, Maryland 20782; George Washington University (S.L.E., M.G.T.), Rockville, MD 20852; University of Miami (R.B.G.), Miami, Florida 33146; National Institute of Diabetes and Digestive and Kidney Diseases (W.C.K.), Phoenix, Arizona 85014; University of Washington (S.M.M.), Seattle, Washington 98185; University of Pittsburgh (T.J.O.), Pittsburgh, Pennsylvania 15260; Pennington Biomedical Research Institute (G.A.B.), Baton Rouge, Louisiana 70808; University of New Mexico (D.S.S.), Albuquerque, New Mexico 87131; Washington University School of Medicine (N.H.W.), St. Louis, Missouri 63110; and Albert Einstein College of Medicine (J.P.C.), Bronx, New York 10461

Context: Vitamin B12 deficiency may occur with metformin treatment, but few studies have assessed risk with long-term use.

Objective: To assess the risk of B12 deficiency with metformin use in the Diabetes Prevention Program (DPP)/DPP Outcomes Study (DPPOS).

Design: Secondary analysis from the DPP/DPPOS. Participants were assigned to the placebo group (PLA) (n = 1082) or the metformin group (MET) (n = 1073) for 3.2 years; subjects in the metformin group received open-label metformin for an additional 9 years.

Setting: Twenty-seven study centers in the United States.

Patients: DPP eligibility criteria were: elevated fasting glucose, impaired glucose tolerance, and overweight/obesity. The analytic population comprised participants with available stored samples. B12 levels were assessed at 5 years (n = 857, n = 858) and 13 years (n = 756, n = 764) in PLA and MET, respectively.

Interventions: Metformin 850 mg twice daily vs placebo (DPP), and open-label metformin in the metformin group (DPPOS).

Main Outcome Measures: B12 deficiency, anemia, and peripheral neuropathy.

Results: Low B12 ($\leq$ 203 pg/mL) occurred more often in MET than PLA at 5 years (4.3 vs 2.3%; P = .02) but not at 13 years (7.4 vs 5.4%; P = .12). Combined low and borderline-low B12 ($\leq$ 298 pg/mL) was more common in MET at 5 years (19.1 vs 9.5%; P < .01) and 13 years (20.3 vs 15.6%; P = .02). Years of metformin use were associated with increased risk of B12 deficiency (odds ratio, B12 deficiency/year metformin use, 1.13; 95% confidence interval, 1.06–1.20). Anemia prevalence was higher in MET, but did not differ by B12 status. Neuropathy prevalence was higher in MET with low B12 levels.

Conclusions: Long-term use of metformin in DPPOS was associated with biochemical B12 deficiency and anemia. Routine testing of vitamin B12 levels in metformin-treated patients should be considered. (*J Clin Endocrinol Metab* 101: 1754–1761, 2016)

Abbreviation: BMI, body mass index.

In 1969, Berchtold et al (1) reported evidence of vitamin B12 malabsorption in patients who had been treated with metformin for as little as 3 months. As early as 1971, Tomkin et al (2) recommended that all patients on long-term metformin therapy have annual serum B12 testing, based on a cross-sectional evaluation.

Since then, cross-sectional, retrospective, and longitudinal observational studies (3–14), as well as case reports (15, 16), have suggested a clinical association between long-term metformin use and vitamin B12 deficiency (17, 18).

However, few prospective placebo-controlled studies have been conducted to define the risk of vitamin B12 deficiency in metformin-treated individuals, and none thus far specifically in prediabetic individuals. Most randomized trials addressing this issue have been small or of short duration (<6 months) and have primarily been in patients with type 2 diabetes (19–21), with a few short-term studies specifically done in gestational diabetes or polycystic ovary syndrome (22–25). The largest prospective placebo-controlled randomized trial to date evaluating vitamin B12 levels with metformin in type 2 diabetes showed that over 4.3 years, metformin reduced vitamin B12 concentration by 19%, increased homocysteine concentration by 5%, and was associated with an 11-fold increased risk in low vitamin B12 levels compared to placebo (19).

The Diabetes Prevention Program (DPP)/DPP Outcomes Study (DPPOS) represents one of the largest and longest studies of metformin treatment. In the DPP/DPPOS, persons at high risk for type 2 diabetes have been continued on randomized metformin treatment for over 10 years. Assessment of vitamin B12 status in the population represented in the DPP/DPPOS has not previously been reported. In addition, the DPP/DPPOS recorded relevant exposure history from participants who were started on nonstudy metformin therapy due to the subsequent development of diabetes during the study and has assessed potential complications of B12 deficiency.

In this analysis from the DPP/DPPOS, vitamin B12 and homocysteine levels were assessed in metformin and placebo-assigned participants at a mean 5 years and 13 years of follow-up. We report here the risk of vitamin B12 deficiency, as well as associated complications (anemia, neuropathy) with metformin use in the DPP/DPPOS.

Subjects and Methods

Study design

The DPP and DPPOS design, eligibility, and baseline characteristics have been reported elsewhere (26, 27). The DPP enrolled 3234 participants with impaired glucose tolerance and fasting blood glucose of 95 to 125 mg/dL (≤125 mg/dL in American Indians) who were at least 25 years of age and had body mass index (BMI) of 24 kg/m^2 or higher (≥22 kg/m^2 in Asian Americans). Enrollment began in July 1996 and ended in May 1999. Participants were randomly assigned to one of three treatments: placebo, an intensive lifestyle program, or metformin 850 mg twice daily. All of the 27 DPP clinical study centers as well as the DPP Coordinating Center had institutional review board approval. All participants gave written informed consent.

Mean follow-up at the end of the DPP was 3.2 years. At the end of the DPP, all participants were offered a group-implemented lifestyle intervention and invited to enroll in the follow-up study, DPPOS. During the DPPOS, those originally assigned to metformin received open-label metformin at the same prior dose of 850 mg twice daily, which was continued until such time as diabetes developed and HbA1c value reached ≥ 7%. At this point, study metformin was discontinued, and the participant referred to his or her own physician for treatment of diabetes, which may have included metformin. Samples used for measurement of vitamin B12 and homocysteine, as a corroborating indicator of vitamin B12 deficiency, were collected an average of 5 years (DPPOS year 1) and 13 years (DPPOS year 9) after initial randomization and analyzed in 2011 (Supplemental Data). Stored serum samples from other time points, including the DPP baseline, were not available. DPPOS year 9 was chosen to coincide with planned neuropathy outcome measures.

Vitamin B12 levels were measured using Tosoh reagent on a Tosoh 1800 analyzer (TOSOH Bioscience, Inc). The assay sensitivity is 50 pg/mL, and the results are traceable to the World Health Organization IS 03/178 reference material. The interassay coefficients of variation obtained on quality control samples with high-, medium- and low levels ranged from 7.2% to 8.8%. Homocysteine levels were measured by a homogeneous enzymatic assay on a Roche Modular P analyzer (Roche Diagnostics, Inc., Indianapolis, IN). The assay sensitivity is 1 μmol/L and the results are traceable to the National Institute of Standards and Technology reference preparation homocysteine. The interassay coefficients of variation obtained on quality control samples with high-, medium-, and low levels ranged from 3.9 to 8.9%.

Hemoglobin and hematocrit were obtained as a safety measure to detect B12 deficiency anemia in placebo and metformin-assigned participants annually in DPP and in DPPOS year 1, but only in participants actively taking study-provided metformin thereafter. Participants identified with anemia were referred to their own physicians for evaluation and treatment.

Low vitamin B12 was defined as ≤ 203 pg/mL, and borderline-low levels were defined as between 204 and 298 pg/mL, inclusive (28, 29). Anemia was defined as hemoglobin < 12 g/dL or hematocrit < 36% (females) and hemoglobin < 13 g/dL or hematocrit < 40% (males). Elevated homocysteine was defined as > 13.7 μmol/L (13). Metformin exposure, documented by counting returned tablets at each study visit, was assessed semi-annually. Study-provided metformin usage was calculated, as well as total metformin years of exposure, which includes both protocol-based and nonprotocol metformin use (ie, prescribed outside of the study for diabetes treatment). Clinical neuropathy was defined as a reduction or absence of light touch sensation to monofilament (Semmes-Weinstein, 10 g) in either foot (< eight of 10 applications detected). The Michigan Neuropathy Screening Instrument (MNSI) was conducted annually and was analyzed for DPPOS years 1 and 9, concurrent with B12 measurements (30).

Statistical analysis

The study population for this analysis comprised participants randomized to placebo (n = 902) or metformin (n = 898) during DPP who had available serum stored from either DPPOS years 1 or 9 measured for vitamin B12 levels. Participants who had undergone bariatric surgery before collection of serum samples (n = 4 at DPPOS year 1, and n = 49 at year 9) were excluded from this analysis. Comparisons of vitamin B12 levels and vitamin B12 deficiency by treatment group were made at each time point using t tests and χ^2 tests. Total metformin-years were computed by adding years of taking any study-provided metformin as well as self-reported use of any metformin prescribed outside of the study. Separate treatment-specific and combined logistic regression models were used to assess vitamin B12 deficiency at DPPOS year 9 by years of total metformin use after adjustment for age, sex, BMI, use of prescription proton pump inhibitors or prescription H2 blockers, diabetes status, weight change during DPP/DPPOS, and treatment arm for the combined sample.

Results

Study population

As described previously (26), the original DPP cohort comprised of two-thirds women and 45% ethnic minorities, with an average age of 51 years at baseline. Characteristics of the participants included in this analysis at DPP baseline and DPPOS years 1 and 9 are shown in Table 1.

Vitamin B12, homocysteine levels, and vitamin B12 deficiency

Levels of vitamin B12 and homocysteine and metformin exposure at a mean 5 years (DPPOS year 1) and 13 years (DPPOS year 9) of follow-up are shown in Table 1. Comparing the metformin to placebo groups at DPPOS year 1, mean B12 levels were 10% lower, and the prevalence of B12 deficiency (4.3 vs 2.4%; $P = .02$) and borderline-low vitamin B12 levels (19.1 vs 9.5%; $P < .01$) were significantly higher. A similar, but nonsignificant pattern was seen for mean vitamin B12 levels and prevalence of deficiency in DPPOS year 9. Somewhat paradoxically, in both treatment groups, mean vitamin B12 levels were greater in DPPOS year 9 compared with year 1, yet the prevalence of vitamin B12 deficiency was also greater in year 9 compared with year 1 in each treatment group. The prevalence of vitamin B12 deficiency was greatest among participants actively taking study-provided metformin and increased over time (5.2 vs. 9.2% after 5 and 13 years, respectively). Among participants with vitamin B12 deficiency in DPPOS year 1, 38 and 45% remained deficient at DPPOS year 9 in the metformin and placebo groups, respectively. Vitamin B12 levels at DPPOS year 1 were correlated with DPPOS year 9 measurements (Spearman correlation coefficient, 0.54). At DPPOS year 1, use

Table 1. Participant Characteristics: Vitamin B12 Status and Metformin Use by DPP Treatment Group and Study Visit

	DPP Baseline[a]	DPPOS Year 1 (Mean 5 y of Follow-up)			DPPOS Year 9 (Mean 13 y of Follow-up)		
	Total	MET	PLA	P Value	MET	PLA	P Value
No. of participants	1800	859	856		753	736	
Age, y	51.2 ± 10.0	56.7 ± 10.1	56.0 ± 9.9	n.s.	65.5 ± 9.6	63.9 ± 9.7	n.s.
Sex, n (%)							
Female	1217 (67.6)	574 (66.8)	586 (68.5)	n.s.	496 (65.9)	499 (67.8)	n.s.
Male	583 (32.4)	285 (33.2)	270 (31.5)	n.s.	257 (34.1)	237 (32.2)	n.s.
Race/ethnicity, % non-white	45.2	43.8	46.0	n.s.	45.1	47.1	n.s.
BMI, kg/m²	33.6 ± 7.04	33.2 ± 6.9	33.0 ± 6.9	.03	32.8 ± 6.7	33.5 ± 6.8	.03
HbA1c, %	6.06 ± 1.02	5.90 ± 0.64	6.02 ± 0.74	<.01	5.99 ± 0.99	6.15 ± 1.10	<.01
Acid suppression inhibitor use, %	10.9	6.2	8.9	.03	11.4	10.1	n.s.
Metformin-years of exposure	0	2.9 ± 1.7	0.1 ± 0.6	<.01	9.5 ± 4.4	1.7 ± 2.8	<.01
Patients with diabetes, %	0	33.2	39.0	<.01	52.3	59.7	<.01
Vitamin B12 level, pg/mL	NM	546 ± 337.2	606.6 ± 352.7	<.01	615.9 ± 503.8	650.0 ± 498	.19
Vitamin B12 deficiency (≤ 203 pg/mL), n (%)	NM	37 (4.3)	20 (2.3)	.02	56 (7.4)	40 (5.4)	.12
Vitamin B12 deficiency or borderline-low (≤ 298 pg/mL), n (%)	NM	164 (19.1)	81 (9.5)	<.01	153 (20.3)	115 (15.6)	.02
Vitamin B12 deficiency among current active users of study metformin, n (%)[b]	NM	29 (5.2)	NA	NA	39 (9.2)	NA	NA
Homocysteine level, μmol/L	NM	10.8 ± 3.6	10.3 ± 2.9	<.01	11.7 ± 4.1	11.4 ± 3.9	.09
Hemoglobin, g/dl	13.8 ± 1.3	13.6 ± 1.3	13.8 ± 1.3	<.01	13.4 ± 1.2	NM	NA
Hematocrit, %	41.0 ± 3.5	40.6 ± 3.7	41.1 ± 3.6	<.01	40.1 ± 3.5	NM	NA
Patients with anemia, %	9.8	14.3	10.5	.02	21.0	NA	NA
Patients with neuropathy, %	NM	9.7	9.9	n.s.	13.3	12.9	n.s.

Abbreviations: MET, metformin group; PLA, placebo group; NA, not applicable; NM, not measured. Values are expressed as mean ± SD or percentage, unless described otherwise.

[a] Participants with B12 measurement at DPPOS year 1, DPPOS year 9, or both.

[b] Any study metformin use since last DPP visit.

of prescription (injectable) B12 was reported by two participants in the metformin group and one participant in the placebo group, and in year 9 by seven participants in the metformin group and three participants in the placebo group.

Vitamin B12 deficiency and borderline-low vitamin B12 were associated with higher circulating homocysteine levels in the metformin and placebo groups, although mean homocysteine was not significantly different between the two treatment groups (Table 2). In the metformin group, among those with low or borderline vitamin B12 levels, 49 and 20%, respectively, had concurrently elevated homocysteine levels in DPPOS year 1 and 55 and 36%, respectively, in year 9 (Table 2). A similar pattern was seen in the placebo group.

Age was not related to vitamin B12 deficiency or vitamin B12 levels. In the metformin group, vitamin B12 deficiency increased over time in all age categories, although the combined prevalence of vitamin B12 deficiency and borderline-low vitamin B12 levels remained stable (Supplemental Data). In the placebo group, vitamin B12 deficiency also increased over time in all age categories, as did the combined prevalence of vitamin B12 deficiency and borderline-low vitamin B12 levels. Median vitamin B12 levels remained stable over time. In each age category ($<$60, 60–69, and 70 years or older), median vitamin B12 levels were consistently lower in the metformin group compared to placebo, and the prevalence of vitamin B12 deficiency or combined borderline-low/vitamin B12 deficiency was consistently higher in the metformin group compared to placebo (Supplemental Data).

Risk of vitamin B12 deficiency based on total metformin exposure

In both metformin and placebo treatment arms, total metformin-years of exposure was the only significant predictor of vitamin B12 deficiency in a multivariate logistic model including age, sex, baseline BMI, diabetes status, weight change, and prescription acid suppression use. The odds ratio (95% confidence interval) associated with vitamin B12 deficiency per year of total metformin use was 1.11 (1.03–1.20) in year 9 in metformin participants and 1.19 (1.07–1.32) in placebo participants (Table 3). In the metformin and placebo participants combined, the odds ratio (95% confidence interval) associated with vitamin B12 deficiency per year of metformin use was 1.13 (1.06–1.20) after adjustment for the confounders listed above as well as treatment assignment.

Associated risks of vitamin B12 deficiency: anemia and neuropathy

As shown in Figure 1, the presence of anemia was not different across vitamin B12 categories at DPPOS year 1. There were nonsignificant increases in anemia in each of the vitamin B12 level groups at DPPOS year 9 ($P = .25$) in the metformin group, perhaps related to the longer exposure to metformin. Note that in DPPOS year 9, anemia was assessed only in participants actively taking study-provided metformin.

Long-term follow-up (DPPOS year 9) showed statistically significantly higher prevalence of neuropathy (abnormal monofilament examination) among metformin group participants with low vitamin B12 levels compared to those with normal or borderline vitamin B12 levels ($P = .03$). This finding, however, was based on a very small number of cases of neuropathy (n = 13 of the 56 metformin participants with low vitamin B12 at DPPOS year 9). Review of MNSI questionnaire and examination data showed no difference in neuropathy symptoms or total MNSI score by B12 levels in the metformin or placebo groups at either time point (data not shown).

Table 2. Homocysteine Levels According to Vitamin B12 Category[a]

	Metformin			Placebo			
	Normal B12	Borderline B12	Low B12	Normal B12	Borderline B12	Low B12	P Value[b]
DPPOS year 1							
Mean Hcy, μmol/L	10.4 ± 2.6	12.1 ± 5.4	14.9 ± 7.2	10.1 ± 2.6	11.7 ± 3.6	13.5 ± 4.7	n.s.
Elevated Hcy, %[c]	11	20	49	8	20	40	n.s.
DPPOS year 9							
Mean Hcy, μmol/L	11.1 ± 3.2	13.6 ± 6.0	16.8 ± 12.8	10.9 ± 2.7	12.6 ± 3.5	16.0 ± 11.3	n.s.
Elevated Hcy, %[c]	15	36	53	14	36	41	n.s.

Abbreviations: Hcy, homocysteine; n.s., not significant.

[a] B12 categories are defined as normal ($>$ 298 pg/mL), borderline (204–298 pg/mL), and low ($\leq$ 203 pg/mL).

[b] P value represents differences between metformin and placebo groups.

[c] Elevated Hcy is defined as homocysteine $\geq$ 13.7 μmol/L.

Table 3. Adjusted Odds Ratios (95% Confidence Intervals) for Vitamin B12 Deficiency per Year of Total Metformin Use at the DPPOS Year 9 Visit

	Metformin	Placebo	All Participants Combined
Unadjusted	1.11 (1.03–1.20)	1.15 (1.05–1.26)	1.13 (1.06–1.20)
Age adjusted	1.11 (1.03–1.20)	1.16 (1.05–1.27)	1.13 (1.06–1.20)
Fully adjusted	1.11 (1.03–1.20)	1.19 (1.07–1.32)	1.13 (1.06–1.20)

Total metformin exposure includes use of protocol-prescribed metformin and use of metformin prescribed outside of the study (metformin and placebo participants). Fully-adjusted model adjusts for age, sex, baseline BMI, prescription acid suppression therapy, diabetes status, weight change at time of measurement, and for treatment assignment in the combined model.

Discussion

In this analysis of vitamin B12 and homocysteine levels in the DPP/DPPOS cohort, we report an increased risk of low vitamin B12 levels with long-term treatment with metformin. At 13 years since randomization, and controlling for age, sex, baseline BMI, prescription acid suppression therapy, diabetes status, and weight change, there was a 13% increased risk of vitamin B12 deficiency per year of total metformin use. Homocysteine levels were likewise increased in the groups with low or borderline-low vitamin B12 levels, supporting the presence of vitamin B12 deficiency at the tissue level.

Furthermore, in those treated with metformin compared to placebo, there was a greater prevalence of anemia at a mean 5 years after randomization. Interestingly, vitamin B12 deficiency was seen even in the absence of anemia. Peripheral neuropathy, a significant clinical consequence of B12 deficiency, was higher among metformin group participants with low B12, although the number of cases was small.

Although this report represents a post hoc analysis, the potential for development of B12 deficiency was recognized in the design of the study, and hematological pa-

rameters were monitored for this reason. Furthermore, the population studied and duration of exposure to metformin offer distinct strengths to the analysis. This is the largest cohort to date in which B12 levels and metformin exposure have been reported. Furthermore, with a mean follow-up of 13 years at the time of sample collection, the DPP/DPPOS represents the longest duration of randomized metformin exposure and vitamin B12 assessments in any population. Most other studies evaluating metformin and vitamin B12 deficiency have been conducted in patients with type 2 diabetes (19–21), with smaller studies evaluating vitamin B12 levels in patients with gestational diabetes or polycystic ovary syndrome (22–25). Of note, the prevalence of vitamin B12 deficiency in the placebo group at 5 years of follow-up (2.4%) is comparable to the National Health and Nutrition Examination Survey (NHANES) report of the prevalence of biochemical vitamin B12 deficiency in individuals with type 2 diabetes not using metformin (2.4%), and the prevalence of vitamin B12 deficiency in our metformin group (4.3%) was similar to that seen in the NHANES cross-sectional evaluation of individuals with type 2 diabetes using metformin (5.8%) (9). Furthermore, cross-sectional studies report a wide range of prevalence of biochemical vitamin B12 deficiency with metformin exposure, ranging from 5.8% to as high as 30% (2, 7–9, 31, 32). Detailed prospective characterization of metformin exposure, with adjustment for various contributors to vitamin B12 deficiency such as we were able to do in this analysis, may allow a more accurate risk assessment.

There are some limitations worth noting. Although B12 status was assessed indirectly via annual hemoglobin and hematocrit testing, vitamin B12 levels were measured post hoc from samples obtained in DPPOS years 1 and 9. Stored serum samples from other time points, including DPP baseline, were not available, and potentially informative red blood cell indices that could demonstrate the macrocytosis typical of vitamin B12 deficiency anemia were not routinely obtained. In addition, after DPPOS year 1, anemia was assessed only in metformin group participants actively taking the study drug, which may limit our ability
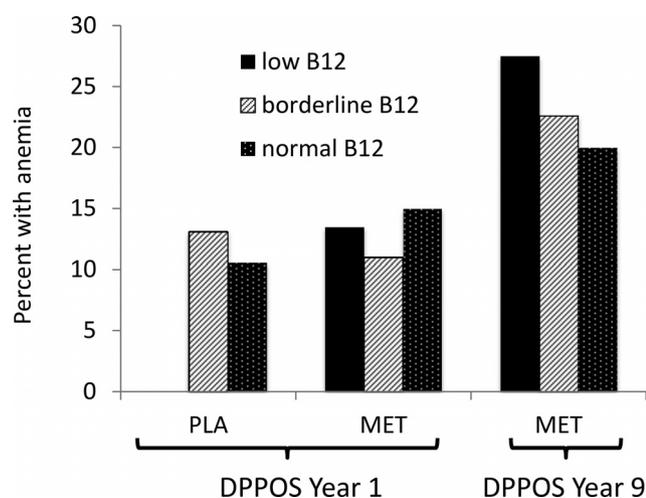
Figure 1. Percentage of participants with anemia by vitamin B12 status, 5 and 13 years after randomization (DPPOS years 1 and 9). PLA, placebo.

to accurately define the relationship between vitamin B12 status and anemia. Furthermore, anemia detected on annual screening may have resulted in evaluation and treatment of B12 deficiency outside of the study, thus reducing the apparent frequency of metformin-associated B12 deficiency. This seems to have been an infrequent occurrence, however, given that so few participants reported the use of prescription (injectable) B12, which is standard therapy for B12 deficiency. Finally, information about the use of over-the-counter vitamin supplements was not collected during the study.

There is significant debate about the clinical significance of biochemical vitamin B12 deficiency vs true tissue deficiency. In our cohort, approximately 50% of participants with low vitamin B12 levels had concurrently elevated homocysteine levels, suggesting true tissue deficiency. However, measurement of additional markers, such as holotranscobalamin, methylmalonic acid, red blood cell-B12, and plasma concentrations of methylation indices, for example, would provide a more comprehensive assessment of true deficiency; they are beyond the scope of our study, and appropriate samples for performing these assays are not available (33). Others have suggested that metformin treatment actually improves intracellular metabolism of vitamin B12, despite low serum levels. In a rodent model, metformin treatment resulted in increased liver accumulation of vitamin B12, thereby decreasing circulating vitamin B12 levels, suggesting that metformin alters tissue distribution and metabolism of vitamin B12, rather than causing true deficiency (24, 34).

Vitamin B12 deficiency associated with metformin use is thought to occur due to vitamin B12 malabsorption. Initial theories included alteration of bile acid metabolism, small intestinal bacterial overgrowth, or effects on intrinsic factor secretion, but a more currently accepted explanation is the interference by metformin on calcium-dependent membrane action responsible for vitamin B12-intrinsic factor absorption in the terminal ileum (1, 35–38). The use of proton pump inhibitors is also thought to contribute to vitamin B12 deficiency (39), although this does not appear to be a factor in our analysis (Table 1).

Our finding of a higher prevalence of monofilament-defined neuropathy in metformin-treated participants with low vitamin B12 levels should be viewed with caution, given the small number of neuropathy cases among those with low vitamin B12 levels (n = 13), which was also not confirmed by the MNSI. There have been case reports of metformin-induced vitamin B12 deficiency presenting as peripheral neuropathy, with evidence suggesting that prolonged exposure (12–15 years) may be required for this to occur (38). In a prospective case control study of diabetic patients referred for treatment of symptomatic peripheral neuropathy, metformin-treated patients had lower vitamin B12 levels and elevated methylmalonic acid and homocysteine levels, along with clinical and electrophysiological evidence of more severe peripheral neuropathy (13). Furthermore, cumulative metformin dose correlated strongly with the differences seen. In contrast, in a retrospective review, metformin use did not predict the risk of anemia or neuropathy, despite finding that metformin use was associated with biochemical vitamin B12 deficiency (4).

Despite the accumulating evidence associating metformin exposure to low vitamin B12 levels, as well as potential clinical sequelae, assessment of B12 levels in individuals treated with metformin has not been incorporated into clinical practice guidelines. Evidence suggests that such monitoring is rarely performed (40).

Given that metformin is widely recommended as a first-line agent for the treatment of type 2 diabetes, the growing population of individuals who receive metformin for other indications (including high risk of diabetes, gestational diabetes, polycystic ovary syndrome), and the chronic nature of treatment of these conditions, understanding the potential adverse consequences of metformin treatment is essential. Long-term follow-up data from DPP/DPPOS support the evidence that metformin is associated with vitamin B12 deficiency, and routine measurement of vitamin B12 for metformin-treated individuals should be considered.

Acknowledgments

Center for Minority Health and Human Disease, the Centers for Disease Control and Prevention, and the American Diabetes Association. Bristol-Myers Squibb and Parke-Davis provided additional funding and material support during the DPP, Lipha (Merck-Santé) provided medication, and LifeScan Inc donated materials during the DPP and DPPOS. The opinions expressed are those of the investigators and do not necessarily reflect the views of the funding agencies. A complete list of centers, investigators, and staff can be found in the Supplemental Data.

Author Contributions: V.R.A., S.L.E., and J.P.C. obtained research data, wrote, reviewed/edited, and contributed to discussion of the manuscript. R.B.G., W.C.K., T.J.O., G.A.B., D.S.S., and N.H.W. reviewed/edited and contributed to discussion of the manuscript. S.M.M. and M.G.T. obtained research data, reviewed/edited, and contributed to discussion of the manuscript.

Clinical Trial Registration No.: NCT00038727; ClinicalTrials.gov.

Disclosure Summary: The authors have no relevant conflicts of interest to disclose.

References

1. **Berchtold P, Bolli P, Arbenz U, Keiser G.** Disturbance of intestinal absorption following metformin therapy (observations on the mode of action of biguanides) [in German]. *Diabetologia.* 1969;5:405–412.
2. **Tomkin GH, Hadden DR, Weaver JA, Montgomery DA.** Vitamin-B12 status of patients on long-term metformin therapy. *Br Med J.* 1971;2:685–687.
3. **Beulens JW, Hart HE, Kuijs R, Kooijman-Buiting AM, Rutten GE.** Influence of duration and dose of metformin on cobalamin deficiency in type 2 diabetes patients using metformin. *Acta Diabetol.* 2015;52:47–53.
4. **de Groot-Kamphuis DM, van Dijk PR, Groenier KH, Houweling ST, Bilo HJ, Kleefstra N.** Vitamin B12 deficiency and the lack of its consequences in type 2 diabetes patients using metformin. *Neth J Med.* 2013;71:386–390.
5. **Haeusler S, Parry-Strong A, Krebs JD.** The prevalence of low vitamin B12 status in people with type 2 diabetes receiving metformin therapy in New Zealand–a clinical audit. *N Z Med J.* 2014;127:8–16.
6. **Ko SH, Ko SH, Ahn YB, et al.** Association of vitamin B12 deficiency and metformin use in patients with type 2 diabetes. *J Korean Med Sci.* 2014;29:965–972.
7. **Nervo M, Lubini A, Raimundo FV, et al.** Vitamin B12 in metformin-treated diabetic patients: a cross-sectional study in Brazil. *Rev Assoc Med Bras.* 2011;57:46–49.
8. **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.
9. **Reinstatler L, Qi YP, Williamson RS, Garn JV, Oakley GP, Jr.** Association of biochemical B_{12} deficiency with metformin therapy and vitamin B_{12} supplements: the National Health and Nutrition Examination Survey, 1999–2006. *Diabetes Care* 2012;35:327–333.
10. **Sato Y, Ouchi K, Funase Y, Yamauchi K, Aizawa T.** Relationship between metformin use, vitamin B12 deficiency, hyperhomocysteinemia and vascular complications in patients with type 2 diabetes. *Endocr J.* 2013;60:1275–1280.
11. **Singh AK, Kumar A, Karmakar D, Jha RK.** Association of B12 deficiency and clinical neuropathy with metformin use in type 2 diabetes patients. *J Postgrad Med.* 2013;59:253–257.
12. **Ting RZ, Szeto CC, Chan MH, Ma KK, Chow KM.** Risk factors of vitamin B(12) deficiency in patients receiving metformin. *Arch Intern Med.* 2006;166:1975–1979.
13. **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.
14. **Kos E, Liszek MJ, Emanuele MA, Durazo-Arvizu R, Camacho P.** Effect of metformin therapy on vitamin D and vitamin B_{12} levels in patients with type 2 diabetes mellitus. *Endocr Pract.* 2012;18:179–184.
15. **Tung ML, Tan LK.** Long term use of metformin leading to vitamin B 12 deficiency. *Diabetes Res Clin Pract.* 2014;104:e75–e76.
16. **Liu KW, Dai LK, Jean W.** Metformin-related vitamin B12 deficiency. *Age Ageing.* 2006;35:200–201.
17. **Liu Q, Li S, Quan H, Li J.** Vitamin B12 status in metformin treated patients: systematic review. *PLoS One.* 2014;9:e100379.
18. **Niafar M, Hai F, Porhomayon J, Nader ND.** The role of metformin on vitamin B12 deficiency: a meta-analysis review. *Intern Emerg Med.* 2015;10:93–102.
19. **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.
20. **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.
21. *Wulffelé 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.
22. **Carlsen SM, Kjøtrød S, Vanky E, Romundstad P.** Homocysteine levels are unaffected by metformin treatment in both nonpregnant and pregnant women with polycystic ovary syndrome. *Acta Obstet Gynecol Scand.* 2007;86:145–150.
23. **Gatford KL, Houda CM, Lu ZX, et al.** Vitamin B12 and homocysteine status during pregnancy in the metformin in gestational diabetes trial: responses to maternal metformin compared with insulin treatment. *Diabetes Obes Metab.* 2013;15:660–667.
24. **Greibe E, Trolle B, Bor MV, Lauszus FF, Nexo E.** Metformin lowers serum cobalamin without changing other markers of cobalamin status: a study on women with polycystic ovary syndrome. *Nutrients.* 2013;5:2475–2482.
25. **Kilicdag EB, Bagis T, Tarim E, et al.** Administration of B-group vitamins reduces circulating homocysteine in polycystic ovarian syndrome patients treated with metformin: a randomized trial. *Hum Reprod.* 2005;20:1521–1528.
26. **Knowler WC, Barrett-Connor E, Fowler SE, et al.** Reduction in the incidence of type 2 diabetes with lifestyle intervention or metformin. *N Engl J Med.* 2002;346:393–403.
27. *Diabetes Prevention Program Research Group,* Knowler WC, Fowler SE, Hamman RF, et al. 10-year follow-up of diabetes incidence and weight loss in the Diabetes Prevention Program Outcomes Study. *Lancet.* 2009;374:1677–1686.
28. **Marks PW, Zukerberg LR.** Case records of the Massachusetts General Hospital. Weekly clinicopathological exercises. Case 30–2004. A 37-year-old woman with paresthesias of the arms and legs. *N Engl J Med.* 2004;351:1333–1341.
29. **Centers for Disease Control and Prevention.** Second National Report on Biochemical Indicators of Diet and Nutrition in the U.S. Population, 2012. http://www.cdc.gov/nutritionreport/pdf/Nutrition_Book_complete508_final.pdf. Accessed January 11, 2016.
30. **Feldman EL, Stevens MJ, Thomas PK, Brown MB, Canal N, Greene DA.** A practical two-step quantitative clinical and electrophysiological assessment for the diagnosis and staging of diabetic neuropathy. *Diabetes Care.* 1994;17:1281–1289.
31. **Calvo Romero JM, Ramiro Lozano JM.** Vitamin B(12) in type 2

diabetic patients treated with metformin. *Endocrinol Nutr*. 2012; 59:487–490.

32. **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.

33. **Obeid R, Jung J, Falk J, et al.** Serum vitamin B12 not reflecting vitamin B12 status in patients with type 2 diabetes. *Biochimie*. 2013; 95:1056–1061.

34. **Greibe E, Miller JW, Foutouhi SH, Green R, Nexo E.** Metformin increases liver accumulation of vitamin *B12 - an experimental study in rats. Biochimie*. 2013;95:1062–1065.

35. **Caspary WF, Zavada I, Reimold W, Deuticke U, Emrich D, Willms B.** Alteration of bile acid metabolism and vitamin-B12-absorption in diabetics on biguanides. *Diabetologia*. 1977;13:187–193.

36. **Berchtold P, Dahlqvist A, Gustafson A, Asp NG.** Effects of a biguanide (metformin) on vitamin B 12 and folic acid absorption and intestinal enzyme activities. *Scand J Gastroenterol*. 1971;6:751–754.

37. **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.

38. **Bell DS.** Metformin-induced vitamin B12 deficiency presenting as a peripheral neuropathy. *South Med J*. 2010;103:265–267.

39. **Long AN, Atwell CL, Yoo W, Solomon SS.** Vitamin B(12) deficiency associated with concomitant metformin and proton pump inhibitor use. *Diabetes Care*. 2012;35:e84.

40. **Pierce SA, Chung AH, Black KK.** Evaluation of vitamin B12 monitoring in a veteran population on long-term, high-dose metformin therapy. *Ann Pharmacother*. 2012;46:1470–1476.