Association of Metformin, Elevated Homocysteine, and Methylmalonic Acid Levels and Clinically Worsened Diabetic Peripheral Neuropathy

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OBJECTIVE — The severity of peripheral neuropathy in diabetic patients varies for unclear reasons. Long-term use of metformin is associated with malabsorption of vitamin B_{12} (cobalamin [Cbl]) and elevated homocysteine (Hcy) and methylmalonic acid (MMA) levels, which may have deleterious effects on peripheral nerves. The intent of this study was to clarify the relationship among metformin exposure, levels of Cbl, Hcy, and MMA, and severity of peripheral neuropathy in diabetic patients. We hypothesized that metformin exposure would be associated with lower Cbl levels, elevated Hcy and MMA levels, and more severe peripheral neuropathy.

RESEARCH DESIGN AND METHODS — This was a prospective case-control study of patients with type 2 diabetes and concurrent symptomatic peripheral neuropathy, comparing those who had received >6 months of metformin therapy ($n = 59$) with those without metformin exposure ($n = 63$). Comparisons were made using clinical (Toronto Clinical Scoring System and Neuropathy Impairment Score), laboratory (serum Cbl, fasting Hcy, and fasting MMA), and electrophysiological measures (nerve conduction studies).

RESULTS — Metformin-treated patients had depressed Cbl levels and elevated fasting MMA and Hcy levels. Clinical and electrophysiological measures identified more severe peripheral neuropathy in these patients; the cumulative metformin dose correlated strongly with these clinical and paraclinical group differences.

CONCLUSIONS — Metformin exposure may be an iatrogenic cause for exacerbation of peripheral neuropathy in patients with type 2 diabetes. Interval screening for Cbl deficiency and systemic Cbl therapy should be considered upon initiation of, as well as during, metformin therapy to detect potential secondary causes of worsening peripheral neuropathy.

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Diabetes is an increasingly prevalent disorder with a range of systemic complications including diabetic peripheral neuropathy (DPN), which occurs in up to 50% of diabetic patients and causes sensory, motor, and/or autonomic dysfunction (1). Several pathogenic mechanisms contribute to DPN severity, including microangiopathy, oxidative stress, polyol flux, mitochondrial dysfunction, insulin deficiency, and advanced glycation end products and ligand activation of their receptor (2–5). The course and severity of DPN are further affected by a wide range of comorbid conditions.

Vitamin B_{12} (cobalamin [Cbl]) deficiency may co-occur with diabetes. Although it is most classically associated with subacute combined degeneration, an exclusive peripheral neuropathy presentation can occur, typically manifesting as axonal neuropathy based on electrophysiology and pathology (6–8). Accumulating evidence suggests that Cbl-associated metabolites methylmalonic acid (MMA) and homocysteine (Hcy) are more sensitive (MMA and Hcy) and specific (MMA) indicators of early symptomatic Cbl deficiency than serum Cbl itself (9,10).

Metformin, a biguanide, is perennially reported as a pharmacological cause of Cbl deficiency (11–13). The responsible mechanism has been controversial; proposed contributors have included competitive inhibition or inactivation of Cbl absorption, alterations in intrinsic factor levels, bacterial flora, gastrointestinal motility, or ileal morphological structure, and interaction with the cubulin endocytic receptor (11,14,15). Biguanides have recently been shown to impair calcium-dependent membrane activity in the ileum, including uptake of the Cbl-intrinsic factor complex (16).

Metformin is recommended by the American Diabetes Association and the European Association for the Study of Diabetes as initial medical therapy for type 2 diabetes at diagnosis (17). Despite its wide use and its known effects on Cbl, metformin has not been systematically studied as a potential iatrogenic cause of or contributor to DPN. The potentially reversible effect of cobalamin deficiency may increase the clinical burden for a population of patients with DPN whose sensory function, gait, and balance frequently are already compromised.

We designed a prospective case-control study to assess the effects of prolonged metformin intake in patients with type 2 diabetes matched for disease duration and disease control. We specifically examined the relationship among metformin use, levels of Cbl and its metabolites, and clinical and electrophysiological markers of peripheral neuropathy severity. We hypothesized first that metformin use would be associated with biochemical evidence of Cbl deficiency (lower serum Cbl levels and elevated MMA and Hcy) and second that metformin use would be associated with more severe peripheral neuropathy. Decreases in Cbl have been shown to depend on the dose and duration of metformin therapy in a previous case-control study (18); this finding led

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us to further hypothesize that biochemical abnormalities and severity of neuropathy would correlate with cumulative lifetime metformin dose.

RESEARCH DESIGN AND METHODS

— Ethical approval for this study was received from the University of Calgary Centre for Advancement of Health. From December 2002 until May 2007, patients with preexisting type 2 diabetes and a primary diagnosis of peripheral neuropathy were assessed within the Neuromuscular Clinic at the University of Calgary. These patients then underwent further clinical, laboratory, and electrophysiological evaluation of their neuropathy. The presence of diabetes was verified by two separate positive results: two prior fasting glucose results of ≥7.1 mmol/l (126 mg/dl) or two oral glucose tolerance tests leading to a 2-h serum glucose of ≥11.1 mmol/l (200 mg/dl) (based on Canadian Diabetes Association guidelines). The age of diagnosis of diabetes and the duration of symptoms of DPN were recorded. History of other systemic illnesses, alcohol use, toxin and medication exposures, and family history of neuropathy was documented to assess for other potential causes of peripheral neuropathy. The duration of metformin therapy and dosage history were determined for each patient by review of their medical record, and these dosages were confirmed verbally by the patients; these data were used to calculate a cumulative lifetime dose of metformin for each patient. Use of other antidiabetic agents was recorded.

All patients underwent laboratory testing including a complete blood count, electrolytes, urea, creatinine, alanine aminotransferase, aspartate aminotransferase, γ-glutamyltranspeptidase, alkaline phosphatase, albumin, total bilirubin, international normalized ratio, thyroid-stimulating hormone, erythrocyte sedimentation rate, antinuclear antibody, extracted nuclear antibody, serum protein electrophoresis, rheumatoid factor, lactate, and serum folate. A1C was measured in all patients. The sensitivity for detection of gammopathy in our center is 2 g/l by serum protein electrophoresis, with immunofixation performed when peaks are found in the range of 2–4 g/l. Serum Cbl levels were measured by cobas e immunoassay analysis (Roche Diagnostics), Hcy levels were measured using high-performance liquid chromatography, and MMA levels were measured using mass spectrometry for all patients. The lower limit of normal for Cbl in our center is 210 pmol/l (285 pg/ml). The upper limit for Hcy is 13.7 μmol/l (1.85 mg/l) in adult men, 9.9 μmol/l (1.34 mg/l) in adult women aged ≤49 years and 12.8 μmol/l (1.73 mg/l) in adult women aged >49 years; here we adopt a conservative upper limit of 13.7 μmol/l (1.85 mg/l) for all participants. The upper limit of normal for MMA in our center is 0.15 μmol/l. All blood testing was performed by Calgary Laboratory Services.

Patients were excluded if potential causes for peripheral neuropathy other than diabetes and Cbl deficiency were identified, if they had previously been treated with metformin and had discontinued therapy, if they had received <6 months of metformin treatment at the time of assessment, if they had impaired glucose tolerance only, or if they had juvenile onset of diabetes or a frank requirement for insulin at diagnosis (i.e., possible type 1 diabetes). Last, patients were excluded if they refused concurrent electrophysiological or laboratory testing. We did not exclude patients with renal failure concurrently using metformin, although renal failure is often considered a contraindication to metformin use because of the potential for lactic acidosis; however, many patients do not discontinue metformin use after diagnosis of renal impairment (19), and lactate levels were normal in all patients.

Complete standardized neurological examinations were performed in all patients with DPN, including tone, power, deep tendon reflexes, sensory function, Romberg testing, gait, and tandem gait. Tandem gait was recorded as the number of heel to toe steps along a straight line the patient could perform to a normal threshold value of 6. Each patient was also scored using the Toronto Clinical Scoring System (TCSS) (20) by an unblinded investigator before knowledge of laboratory results. The TCSS was developed as a clinical screening tool for the presence and severity of DPN that emphasizes sensory deficits. Although it introduces some subjectivity in scoring, it has been validated by sural nerve fiber density. We also determined the Neuropathy Impairment Score (NIS), a scale scoring weakness of groups of muscles of the head and neck, upper limbs, and lower limbs; tactile, vibratory, and joint position sensation; pinprick sensation of index fingers and great toes; and reflexes, for each patient (21).

Electrophysiological assessment was performed after clinical assessment and before knowledge of the laboratory results using a Dantec Datapoint (Dantec Dynamics, Bristol, U.K.). Sensory and motor nerves of the nondominant upper and lower extremities were tested within 3 months of clinical assessment. Motor nerve conduction studies (NCSs) were performed using stimulation of the median nerve (wrist and elbow), ulnar nerve (wrist, below elbow, and above elbow), peroneal nerve (ankle and below fibular head and above fibular head locations), and tibial nerve (ankle and popliteal fossa locations). For each motor nerve, distal motor latencies, compound motor action potentials, and conduction velocities were obtained or calculated. F wave latencies were obtained from median, ulnar, peroneal, and tibial nerves. Sensory NCSs were performed using the median (digits 2 and 4), ulnar (digits 4 and 5), superficial radial, superficial peroneal, and sural nerves with sensory nerve action potentials (SNAPs), onset latency, and conduction velocity obtained or calculated. Temperatures were maintained at ≥32°C for the upper extremities and ≥30°C for the lower extremities during NCS testing. Although all participants completed electrophysiological testing, some participants do not have complete data for all individual nerves.

After all clinical, electrophysiological, and laboratory testing, monthly intramuscular Cbl was prescribed for those patients with abnormal Cbl, MMA, or Hcy levels. Sural nerve biopsies were done only in clinical situations when vasculitis or another serious cause of peripheral neuropathy was suspected.

Group equivalence for patient age, duration of type 2 diabetes, duration of peripheral neuropathy symptoms, A1C, and alcohol exposure were compared by independent-samples *t* tests; sex and proportion using other antidiabetic agents were compared by χ^2 test. Elements of the past medical history (e.g., iron deficiency anemia and hereditary spherocytosis) were broadly classified (e.g., hematological disease) and are summarized in Table 1; these were not compared statistically because of their heterogeneity. These elements of the history are detailed in the appendix (available online at http://care.diabetesjournals.org/cgi/content/full/dc09-0606/DC1). The primary outcome measures were Hcy, MMA, and Cbl blood levels, clinical neuropathy severity (TCSS and NIS), and electrophysiological markers of neuropathy; of the latter, we chose to test sensory NCS in the lower extremity (con-

Table 1—*Patient characteristics*

	Metformin-treated	Non–metformin-treated	*P*
n	59	63	
Demographic variables			
Age (years)	66.6 ± 11.9	64.8 ± 12.0	NS*
Female sex (%)	24 (41)	29 (46)	NS
Disease severity			
Duration of diabetes (years)	5.5 ± 3.3	4.7 ± 2.9	NS
Duration of peripheral neuropathy symptoms (years)	3.8 ± 2.1	3.8 ± 2.4	NS
A1C (%)	6.7 ± 1.0	6.8 ± 1.1	NS
Other diabetes pharmacotherapy			
Glyburide use	38 (64)	22 (35)	<0.001†
Gliclazide use	18 (31)	15 (24)	NS
Insulin therapy	7 (12)	29 (46)	<0.001†
Clinical history			
Alcohol exposure (drinks/week)	3.0 ± 4.5	2.2 ± 4.0	NS
History of first-degree relatives with peripheral neuropathy	4 (5)	0	
Rheumatological disease (osteoarthritis or rheumatoid arthritis)	7 (12)	4 (6)	
Elevated creatinine with renal failure	6 (10)	7 (11)	
Thyroid disease	11 (19)	16 (25)	
Hematologic disease	5 (8)	0	
Cancer	7 (12)	7 (11)	
Other illness	42 (71)	29 (46)	

Data are means ± SD or *n* (%). *NS at the $\alpha = 0.05$ level. †Significant at $\alpha = 0.05$ level for χ^2 test.

duction velocity and SNAP for superficial peroneal and sural nerves), as we felt these would be most in keeping with exacerbation due to Cbl deficiency. These data did not follow a normal distribution (by Shapiro-Wilk test), and comparisons were made using a Mann-Whitney *U* test. Proportions of patients with deficiency of Cbl and upregulation of Hcy and MMA were compared using χ^2 tests. Bivariate correlations of clinical and laboratory variables with cumulative metformin dose were calculated using a Spearman ρ test. Last, a linear regression analysis was performed using the NIS total score as the dependent variable and age, duration of diabetes, A1C, and presence of metformin exposure as explanatory variables.

RESULTS — Of 226 patients with type 2 diabetes and peripheral neuropathy assessed for eligibility, 104 patients were excluded; 55 had brief (<6 months) metformin exposure, 46 patients discontinued metformin before assessment, and 3 were unable to perform laboratory or electrophysiological testing. Of the 122 patients who were eligible for analysis, 59 (48%) received metformin therapy for >6 months (mean cumulative exposure 3,389.5 g, $\sigma = 2{,}560.6$ g); the remaining patients had no prior metformin exposure. There were no significant differences in demographic variables (age and sex) or disease severity (duration of type 2 diabetes, A1C, and duration of peripheral neuropathy symptoms) between the two groups (Table 1). A significantly higher number of patients in the metformin-treated group were concurrently treated with glyburide and significantly fewer with insulin (Table 1). There were no other notable differences in prescription medication use, presence of other systemic conditions, or alcohol exposure among groups (Table 1; details are available in the online appendix).

Analysis of laboratory testing is summarized in Table 2. Median serum Cbl was significantly lower in the metformin-treated group (231 vs. 486 pmol/l; $U = 299.0$, $P < 0.001$) with frank deficiency in 18 patients (31%) compared with 2 (3%) in the non–metformin-treated group ($P < 0.001$). Cumulative metformin dose was inversely correlated with serum Cbl (Spearman $\rho = -0.41$, two-tailed $P = 0.001$). Median fasting serum Hcy was significantly higher in the metformin-treated group (11.6 vs. 8.4 μmol/l; $U = 454.0$, $P < 0.001$) with upregulation of Hcy in 15 patients versus 1 patient ($P < 0.001$). Median MMA was significantly higher in the metformin-treated group (0.18 vs. 0.11 μmol/l; $U = 306.5$, $P < 0.001$) and upregulated in 43 of these patients compared with 7 patients with no metformin exposure ($P < 0.001$). Cumulative metformin dose was positively correlated with fasting serum Hcy (Spearman $\rho = 0.50$, two-tailed $P < 0.001$) and fasting serum MMA (Spearman $\rho = 0.37$, two-tailed $P = 0.005$) (Fig. 1).

Clinical and paraclinical markers of neuropathy severity are summarized in Table 2. The median TCSS total score was higher in the metformin-treated group (10 vs. 5; $U = 527.0$, $P < 0.001$), with a strong positive correlation to increasing cumulative metformin dose (Spearman $\rho = 0.80$, two-tailed $P < 0.001$). Median NIS total score was significantly higher in the metformin-treated group (10 vs. 4; $U = 408.0$, $P < 0.001$) and also had a strong positive correlation with increasing cumulative metformin dose (Spearman $\rho = 0.79$, two-tailed $P < 0.001$) (Fig. 1). Left sural (metformin-treated $n = 41$; non–metformin-treated $n = 51$) and superficial peroneal nerves (metformin-treated $n = 39$; non–metformin-treated $n = 49$) had lower median SNAP and slower median conduction velocity in the metformin-treated group; however, these measures were not significantly different between groups after corrections for multiple comparisons (Table 2).

In the linear regression analysis, using the enter method, a significant model emerged ($F_{4, 117} = 47.7$, $P < 0.005$) with adjusted $R^2 = 0.61$. Metformin exposure ($\beta = 0.55$, $P < 0.001$) and duration of diabetes ($\beta = 0.41$, $P < 0.001$) were the only significant explanatory variables in this model. Collinearity diagnostics did not suggest a problem with multicollinearity in this model (variance inflation factor <1.5 for all included variables).

CONCLUSIONS — We found evidence that patients with type 2 diabetes, peripheral neuropathy, and >6 months' exposure to metformin had lower serum Cbl, higher serum Hcy and MMA, and higher scores on the NIS and TCSS, indicating clinically more severe peripheral neuropathy compared with similar patients with no metformin exposure. These abnormalities were correlated strongly with cumulative metformin exposure. A linear regression analysis including age,

Table 2—*Markers of Cbl deficiency and neuropathy severity*

	Metformin-treated	Non–metformin-treated	*P*	Correlation with cumulative metformin dose	*P*
n	59	63			
Biochemical markers of Cbl deficiency					
Serum Cbl (pmol/l)	231 (343)	486 (863)	<0.001*	−0.41	0.001†
Cbl deficiency (<210 pmol/l)	18 (31%)	2 (3%)	<0.001*		
Fasting serum Hcy (μmol/l)	11.6 (17.7)	8.4 (24.9)	<0.001*	0.50	<0.001†
Hcy upregulation (>13.7 μmol/l)	15 (25%)	1 (2%)	<0.001*		
Fasting serum MMA (μmol/l)	0.18 (0.47)	0.11 (0.14)	<0.001*	0.37	0.005†
MMA upregulation (>0.15 μmol/l)	43 (73%)	7 (11%)	<0.001*		
Clinical markers of neuropathy severity					
TCSS total score	10 (17)	5 (12)	<0.001*	0.80	<0.001†
NIS total score	10 (32)	4 (12)	<0.001*	0.79	<0.001†
Electrophysiological markers of neuropathy severity					
Left sural nerve SNAP amplitude (μV)	3.0 (11.4)	4.4 (12.9)	0.038		
Left sural nerve sensory conduction velocity (m/s)	33.3 (15.0)	33.0 (15.7)	0.69		
Left superficial peroneal nerve SNAP amplitude (μV)	2.5 (7.1)	3.6 (7.8)	0.12		
Left superficial peroneal nerve sensory conduction velocity (m/s)	34.2 (18.4)	36.8 (18.8)	0.071		

Data are median (range) or *n* (%). *Significant at Bonferroni corrected $\alpha = 0.05$ level using Mann-Whitney *U* testing. †Two-tailed correlation coefficient significant at Bonferroni corrected $\alpha = 0.05$ level using Spearman ρ testing.

duration of diabetes, A1C, and metformin exposure to predict clinical status (NIS total score) showed that metformin exposure and duration of diabetes were significant explanatory variables. We were unable to demonstrate significant group differences in sural or superficial peroneal SNAP or conduction velocity.

Metformin-associated Cbl deficiency may contribute to the clinical burden of DPN; this contribution is both detectable and ameliorable. This deficiency and concomitant increase in serum Hcy have been demonstrated in a randomized controlled trial (22), and this relationship depends

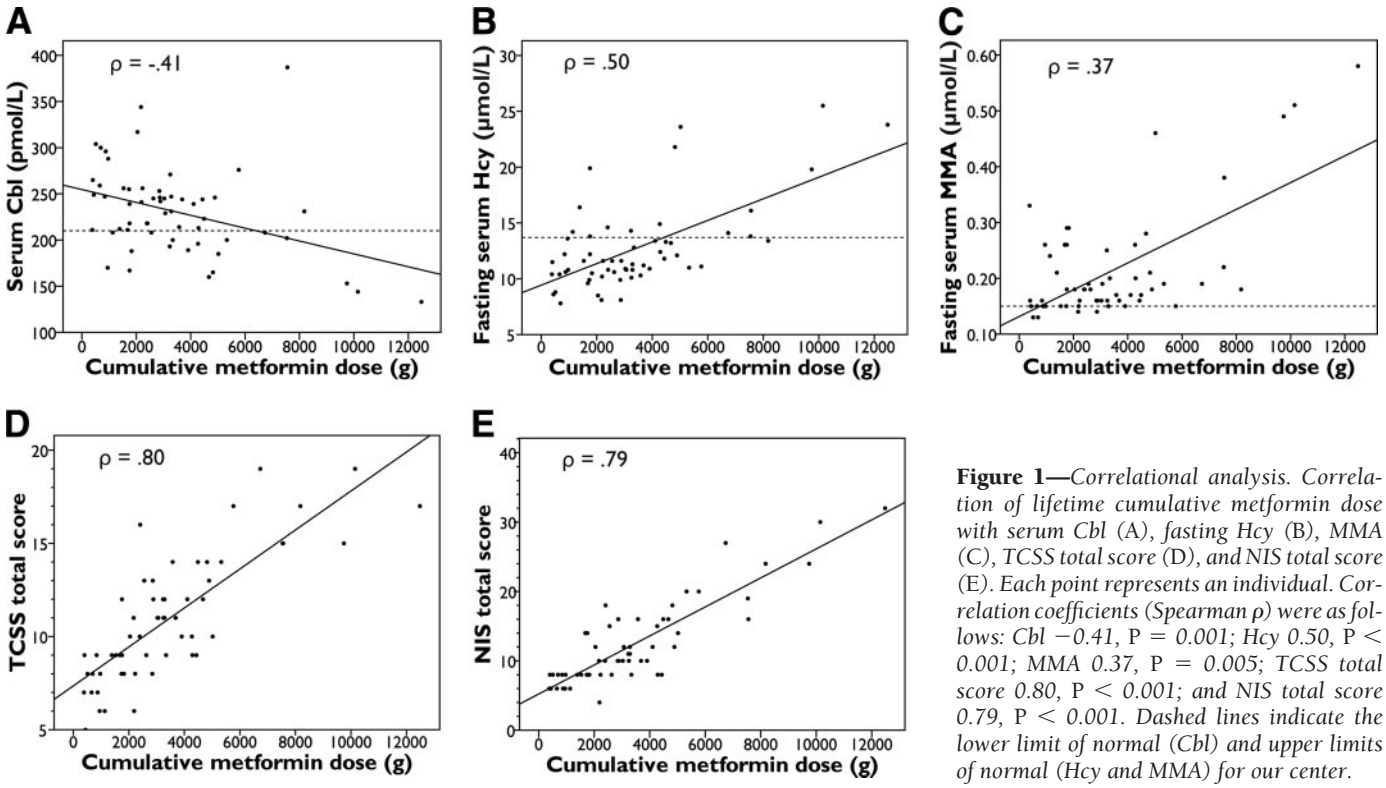

Figure 1—*Correlational analysis. Correlation of lifetime cumulative metformin dose with serum Cbl* (A)*, fasting Hcy* (B)*, MMA* (C)*, TCSS total score* (D)*, and NIS total score* (E)*. Each point represents an individual. Correlation coefficients (Spearman ρ) were as follows: Cbl −0.41,* P *= 0.001; Hcy 0.50,* P *< 0.001; MMA 0.37,* P *= 0.005; TCSS total score 0.80,* P *< 0.001; and NIS total score 0.79,* P *< 0.001. Dashed lines indicate the lower limit of normal (Cbl) and upper limits of normal (Hcy and MMA) for our center.*

on dose and duration of metformin therapy (18). However, the potential for clinical sequelae has been discussed only rarely (11,16) and has not previously been studied formally. The present findings therefore add clinical relevance to the existing literature on metformin-associated Cbl deficiency. Given the prevalence of type 2 diabetes and of metformin use, these effects have the potential to be widespread.

The present findings should not be seen to discourage treatment of diabetic patients with neurological impairment with metformin; in addition to its effects on metabolic control, metformin has been shown to have beneficial effects on advanced glycation end product formation in peripheral nerves (23) and may prevent apoptosis involved in diabetes-associated neurodegenerative processes (24). Instead, we recommend screening for features of Cbl deficiency in diabetic patients receiving long-term metformin therapy. The American Academy of Neurology recommends serum Cbl and metabolites (MMA with or without Hcy) as investigations with a high diagnostic yield in distal symmetric polyneuropathy (10); this yield may be further increased in the present population given their predilection for comorbid Cbl deficiency. The optimal screening frequency remains to be determined, but baseline tests at initiation of metformin therapy and at intervals of no more than 1–2 years seem prudent, because metformin may begin to depress serum Cbl levels after as short a time as 3 months (15).

It is unclear whether Cbl supplementation will prevent clinical worsening in this group, but supplementation carries a low risk of toxicity. Current therapy consists of intramuscular Cbl replacement therapy and possible long-term Cbl and folate therapy; oral Cbl supplementation may be as effective as intramuscular therapy, although long-term outcomes have not yet been examined in patients with DPN (25). Oral calcium supplementation has also been effective in reversing bioavailable Cbl deficiency in metformin-treated patients (16).

Further studies should better define differences in the electrophysiological profile of these groups. Potential central nervous system complications of Cbl deficiency, including myelopathy and cognitive impairment, should be considered as contributors to clinical status. The relationship among Cbl deficiency, elevated Hcy and MMA levels, and peripheral neuropathy is controversial and remains to be proven, but both Cbl deficiency and elevation of its serum metabolites are associated with the presence of a sensorimotor peripheral neuropathy (7).

Our findings are presented with some limitations. Although we identified patients prospectively, they were not randomly selected from a population with type 2 diabetes with or without DPN. Our sample size was not based on a predetermined power analysis. We did not identify a separate group of patients with asymptomatic DPN. We excluded patients with type 1 diabetes because of their expected limited metformin exposure and the potential for distinct pathophysiological mechanisms. Although A1C was not significantly different between groups at the time of evaluation, we did not examine measures of metabolic control over time. Investigators were blinded to the laboratory results until clinical and electrophysiological studies were completed but were not blinded to use of metformin therapy.

Metformin-treated patients were more commonly treated with glyburide and less commonly with insulin therapy; insulin may be beneficial in diabetic patients with peripheral neuropathy because of mechanisms other than glycemic correction (3). Undetected group differences may also exist; additional factors such as inadequate dietary intake might explain in part the lower Cbl levels in our metformin-exposed patients, and it is possible that patients using metformin may have more severe diabetes despite the similar duration of type 2 diabetes and similar A1C levels among groups. All participants had normal folate status, but pyridoxine levels were not measured. Laboratory testing for pernicious anemia (by the Schilling test) was not available during this investigation, and therefore the prevalence of this alternate cause of Cbl deficiency in our population is not known. Our multiple regression analysis should be interpreted with caution as not all variables potentially relevant to the NIS total score were measured in our population, and our participants were grouped in a nonrandom manner.

The current findings suggest an association among metformin, elevated Cbl metabolites, and exacerbation of DPN, but further work is needed to prove a direct causal relationship and its mechanism. Metformin may exacerbate peripheral neuropathy as a result of other unknown mechanisms; a clear understanding of its role necessarily awaits further research on the pathogenesis of DPN. Despite these limitations, we believe that metformin exposure is a potential iatrogenic contributor to the severity of peripheral neuropathy in the population described. Recognition of this readily identifiable and potentially treatable component of disease might improve quality of life for this large population of diabetic patients.

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