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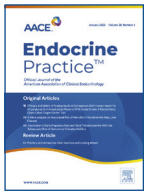

Original Article

Effect of Metformin Use on Vitamin B12 Deficiency Over Time (EMBER): A Real-World Evidence Database Study

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ABSTRACT

Objective: To examine the extent to which metformin increases the risk of vitamin B12 deficiency and borderline deficiency over time in participants with type 2 diabetes mellitus (T2DM).
Methods: Using the All of Us database, adults aged ≥18 years with T2DM and a documented history of metformin use were included for the evaluation of B12 deficiency. Those with B12 deficiency before metformin use were excluded. Adjusted logistic regression models were used to evaluate the association between metformin use and long-term metformin use (≥4 years) and the risk of B12 deficiency. We conducted a subgroup analysis comparing differences in borderline B12 deficiency in metformin and non-metformin users.
Results: Of 36 740 participants with T2DM, 6221 (16.9%) had documented metformin use. The mean age of metformin users was 65.3 years. B12 deficiency was confirmed in 464 (7.5%) metformin users, and 1919 of 30 519 participants (6.3%) did not use metformin. Metformin users had a 4.7% increased risk of developing B12 deficiency compared with nonmetformin users ($P = .44$). Each additional year of metformin use was associated with 5% increased likelihood of deficiency ($P < .05$). Metformin use for ≥4 years resulted in a 41.0% increased odds of B12 deficiency, compared with those who used <4 years of metformin ($P < .05$). Metformin use increased the odds of borderline B12 deficiency by 27.0% ($P < .05$).
Conclusion: Long-term metformin use was associated with an increased risk of B12 deficiency in patients with T2DM, with compounding risk over time.

Introduction

Treatment for type 2 diabetes mellitus (T2DM) generally includes comprehensive lifestyle modification in addition to metformin use, which is frequently used for a patient's entire lifetime.[1,2] It is estimated that over 20 million people are prescribed metformin in the United States, representing a majority of the estimated 27 million people diagnosed with T2DM.[3-9] Metformin use has been associated with reductions in the serum cobalamin (vitamin B12) level, defined as <400 pg/mL.[10-16] Reductions in the vitamin B12 levels are both time- and dose-dependent, with the most significant risk of deficiency associated with long-term and high-dose use.[17-19] In addition, the duration of metformin use and total daily dose are important predictors of reduced serum vitamin B12 levels and vitamin B12 deficiency.[11-13] Other factors, such as older age, female sex, vegetarian diet, and concomitant use of proton pump inhibitors (PPIs), have been found to further increase the risk of B12 deficiency.[11,13,19] Vitamin B12 status is most often evaluated using the serum vitamin B12 levels; however, high methylmalonic acid or low homocysteine levels can also be valuable, particularly in assessing those with borderline serum levels.[12,13,19]

Abbreviations: AoU, All of Us; CI, confidence interval; EHR, electronic health record; NIH, National Institutes of Health; OR, odds ratio; PPI, proton pump inhibitor; T2DM, type 2 diabetes mellitus.

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Metformin users with significant vitamin B12 deficiency have been shown to have an increased incidence of neuropathy.[11,19] This is of particular concern because vitamin B12 deficiency–related neuropathy may mimic diabetic neuropathy and is often irreversible.[11,16,19,20] Unmitigated neuropathic complications may progress to permanent peripheral or autonomic nerve damage, resulting in pain, numbness, weakness in the extremities, or cardioneuropathies.[13,19-21] An estimated 50% of people with T2DM and peripheral neuropathy will further develop a foot ulcer in their lifetime, which may lead to amputation.[22] For these reasons, the early identification of deficiency and borderline deficiency is vitally important. Symptoms of neuropathy in people with diabetes are also often associated with poorer reported quality of life, often due to the unpredictability of neuropathic symptom occurrence.[23] Oral vitamin B12 supplementation has been found to overcome metformin-associated reduction in the serum vitamin B12 levels and to have an impact on peripheral neuropathy measures.[19,24] Vitamin B12 supplementation may also contribute to the treatment of borderline deficiencies because symptoms can arise ahead of overt serum deficiency.[19] However, unnecessary oral vitamin B12 supplementation may negatively affect people with T2DM due to increased pill burden and polypharmacy, which may result in reduced adherence overall to medication.[25]

Although much is known regarding the relationship between metformin use and physiologic vitamin B12 status, the population prevalence and onset of metformin-induced vitamin B12 deficiency among people with T2DM are yet to be fully elucidated. Although previously published literature on the topic includes a number of retrospective studies, clinical trials, systematic reviews, and meta-analyses, the present study is unique because of its large sample size and use of real-world electronic health record (EHR) data, which enabled the examination of the long-term impact of metformin on a diverse study population. In addition, metformin is the standard of care for most patients with diabetes, which presents a significant challenge to designing placebo-controlled clinical trials. Finally, clinical guidelines do not provide clear recommendations for the initial screening and frequency of monitoring of vitamin B12 status in patients who use metformin.

Our primary aim was to examine the extent of B12 deficiency in patients with T2DM as a result of metformin use over time. Our secondary aim was to evaluate the relationship between metformin use and serum vitamin B12 borderline deficiency. An improved understanding of these areas may assist in clinical decision-making with regard to identifying patients most likely to benefit from screening to mitigate the effects of metformin-induced deficiency.

Highlights

- The prevalence of metformin-induced B12 deficiency has not been well described
- Metformin use for ≥4 years increases the risk of B12 deficiency by 41.0%
- For every additional year of metformin use, the risk of B12 deficiency increases by 5%

Clinical Relevance

Consistent testing for Vitamin B12 deficiency is currently not recommended for people with type 2 diabetes using metformin. Here, we demonstrated that metformin users (particularly those using metformin for 4 or more years) are at an increased likelihood of reporting either borderline B12 deficiency or B12 deficiency itself, adjusting for other factors. Future investigators may want to assess whether this has an appreciable impact on negative clinical outcomes, such as neuropathy.

Methods

The All of Us Research Platform

The All of Us (AoU) Research Program is a collaborative effort led by the National Institutes of Health (NIH) as part of the Precision Medicine Initiative.[26] The NIH AoU Research Program is open to enrollment for all eligible adults living in the United States. Participants may sign up directly from the NIH "All of Us" Research Program website or through a participating health care organization. This unique database is composed of EHRs, survey data, biosamples, genomic data and other information for participants in every U.S. state. The AoU intentionally oversamples from racial and ethnic minorities with a focus on those who are historically underrepresented in biomedical research and has a goal of eventually enrolling over 1 million diverse participants. At the time this work was completed, the database contained EHRs from more than 300 000 participants. A combination of EHR and survey-based data from the AoU research database was used for this research.[26] For this study, we used the person, survey, measurement (laboratory data), conditions, and drug data tables.

This analysis was performed using the AoU Researcher Workbench, a cloud-based platform where researchers may access and analyze AoU data. The Researcher Workbench contains user interfaces to generate study cohorts ("Cohort Builder") and datasets for analysis ("Dataset Builder") and Workspaces to analyze data using the Jupyter platform ("Notebooks") using either R or Python programming languages.

Study Population

We identified participants aged ≥18 years at the time of initial AoU enrollment with the Systematized Nomenclature of Medicine diagnosis code 440545006 or 313436004 (T2DM with or without complications) using the conditions data table. These participants must have had at least 1 indication of a serum vitamin B12 measurement result from the measurements data table after starting metformin. Serum vitamin B12 measurements were identified using Logical Observation Identifiers Names and Codes 2132-9, 14685-2, 16695-9, and LP31689-0. We further divided the participants into 2 groups: (1) those with metformin drug data and (2) those without. Metformin use was identified using the RXNORM code 6809. For those reporting metformin drug data, we included those with at least 3 reported orders within 1 year. We excluded any metformin users who did not have a B12 measurement after their first metformin order. We additionally excluded participants with B12 measurements whose units were either not applicable or invalid (eg, "no matching concept" and "inches/US"). In addition to assessing the association between the number of years on metformin and B12 deficiency, we compared "long-term" metformin users with "short-term" metformin users. Yearly metformin use was defined by the presence of at least 1 metformin order in a calendar year (consecutive or nonconsecutive) and totaled for all years of reported use. "Long-term" metformin use was defined as reported use for ≥4 years.[11,13]

Primary Outcome: Vitamin B12 Deficiency

The primary outcome of this study was vitamin B12 deficiency, defined as <200 pg/mL. We further defined the categories of

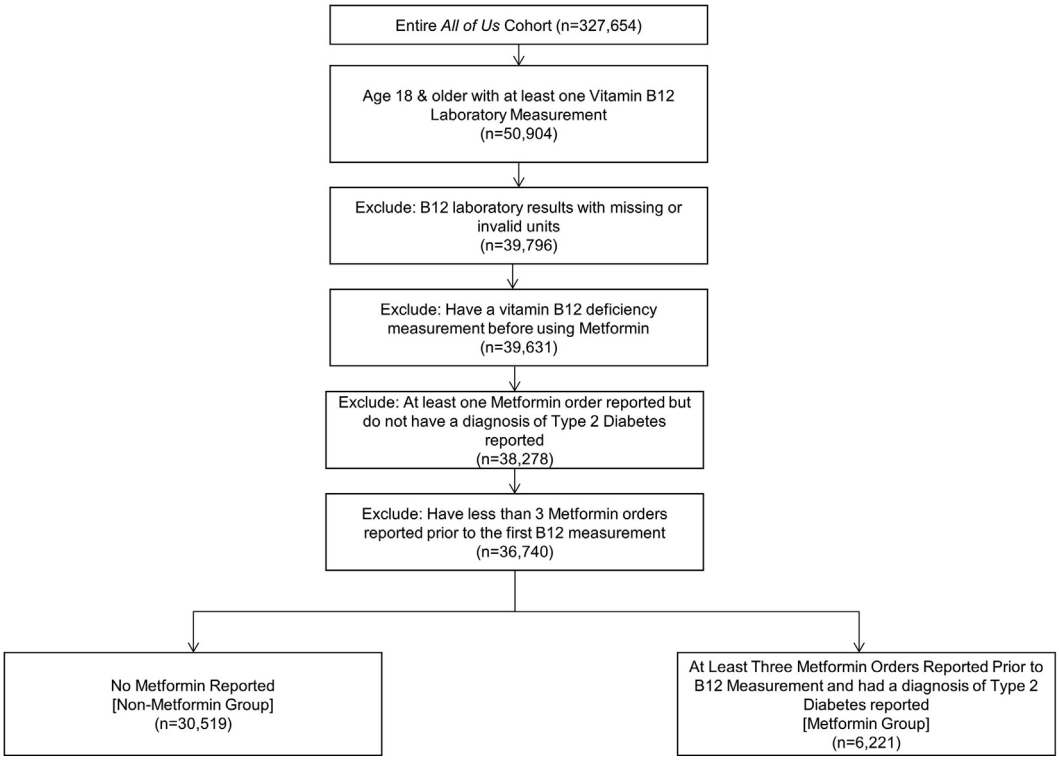

Figure. Cohort selection diagram. Flowchart displaying the final study cohort selection criteria using the *All of Us* database.

borderline (200-400 pg/mL), normal (>400 to 1000 pg/mL), high (>1000 to 2000 pg/mL), and extreme (>2000 pg/mL) B12 levels, according to published literature.[27,28]

Covariates

Patient-level sociodemographic data included age, race, ethnicity, sex, education level, marital status, insurance status, annual income, self-reported overall health, vitamin B12 supplement use, PPI use, and years of metformin, vitamin B12 supplements, and PPI use. Vitamin B12 supplement use was defined as any order for an oral or injectable vitamin B12 supplement 1 year before or after metformin use using the Anatomical Therapeutic Chemical code B03BA. PPI use was included in this study as any reported order of dexlansoprazole, esomeprazole, lansoprazole, omeprazole, pantoprazole, or rabeprazole before or during metformin use. A full list of the RXNORM codes for these drugs and codes used for other cohort definition criteria with their corresponding OMOP concept IDs can be found in the Supplementary Table. We used EHR diagnosis data to derive a Charlson Comorbidity Index score for each participant using a previously published algorithm.[29]

Statistical Analysis

Descriptive statistics were evaluated using means, medians, standard deviations, minimums, and maximums for continuous variables and counts with frequencies for categorical variables. Comparative statistical tests were performed for the included demographic variables first between long-term and short-term metformin users and again between metformin and non-metformin users. Nonparametrically distributed continuous variables were compared using the Mann-Whitney *U* test; normally distributed continuous variables were compared using Student's *t* test. Categorical variables were compared using the chi-square tests. To assess for the association between the length of time on metformin use and B12 deficiency, 2 logistic regression models were fitted: (1) a model assessing the impact of the total number of years of metformin on B12 deficiency and (2) another assessing the impact of long-term metformin use (at least 4 years) on B12 deficiency. As a secondary aim, we tested the association between metformin use status (yes/no) and B12 deficiency using an adjusted logistic regression model. As another secondary aim, the same logistic regression models were fitted to assess the impact on B12 borderline deficiencies. All models were adjusted for potential confounding variables including age, sex, race, ethnicity, education, marital status, insurance status, annual income, overall health, number of years of PPI use, and number of years of B12 supplement use. All statistical analyses were performed with an alpha level of 0.05 using R in the online Jupyter platform notebook supported by the NIH AoU platform.[29] This study was exempt from Institutional Review Board approval because of the use of deidentified data.

Results

A total of 36 740 participants were included in the final analysis after applying exclusion criteria (Fig.). Of these, 6 221 (16.93%) qualified as metformin users (MET group), and 30 519 (83.07%) did not (non-MET group). The MET group had a higher mean age (65.3 vs 60.1 years, $P < .05$), more Black or African American participants (28.9% vs 20.7%, $P < .05$) and Hispanic or Latino participants (20.1% vs 13.7%, $P < .05$), and a higher proportion of males (38.4% vs 32.1%, $P < .05$) than the non-MET group (Table 1). The MET group had fewer participants who were college graduates than the non-MET group (31.4% vs 45.8%, $P < .05$). Fewer participants in the MET group were also married in comparison (39.3% vs 42.8%, $P < .05$) but were approximately equal in proportion of those reporting any health

Table 1
Cohort Demographic Characteristics

Variable	Full cohort (N = 36 740)		Metformin (N = 6221)		Non-metformin (N = 30 519)		*P* value
	N	%	N	%	N	%	
Age (categorical)							<.05
18-24	192	0.5	NR[a]	0.08	187	0.61	
25-39	4084	11.1	179	2.88	3905	12.8	
40-49	4316	11.7	409	6.57	3907	12.8	
50-64	11 033	30.0	2105	33.8	8928	29.3	
65-74	9568	26.0	2117	34	7451	24.4	
≥75	7547	20.5	1406.0	22.6	6141	20.1	
Race							
Asian American or Pacific Islander	817	2.2	139	2.23	678	2.22	<.05
Black or African American	8124	22.1	1799	28.9	6325	20.7	
White	20 982	57.1	2747	44.2	18 235	59.7	
Other	669	1.8	126	2.03	543	1.78	
Ethnicity							
Hispanic or Latino	5442	14.8	1253	20.1	4189	13.7	<.05
Sex							
Female	24 054	65.5	3742	60.2	20 312	66.6	<.05
Education							
Less than a high school degree or equivalent	3223	8.8	866	13.9	2357	7.72	<.05
Grade 12 or GED	6690	18.2	1390	22.3	5300	17.4	
College years 1-3	10 110	27.5	1857	29.9	8253	27	
College graduate or advanced degree	15 928	43.4	1956	31.4	13 972	45.8	
Marital status							
Married	15 511	42.2	2442	39.3	13 069	42.8	<.05
Living with partner	1828	5.0	234	3.76	1594	5.22	
Divorced	6404	17.4	1246	20	5158	16.9	
Never married	8017	21.8	1231	19.8	6786	22.2	
Separated	1301	3.5	271	4.36	1030	3.37	
Widowed	2792	7.6	632	10.2	2160	7.08	
Insurance status							
Yes	34 746	94.6	5857	94.1	28 889	94.7	.046
Annual income							
<$10 000	4813	13.1	993	16	3820	12.5	<.05
$10 000-$25 000	5255	14.3	1130	18.2	4125	13.5	
$25 000-$35 000	2686	7.3	507	8.15	2179	7.14	
$35 000-$50 000	3055	8.3	542	8.71	2513	8.23	
$50 000-$75 000	3929	10.7	597	9.6	3332	10.9	
$75 000-$100 000	2818	7.7	371	5.96	2447	8.02	
$100 000-$150 000	3325	9.1	381	6.12	2944	9.65	
$150 000-$200 000	1448	3.9	129	2.07	1319	4.32	
>$200 000	2074	5.6	166	2.67	1908	6.25	
Self-reported overall health							
Excellent	5687	15.5	571	9.18	5116	16.8	<.05
Very good	11 558	31.5	1565	25.2	9993	32.7	
Good	11 939	32.5	2400	38.6	9539	31.3	
Fair	5676	15.4	1309	21	4367	14.3	
Poor	996	2.7	208	3.34	788	2.58	
Vitamin B12 supplement use							
Yes	9745	26.5	2009	32.3	7736	25.3	<.05
PPI use							
Yes	21 509	58.5	4451	71.5	17 058	55.9	<.05
Charlson Comorbidity Index score (mean and SD)	4.5 (3.7)		7.1 (3.7)		3.9 (3.5)		<.05

Abbreviations: GED = General Educational Development; PPI = proton pump inhibitor; SD = standard deviation.
[a] NR indicates not reportable because of the sample size reporting restrictions.

insurance coverage ($P = .046$). Metformin was used for a mean of 6.2 years among participants in the MET group (min, 1.0 years; median, 5.0 years; and max, 25.0 years) from 1995 to 2022.

Self-Reported Outcomes: Physical Health and Medication History

Fewer participants in the MET group reported having "Excellent" or "Very Good" overall self-reported physical health status (9.2% vs 16.8%, $P < .05$; 25.2% vs 32.7%, $P < .05$). Participants in the MET group reported a higher mean Charlson Comorbidity Index score (7.1 vs 3.9, $P < .05$). Before the first B12 measurement reported, participants in the MET group reported a higher mean number of vitamin B12 supplement orders (3.7 vs 2.5, $P < .05$), with a slightly higher mean number of years of B12 supplement use (0.8 vs 0.6, $P < .05$). The participants in the MET group also had a higher mean number of PPI orders before the first B12 measurement (21.7 vs 11.4, $P < .05$), with a higher mean number of years of PPI use (3.9 vs 2.2 years, $P < .05$).

Table 2
Serum Vitamin B12 Level Category Stratified by Metformin Use

Level	Metformin		No metformin	
	N	%	N	%
Extreme (>2000 pg/mL)	37	0.59	200	0.66
High (>1000 to 2000 pg/mL)	777	12.5	3740	12.3
Normal (>400 to 1000 pg/mL)	2227	35.8	12 961	42.5
Borderline (200-400 pg/mL)	2716	43.7	11 699	38.3
Deficient (<200 pg/mL)	464	7.46	1919	6.29

Table 3
Adjusted Logistic Regression Results on the Risk of Vitamin B12 Deficiency by the Duration of Metformin Use, Presence of Metformin, and Long-Term Metformin Use

Predictor variable	Odds ratio	Standard error	*P* value	95% confidence interval
Years of metformin use[a]	1.05	0.013	<.05	1.03-1.08
Metformin use[b]	1.05	0.06	.44	0.93-1.18
Long-term metformin use[c]	1.35	0.12	<.05	1.11-1.80

[a] The years of metformin use were evaluated as a continuous variable.
[b] Metformin use was either yes or no.
[c] Long-term use was defined as ≥6 reported years of metformin use.

Baseline Vitamin B12 Measurements

The MET group had more participants with serum vitamin B12 deficiency than the non-MET group (7.46% vs 6.29%, $P < .05$), in addition to borderline deficiency results (43.7% vs 38.3%, $P < .05$). Participants in the non-MET group had a higher number of vitamin B12 results considered "Normal" (35.8% vs 42.5%, $P < .05$) (Table 2).

Impact of Metformin on Vitamin B12 Level

Compared with the participants in the non-MET group, those in the MET group as a whole had a non-significant 5% increased odds of 1 or more vitamin B12 deficiency lab results (odds ratio [OR], 1.05; 95% confidence interval [CI], 0.93-1.18; $P = .44$) (Table 3). Of the participants in the MET group, 4340 (69.76%) were considered to be long-term users ($P < .05$). Of these, 8.43% had a vitamin B12 deficiency measurement, compared with 5.21% among the short-term users. Each year of MET use was found to increase the odds of a vitamin B12 deficiency result by 5.0% (OR, 1.05; 95% CI, 1.03-1.08; $P < .05$). Furthermore, long-term metformin use was associated with a 41% increased likelihood of a vitamin B12 deficiency lab result compared with short-term metformin use (OR, 1.41; 95% CI, 1.11-1.80; $P < .05$).

Participants in the MET group were also at a 27% significantly increased odds of experiencing a vitamin B12 borderline deficiency result compared with those in the non-MET group (OR, 1.27; 95% CI, 1.20-1.35; $P < .05$). Each year of metformin use was associated with a 2.2% increased odds of a vitamin B12 borderline deficiency result (OR, 1.02; 95% CI, 1.01-1.04; $P < .05$). Compared with short-term users, long-term metformin users were at a 20% increased odds of a vitamin B12 borderline deficiency result (OR, 1.20; 95% CI, 1.07-1.35; $P < .05$).

Discussion

This preliminary analysis represents the first use of a large real-world database of participants in the United States to examine the relationship between metformin use and vitamin B12 deficiency.

The relationship between metformin use and vitamin B12 deficiency was evident when we compared the vitamin B12 levels of the participants in the MET group with those of the participants in the non-MET group, where those in the MET group demonstrated consistently higher proportions of borderline deficient or deficient vitamin B12 levels. This finding is consistent with the published literature, including a 2019 meta-analysis that evaluated the association between metformin use and the vitamin B12 levels of more than 8000 patients from 31 studies. In this study, the authors concluded that patients taking metformin were twice as likely to have an increased risk of vitamin B12 deficiency with a significantly greater percentage decrease in the serum vitamin B12 levels.[16] Therefore, the possibility of a drug-induced problem should be considered when monitoring therapy or investigating the cause of B12 deficiency among people who take metformin. In people with additional risk factors such as older age, vegetarian diet, or PPI use, screening for vitamin B12 deficiency even in the absence of symptoms or clinical suspicion may be warranted.

A critical finding of our study was the association between the duration of metformin use and risk of vitamin B12 deficiency wherein participants with a long-term history of metformin use were more likely to experience deficiency, and each additional year of use resulted in further increased risk. This adds to what is known from previously published trials and meta-analyses that indicate that metformin-associated vitamin B12 deficiency is a time-dependent phenomenon that is hypothesized to be related to liver stores of vitamin B12 that take years to be depleted in most people. This may also explain the lack of significant association between metformin use itself and deficiency. The strong association between the longer duration of metformin use and risk of deficiency can help to inform recommendations for cost-effective screening, monitoring, and treatment. Although these measures, including B12 supplementation, are likely to be low risk, it is important to consider direct and indirect patient costs. It is necessary to ensure that decisions regarding laboratory testing and patient-related considerations, such as increased pill burden, are informed by evidence.

Our study demonstrated that the risk of vitamin B12 deficiency was strongly associated with an increase in age. Although older age is often associated with vitamin B12 deficiency because of the increased risk of atrophic gastritis-associated food-cobalamin malabsorption and pernicious anemia in elderly patients, the use of metformin has shown to compound the severity of vitamin B12 deficiency.[16,30,31] In a retrospective study that examined 1996 institutionalized elderly patients already diagnosed with vitamin B12 deficiency, the serum vitamin B12 levels of patients taking metformin were significantly lower than those of patients who were not taking metformin. In addition, a subanalysis of this study showed that vitamin B12 deficiency was 3 times more prevalent among elderly patients who took metformin for more than 2 years.[32] This association appears to be consistent, where other studies have noted an increase in the risk of B12 deficiency among patients who took metformin for more than 4 years.[10,15,33]

Our findings further support the association between metformin use and vitamin B12 levels. The clinical sequelae of borderline vitamin B12 deficiency are less well described but usually considered similar to frank vitamin B12 deficiency.[34] Our study population had a much larger cohort of metformin users with borderline vitamin B12 deficiency than with frank vitamin B12 deficiency, which could suggest a large population with some symptoms of vitamin B12 deficiency. Our results could also indicate that some publications underreport the impact of metformin on the vitamin B12 levels. Although not all patients with borderline vitamin B12 deficiency may progress to frank vitamin B12 deficiency, these

results merit further discussion regarding regular monitoring of the B12 levels in patients taking metformin.

Our retrospective study has several limitations. First, 83.0% of our cohort with T2DM did not have a record of metformin use, which may introduce selection bias. This is likely because drug use data in AoU are derived from EHR (health system) data; hence, participants with metformin dispensed outside of the patient's health system (eg, a retail pharmacy) would not be reported in the AoU dataset. We hypothesize that the majority of AoU participants obtained their metformin in this manner. In addition, the MET cohort had a higher proportion of male participants and higher rate of vitamin B12 supplement use, which may attenuate an association directly related to metformin use. It is difficult to speculate on the likely reasons for an increased prevalence of supplement use; however, provider recommendation and counseling may have played a role. On the other hand, the MET group was, on average, older with lower self-reported health and higher comorbidity index scores and had higher rates of PPI use. Together, these factors make the assessment of risk difficult when comparing the MET and non-MET cohorts. We assumed that if a participant reported one order of metformin per year, then that patient took metformin for the entire year. Volunteer participants in the AoU program may also differ from the majority of people with T2DM, which may introduce bias in this study that we could not account for. Finally, our results cannot be assumed to be national estimates because weighted estimates are not included in the AoU data.

Conclusion

There is an association between long-term metformin use and serum vitamin B12 deficiency among adults with T2DM. Future research should include further analysis of the time course of metformin-associated serum vitamin B12 reductions and deficiency, examination of the relationship between metformin-associated vitamin B12 deficiency and neuropathies, and evaluation for demographic disparities in screening, monitoring, and treatment of low vitamin B12 levels in patients taking metformin.

Disclosure

The authors have no multiplicity of interest to disclose.

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