Diabetes, Metabolic Syndrome and Obesity: Targets and Therapy Dovepress
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REVIEW

Metformin and Micronutrient Status in Type 2 Diabetes: Does Polypharmacy Involving Acid-Suppressing Medications Affect Vitamin B12 Levels?

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Abstract: Metformin is the first-choice drug in uncomplicated type 2 diabetes (T2DM) and is effective in improving glycaemic control. It is the most widely prescribed oral antidiabetic medicine and has a good safety profile. However, there is an abundance of evidence that metformin use is associated with decreased Vitamin B12 status, though the clinical implications of this in terms of increased risk of diabetic peripheral neuropathy are debated. There is growing evidence that other B vitamins, vitamin D and magnesium may also be impacted by metformin use in addition to alterations to the composition of the microbiome, depending on the dose and duration of therapy. Patients using metformin for prolonged periods may, therefore, need initial screening with intermittent follow-up, particularly since vitamin B_{12} deficiency has similar symptoms to diabetic neuropathy which itself affects 40–50% of patients with T2DM at some stage. Among patients with T2DM, 40% are reported to experience symptomatic gastroesophageal reflux disease (GORD), of whom 70% use oral antidiabetic medications. The most common medications used to treat GORD are proton pump inhibitors (PPIs) and antagonists of histamine selective H_2 receptors (H_2RAs), both of which independently affect vitamin B_{12} and magnesium status. Research indicates that co-prescribing metformin with either PPIs or H_2RAs can have further deleterious effects on vitamin B_{12} status. Vitamin B12 deficiency related to metformin and polypharmacy is likely to contribute to the symptoms of diabetic neuropathy which may frequently be under-recognised. This review explores current knowledge surrounding these issues and suggests treatment strategies such as supplementation.

Keywords: H2 antagonists, diabetes, neuropathy, folic acid, magnesium, microbiome

Introduction

Diabetes has a global impact on health, with 415 million known adult cases, of which 91% have type 2 diabetes (T2DM).[1] The prevalence and cost of diabetes are increasing and contribute significantly to total national healthcare costs, accounting for 10% of the entire National Health Service budget in the UK.[2] For example, in 2017, in the US, it was estimated that the total cost of diagnosed diabetes was $245 billion. Of this sum, $176 billion was assigned to direct medical charges and $69 billion to productivity losses. This represented a 41% increase from the 2012 estimate of $174 billion. Furthermore, a diagnosis of diabetes, results on average, in medical expenditures that are approximately 2.3 times more than those typically incurred in its absence.[1]

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T2DM is a chronic, complex and heterogeneous condition. It is one that has numerous pathophysiological abnormalities, with a varying predisposition to complications and an assorted clinical response to therapeutic intervention. It requires ongoing medical supervision, with a high degree of self-management to monitor blood glucose levels, blood pressure and lipid profiles. It also necessitates multifactorial strategies to minimise the risk of possible acute and long-term complications such as neuropathy, retinopathy, and nephropathy and cardiovascular morbidities which are increased two-to-threefold in these patients and are subsequently a major cause of death.[3] Peripheral neuropathy is a common complication of both T1DM and T2DM, and in 40–50% of these individuals, a detectable condition develops within a decade of diagnosis of the primary condition. Commonly, it initially presents as a loss of sensation which can develop into neuropathic ulcers, and ultimately result in amputation. Patients frequently experience distressing and chronic neuropathic pain and paresthesia which can severely limit quality of life [4] and are thought to account for 27% of the medical costs associated with diabetes.[5]

Metformin is a first-line medication typically used in T2DM treatment, particularly in patients who are overweight or obese.[6] In the UK, metformin was prescribed to 45% of newly diagnosed T2DM patients in 2000 and this increased to 91% by 2013.[2] Metformin-like compounds occur naturally in the plant *Galega officinalis*, and whilst it was used in medieval Europe to treat a variety of conditions, the plant is highly toxic with a potential to induce hypotension. The blood glucose-lowering effect of the plant was first described in 1656 by Thomas Culpeper, but at that time it was difficult to identify the ingredient that was responsible for these effects. Subsequently, it was found that the herb is rich in two compounds, guanidine and galegine. Whereas guanidine was shown to exhibit anti-diabetic effects in animals, its toxicity made it unsuitable for clinical use, and whilst galegine had a lower toxicity, it failed to deliver the same significant anti-diabetic activity. In 1945, during the development of paludrine, the antimalarial drug,[7] it was noticed that a portion of the new molecule had a distinct resemblance to galegine and within two years paludrine had been demonstrated to exert a reduction in blood glucose concentrations in animal studies. Contemporaneously, studies of synthetic guanidine analogues discovered a compound referred to as "flumamine" and suggested that it might act in patients with malaria by lowering blood glucose, thereby reducing the ability of the malarial parasite to survive.[8] Subsequently, the anti-diabetic effect of flumamine – which is today known as metformin – was investigated in animals [9] and showed it had a powerful glucose-lowering effect which led investigators to suggest that because metformin appeared to be a "glucose eater" it should be called "Glucophage" and also reported that its precise mechanism of action remained relatively unknown,[10] an observation that is true today, despite many ensuing investigations.

It is now becoming clear that not all of the effects of metformin can be attributed to the reduction of glucose production in the liver as originally thought and evidence is emerging for a key role exerted in the gut as detailed later in this review.[11] There also appear to be variations in the understanding of how the drug works at a molecular level, dependent upon dose administered and whether treatment is of an acute or chronic nature. Metformin has been demonstrated to have both AMP-activated protein kinase (AMPK)-dependent and AMPK-independent actions, and also inhibit mitochondrial respiration possibly via inhibition of mitochondrial glycerophosphate dehydrogenase and/or through the involvement of a lysosomal mechanism.[12,13]

Subsequent to the discovery of metformin, other trials were published on the structurally similar biguanides, namely phenformin and buformin; however, the two drugs were quickly withdrawn from clinical use as a result of inducing lactic acidosis.[14,15] Consequently, there was concern that this might be a class effect which impacted upon the immediate extensive use of metformin. However, other research demonstrating the benefits of metformin, without similar side effects, provided reassurance of the safety of the drug,[16,17] with the strongest supportive evidence provided by the United Kingdom Prospective Diabetes Study (UKPDS).[13] This investigation demonstrated the benefits of the drug not only in lowering cardiovascular mortality but also in increasing overall survival rate of those diabetic patients who were obese [18] and has more recently been shown to reduce cardiovascular morbidity in individuals with non-alcoholic fatty liver disease.[19]

Metformin is thought to exert a number of biological actions, reducing insulin resistance and lowering gluconeogenesis, and thereby appears to relieve some of the metabolic defects observed in T2DM.[20] It also delivers a cardioprotective effect as well as anti-inflammatory and anti-atherothrombotic effects,[21–25] reducing the risk of liver cancer in a dose-dependent manner [26] with a recent

umbrella review of meta-analyses and systematic reviews finding strong associations between metformin use and reduced pancreatic and colorectal cancer risk.[27] Unfortunately, despite having many beneficial effects for overall health, metformin is not universally well tolerated with the adverse effects primarily reported to be the result of gastrointestinal (GI) intolerance. Approximately 88% of newly diagnosed T2DM individuals reported one or more symptoms of the GI tract,[28] with the most common reported being diarrhoea (62%), heartburn (52%), nausea (47%), abdominal pain (36%), bloating (35%) and retching (21%),[29] with the mechanisms for these side effects a result of the actions of the drug in the GI system.[30] However, these are infrequent, mild and typically dose-related and can cease after reduction in the dose administered or removal of the drug.

This review investigates the possible impact that metformin has on the status of certain vitamin and minerals as well as that of the microbiome. The latter area is of particular current relevance given the rapidly expanding research and understanding of the effects gut flora can exert on wellbeing. It further investigates whether there might be a connection between metformin and other medications commonly co-prescribed to diabetics for associated comorbidities and how this could confound the diagnosis of the complication of diabetic neuropathy.

Methods

Studies published in peer-reviewed journals were considered for inclusion in the current review. The methods used and results obtained from the included papers were required to be well described with appropriate data collection and analysis performed. The following databases were searched MEDLINE via Pubmed, Embase, Scopus, CINAHL for the period 1978 to June 2019. The search was restricted to articles in the English language, studies performed on human subjects, but were not limited by study design. The lists of references found in these papers were assessed for relevant additional sources. The following literature search used the terms (including closely related words and synonyms): metformin combined with microbiome and/or Vitamins A, C, D, E, B_1, B_2, B_3, B_5, B_6, B_{12}, and folic acid, iron, magnesium, selenium, zinc, H_2RAs, PPIs and polypharmacy. This review will focus on the impact of metformin on Vitamins B_1, B_{12}, and D, folic acid, magnesium and the microbiome due to literature suggesting there to be an interaction between these subjects and metformin, with a lack of evidence relating to the remainder of the micronutrients searched.

Review of Studies Examining How Metformin Affects the Status of Specific Micronutrients and the Microbiome

Vitamin B1

Vitamin B1 (thiamine) acts as a coenzyme in neurotransmission and low levels of this nutrient may have a role in the development of certain diabetic complications.[31] Phenformin has been reported to exhibit anti-thiamine activity in animals,[32–34] and as a result, it has been suggested that a possible anti-thiamine effect of metformin might contribute to some cases of metformin-associated lactic acidosis, a rare condition, but one that has been shown to have a mortality rate of 18–25%.[35–37] It has been proposed that metformin and phenformin inhibit thiamine absorption through inhibition of the human thiamine transporter (THTR2) in the small intestine,[38] a finding recently confirmed to be clinically relevant,[39] particularly in at-risk populations. In patients with T2DM, plasma thiamine concentrations have been demonstrated to be reduced.[40,41] The magnitude of these reductions can be as large as 76% and 75%, respectively, compared to levels in healthy volunteers with renal clearance of thiamine increased 16-fold in T2DM patients.[40,41] Low thiamine concentrations have been correlated to higher concentrations of soluble vascular adhesion molecule-1 (sVCAM-1), a surrogate marker of potential vascular disease in some, but not all studies.[40,41]

Folic Acid

One hundred and fifty compounds make up the pteroilglutamate family, commonly referred to as folate, and are involved in purine base synthesis of DNA as well as being important co-factors in the metabolism of homocysteine (Hcy) to methionine.[42] Research has found that folate concentrations in plasma and red cells in diabetic individuals with both proliferative diabetic retinopathy and non-proliferative retinopathy were significantly lower than both healthy subjects and diabetic patients without retinopathy.[43] A randomised control trial involving 16 weeks of metformin administration to patients with T2DM has been shown to result in reduced folate and vitamin B12 levels, which typically result in a modest increase in Hcy,[44] a result that was supported subsequently.[45] Individuals with T2DM have elevated risk of cardiovascular disease which is exacerbated by the

presence of hyperhomocysteinemia which has been attributed to reduced folate concentration in addition to the vitamin B12 deficiency as detailed later.[46–49] Indeed, two studies have demonstrated that folate supplementation reduces endothelial dysfunction in T2DM thought to be associated with hyperhomocysteinemia.[50,51] A recent meta-analysis found that folate supplementation in young and elderly populations of varying insulin resistance status might be beneficial in terms of improving glucose homeostasis and reducing insulin resistance, though there is insufficient data to determine whether it can reduce the development of T2DM.[52] Interestingly, folate supplementation in isolation was found to be approximately one-third as effective as metformin in reducing insulin resistance and preventing T2DM[52] in the populations examined.

Vitamin B_{12}

Vitamin B_{12} (cobalamine) is a water-soluble vitamin and a cofactor for the conversion of Hcy to methionine and is a co-enzyme involved in the synthesis of purine bases and pyrimidine. Older individuals (median age 76 years) with T2DM are more likely to be deficient in micronutrients such as Vitamin B12 than their younger counterparts (median age 59 years).[53] A recent study on older adults (>50 years) found that vitamin B12 and folate deficiency were high and metformin use was the largest predictor of vitamin B12 deficiency.[54] Metformin has been demonstrated to impair vitamin B_{12} absorption,[44,55–59] and concentrations of the vitamin in serum have been inversely correlated with both dose and length of therapy with the medication.[60] The possible mechanisms that result in metformin-induced deficiency of vitamin B_{12} in patients with T2DM are highlighted in Box 1.

Box 1 Suggested Mechanisms That Result in Metformin-Induced Deficiency of Vitamin B_{12} in Patients with T2DM

Changes in motility of the small bowel that might stimulate bacterial overgrowth	Alterations in levels of intrinsic factor (IF) that cause inactivation or competitive inhibition absorption of vitamin B_{12}	Interplay with the cubulin endocytic receptor	Inhibition of calcium associated vitamin B_{12} -IF complex absorption in the terminal ileum

Note: Data from these studies.[56–61]

The literature suggests that anything from 10% to 30% of those prescribed prolonged metformin experience malabsorption of vitamin B_{12}, with 6–9% of individuals shown to progress to deficiency.[49,60–63] Risk factors associated with developing metformin-related vitamin B_{12} deficiency include vegetarian diet, older age, metformin use for 3 years and a higher metformin dose,[60] with a more recent study additionally finding that males had a higher incidence than females.[64] One significant study demonstrated that after 16 weeks of treatment with metformin, diabetic patients had lower vitamin B_{12} concentrations in comparison to participants not receiving this drug or non-diabetic controls.[50] Subsequent studies have reported that metformin-treated patients experienced statistically significant reduced vitamin B_{12} concentrations compared to those not prescribed the drug,[62] in geriatric patients [65] and in individuals with or without microalbuminuria.[66] The authors suggested that physicians should check the vitamin B_{12} concentrations at baseline, and then continue to monitor these levels and assess the nutritional status of those prescribed metformin, and recommended supplementation when appropriate.[65]

As well as observing a reduction in folate status, Sahin et al also demonstrated that as little as 6 weeks' treatment with metformin reduced vitamin B_{12} levels and increased homocysteine (Hcy) concentration.[45] A longer-term placebo-controlled study found that vitamin B12 deficiency absolute risk was 11.2% greater in the group treated with metformin and the number needed to harm was 8.9 per 4.3 years.[67] Similar observations have been reported in New Zealand and Brazilian cohorts of T2DM patients taking metformin, with age, dose, and duration of metformin treatment identified as important factors in the depletion of vitamin B_{12}.[68,69] However not all researchers have demonstrated an inverse association between vitamin B_{12} status and metformin treatment as exemplified by one small study performed in Qatar.[70] In contrast, a recent meta-analysis indicated that after up to 4 months of use of metformin, diabetic patients' vitamin B_{12} levels were reduced by an average of 57 $\rho mol.l^{-1}$. It concluded that a significant proportion of people with T2DM could develop B_{12} deficiency or borderline values as a consequence of metformin treatment.[71] The likelihood that there may be a genetic perspective in the deficiency of vitamin B_{12} induced by metformin has been explored by one group with polymorphisms in genes such as methylene tetrahydrofolate reductase (MTHFR) suggested as possible candidates.[72]

Chronic metformin treatment first became known as a pharmacological cause of vitamin B_{12} deficiency within a decade of it coming into use and more recently decreased serum folate concentration has also been associated with its administration.[46,73–75] It is therefore suggested that metformin use may be an agent of iatrogenic potential inducing peripheral neuropathy exacerbation in individuals with T2DM who have depressed Vitamin B12, as a result of elevated fasting methylmalonic acid (MMA) and tHcy levels.[76–79] One group has identified these factors as also contributing to diabetic retinopathy.[80] A means of resolving this situation is provided by studies which have shown that malabsorption of vitamin B_{12} induced by metformin can be reversed by oral supplementation of vitamin $B_{12,}$ even whilst metformin treatment is continued.[56,81–84] In these circumstances, it is necessary to avoid folic acid supplementation prior to Vitamin B12 treatment, because whilst folic acid might reverse in part some of the haematological abnormalities that result from deficiency of vitamin B_{12}, it cannot deliver similar effects with respect to neuropsychiatric symptoms. A recent study of older people (>50) found that multivitamin use was associated with higher serum B12 levels in both those with and without diabetes and in metformin users was associated with serum B12 levels that were 50% higher than those not supplementing.[54] At present, it is not known if supplementation with calcium could potentially prevent metformin-induced deficiency of vitamin B_{12}. Hence this supplementary approach should not be considered for this purpose until this potential role been further elucidated.[85] However, the rationale to support this observation is well characterised, since the hydrophobic tail of metformin can penetrate cell membranes, thereby altering potentials and hence displacing divalent cations such as calcium which can affect adhesion of a number of substances to the membrane. In all DNA synthesising cells, the cell surface TCII receptors are calcium-dependent, and the drug may interfere with the delivery of vitamin B12 into them.[59]

Vitamin D

A classical function of vitamin D is considered to be the regulation of calcium absorption and management of homeostasis of that mineral; however, there is growing evidence of non-classical effects including influencing cell proliferation, differentiation, apoptosis, immune function, genome stability and neurogenesis.[86] Research indicates that vitamin D deficiency is linked with obesity and that it is also associated with both an elevated insulin resistance and a decreased insulin secretion.[85,87] In a study of Dutch community-dwelling geriatric outpatients, after adjustment for gender and age, statistically significant negative associations were found between use of metformin and vitamin D status, with those non-supplement users of vitamin D having levels of the vitamin 14.4% lower compared to controls.[88] The authors of a separate cross-sectional study suggest the impact of metformin on lowering vitamin D levels may have been confounded by use of supplements at the time of diagnosis.[89] In contrast, some recent studies have found vitamin D status to have been unaffected by metformin use[62] and a placebo-controlled study found no effects of the drug at 4 or 16 months use in people with T2DM.[90]

Magnesium

As the fourth most abundant cation in the body, magnesium plays an important physiological role due to its role as a cofactor in over 400 enzymatic reactions, including those involved in energy metabolism, such as carbohydrate oxidation, glucose transport mechanisms, as well as insulin secretion, activity and binding.[91] Oral supplementation of magnesium has been shown to improve glucose handling and insulin sensitivity as well as reducing the progression to diabetes from pre-diabetes.[91] In T2DM, magnesium status also appears to influence the progression of common comorbidities such as hypertension, atherosclerosis and chronic kidney disease.[92] Furthermore, in these patients, hypomagnesemia can result in accelerated renal decline and contributes to a poorer disease progression and prognosis.[93] Serum levels of magnesium have been shown to be associated with microvascular complications in T2DM and status and intake of this mineral also influences blood glucose control in these patients [94–96] This is further complicated as it appears there are many underlying causes of hypomagnesemia, both unrelated and related to T2DM.[97] However, reports suggest that in comparison to those without T2DM, hypomagnesemia occurs at an increased frequency of 13.5–47.7% amongst patients with the condition, as opposed to 2.5–15% in their non-diagnosed counterparts.[98]

Several reports with limited patient numbers from around 30–40 years ago first suggested patients treated with metformin experienced reduced plasma magnesium levels.[99] But these findings conflicted with another small study which reported that metformin increased total intra-erythrocyte concentration of magnesium and decreased urinary magnesium elimination.[100] A more recent study retrospectively evaluated the relationship between hypomagnesemia and

medication in a hospital in-patient cohort. In only 16% of the 181 patients diagnosed with hypomagnesemia could specific causes such as vomiting or chronic diarrhoea-be identified.[101] Of the remainder, 79% had taken proton pump inhibitors and/or metformin and/or diuretics. The authors proposed proton pump inhibitors as possible causative agents of hypomagnesemia, especially when used for over one year or prescribed in conjunction with other medications, such as diuretics or metformin that might also lower magnesium status. Subsequently, a large cohort study of nearly 400 T2DM patients investigated the possible effect of medication on magnesium status.[102] Here, 62% of patients using metformin were reported to have reduced plasma magnesium levels, as were 45% of those using proton pump inhibitors and 8% of β-adrenergic receptor agonist users. However, of all the medications examined, metformin was most strongly statistically, significantly correlated with reduced plasma magnesium concentration.[102] In another recent review, 700 veterans were divided into seven cohorts according to a diagnosis of diabetes and the medications they were prescribed and compared with a control group.[103] Those with diabetes had significantly reduced magnesium concentrations compared to counterparts without the diagnosis. A significant relationship between lower magnesium levels was observed for those with hypertension, cardiovascular disease, or using metformin. The authors noted that greater monitoring and possible supplementation with magnesium are required in PPI and Metformin users as polypharmacy is associated with hypomagnesia which may explain some of the side effects of these drugs.[104]

Microbiome

The recommended oral daily doses of metformin typically range between 1 and 2 g per day. Hence, given that the reported bioavailability of the drug is around 50%-60%, there is still a level of faecal recovery of 20%-30%, suggesting a significant proportion remains available to interact with GI microbiota.[105] Despite there being high inter-individual and inter-population variation in gut microbiota composition, metformin could cause significant alterations to gut bacteria.[11] The enzyme glycerophosphate dehydrogenase has an important role in carbohydrate metabolism in bacteria. The inhibition of this enzyme by metformin impairs the production of nicotinamide adenine dinucleotide (NAD+) required for gluconeogenic reactions.[106] This activity could, therefore, affect many commensal microorganisms, such as *Bacillus subtilis* which utilise this enzyme, thereby resulting in an overproduction in the colon of d-lactate.[107] Other colonic organisms which utilize sugars residing in the colon such as Lactobacillus, Bifidobacterium and Eubacterium species might also contribute to d-lactate overproduction [108] and other colon microbiota can also convert d-lactate to l-lactate, thereby contributing to the plasma lactate pool.[109] Additionally, unabsorbed glucose or glucose polymers that appear in the colon provide a substrate for lactate-producing bacteria [110] which contribute to further elevated levels of both d- and l-lactic acid that can be absorbed into the circulation. Hence, it is probable that the accumulation of lactic acid in the colon might result in all or some of the GI side effects of metformin, particularly in users that consume diets high in sugars and starch.[111] Moreover, the GI predominant microbiome in type 2 diabetics appears to be rich in microbial species that harvest sugars.[112] Other functional changes characterized by the bacterial dysbiosis typical of the condition are elevated branched-chain amino acid metabolism, along with elevated methane metabolism, enhanced degradation of xenobiotics and stimulated sulphate reduction.[113] It is known that perturbations in the microbiome can influence the production and absorption of nutrients, especially B vitamins, and hence it is, therefore, possible, as discussed above, these effects of metformin on the gut flora may contribute to the observed effects on vitamin B_{12} status, with other possible consequences yet to be identified. In contrast, a recent metagenomic analysis suggests the adverse intestinal effects of metformin may also be the result of a relative increase in escherichia abundance.[114] Further complicating the relationship between metformin and gut microbiota is a recent study on mice that found that some of the antidiabetic effects of metformin may be mediated by positive alterations to the microbiome. Indeed, it has been found that 1 g/day of metformin can shift the microbiota after as few as 3 days of administration, resulting in reduced metabolic dysfunction.[115] It is thought that metformin alters the biota towards short-chain fatty acids (SCFAs) producing bacteria, thereby increasing glucagon-like peptide (GLP-1) or peptide YY (PYY) improving glucose homeostasis and improving gut barrier integrity.[116] Therefore, alterations to the gut microbiota appear to mediate some of the positive and potentially negative aspects of metformin administration.

In order to investigate the relationship between metformin and the microbiota, one group of researchers developed a modulator of the microbiome to attempt to shift the GI microflora of that observed in T2DM to one more typical of healthy individuals.[112] The modulator contains

specific ingredients that are proposed to stimulate populations of those commensal microbiota that produce short-chain fatty acids (SCFA) as opposed to lactic acid, slowing down the absorption of small molecules by intensifying the viscosity of the contents of the lumen, strengthening the barrier of the mucosa, sequestering the production of bile salts and acids, and delivering a high level of antioxidant protection that buffers oxidative stress.[112] It was delivered as a dissolvable powder along with metformin and compared with a placebo formulation containing the same dose of drug. The metformin/modulator combination delivered a significantly enhanced tolerance to metformin compared to the placebo formulation. It also delivered mean fasting glucose levels 20% lower than with the metformin-placebo formulation, indicating no reduction of the antidiabetic effects of the drug. Albeit a small-scale study (n=10), it appears that the addition of a modulator of the GI microbiome to metformin may increase tolerance of the drug, particularly in metformin intolerant patients. However, one challenge of this study is that the modulator has many active ingredients, which makes it difficult to ascribe which is/are having the effect and thus the mechanism of action remains speculative.

Discussion

Diabetes, Neuropathy and Vitamin B_{12}

Vitamin B_{12} is key in nucleic acid methylation, S-adenosylmethionine and haemoglobin synthesis, and fat and protein metabolism.[117–119] Vitamin B_{12} deficiency signs and symptoms appear to be very similar to diabetic neuropathy and are considered to be clinically indistinguishable (Box 2).

Box 2 Signs and Symptoms of Vitamin B_{12} Deficiency and Diabetic Neuropathy

Lowered ability to detect vibratory sensations	Paraesthesia	Abnormal reflexes	Axonal degeneration and demyelination
Reduced sensory nerve conduction velocities	Diminished proprioception	Urinary and faecal incontinence	
Loss of cutaneous sensation	Muscle weakness	Loss of vision	

Note: Data from these studies.[120–122]

Most patients with diabetes are reported to experience nervous system damage ranging from mild to severe forms that resemble the signs and symptoms detailed in Box 1.[120,121] A recent meta-analysis of 29 studies of over 50,000 patients put the prevalence of neuropathy at 31.5% (95% confidence interval 24.4−38.6%) in people with T2DM), but it is estimated to develop in 50% of patients in their lifetime.[122] Severe painful peripheral neuropathy in those with T2DM can increase the medical costs of treatment by four-fold compared to those with T2DM but no neuropathy.[123] Greater attention to the role of deficiency of vitamin B_{12} in diabetic neuropathy in recent years has been supported by the 2017 American Diabetic Association position statement where serum B_{12} investigation is recommended to be considered to exclude non-diabetic causes.[124] Despite many foods being fortified with vitamin B_{12} in Ireland, a recent study found that approximately 12% of the general, older (>50 years) population were low or deficient in vitamin B12 and vitamin B12 injection and/or supplementation were the greatest predictors of plasma vitamin B12 concentration.[54] Supplementation of vitamin B_{12}, singularly or together with other interventions, has been shown to improve many symptoms of this condition. These include enhanced cutaneous sensitivity, reduced paraesthesia, pain and autonomic symptoms, as well as improvements in ulnar median/motor sensory nerve conduction velocity and lower-extremity epidermal nerve fibre density.[125–131] As early as 1969, Berchtold et al reported that patients prescribed 2–3 months of metformin treatment exhibited malabsorption of vitamin B_{12}.[75] Vitamin B_{12} deficiency has been reported to occur at a rate of between 5% and 36% of metformin users, with this broad incidence range attributed to cumulative drug-induced B_{12} depletion as a result of the duration and dosage of the medication.[132–138] Furthermore, studies have shown that lower levels of vitamin B_{12} and deteriorating diabetic neuropathy in patients treated with metformin than in those managed with other medications commonly prescribed in the condition.[74,139] Some observers suggest that this neuropathy may, in part, be the result of depletion of B_{12}, and this might be due to elevated levels of homocysteine and MMA which can damage neurones[140]. This is not surprising since although metformin is the primary biguanide used in the treatment of T2DM, both phenformin and buformin have been demonstrated to negatively affect vitamin B_{12} levels.[141,142] Thus, a number of authors have examined the possibility that metformin might be the cause of a vitamin B_{12} deficiency and subsequently directly responsible for peripheral neuropathy in T2DM patients. One review in 2017 that examined evidence from eight studies on the subject concluded that the wide

variety of study designs, durations and small sample sizes made it difficult to reach a definitive conclusion.[143] One complicating factor is the ubiquity of metformin use in individuals with T2DM. Since most studies are cross-sectional, it can be difficult to find an appropriate control with T2DM that is not being prescribed metformin. A more recent study performed in Saudi Arabia identified vitamin B_{12} deficiency to be four times higher in metformin users compared to T2DM patients not using the drug and suggested that metformin doses of >2000 mg per day and duration of use of >4 years to be especially important risk factors, but it too failed to establish a direct link between use of the drug, induction of vitamin deficiency and peripheral neuropathy.[144] A more recent meta-analysis by Yang et al examined metformin use and neuropathy and found no association, highlighting similar issues in terms of the lack of randomised controlled trials, small sample size and varying study design as described above.[145] However, in contrast, a study performed in India concluded metformin use to be associated with vitamin B_{12} deficiency and clinical neuropathy in T2DM patients.[139] It must be stated that metformin is a very effective drug in terms of improving glycaemic control, which itself reduces peripheral nerve damage and indeed several animal studies have indicated that it can have non-glycaemic related anti-neuropathic and neuroprotective effects.[143] It seems likely that these protective effects could be optimised if metformin-induced vitamin B12 deficiency could be avoided.

Monitoring of Vitamin B_{12} in Patients Prescribed Metformin

Given the possible negative impact of metformin on vitamin B_{12} levels, monitoring of the status of individuals receiving this treatment might appear prudent.

The vast majority of studies across a range of countries have found an association between metformin use and vitamin B12 deficiency[71,144–146] and potentially neuropathy,[147] though there are some exceptions.[148] The authors of these studies recommend that vitamin B12 status be assessed, both before and regularly after commencement of metformin treatment,[74,84,144,146] and for peripheral neuropathy to be assessed even in the absence of B12 deficiency.[147] For T2DM patients with borderline deficiency, screening is improved by serum homocysteine or MMA measurements as these makers are more sensitive and specific,[71] though both of these assays are significantly more expensive than serum B12.[149] Results from several meta-analyses identified that there are groups with an increased risk of B12 deficiency, namely those with pre-existing moderately low vitamin B12 levels[71] or those aged ≥60 years, and/or on ≥2000 mg/day metformin and/or ≥3 years of treatment.[145] However, they found that even those aged ≤60 years, taking ≤2000 mg/day metformin and for ≤1 year had significantly reduced vitamin B12, indicating that this reduction started early in treatment for all ages and even with low doses.[145] A complicating factor in determining metformin-induced deficiencies is that there are large vitamin B12 stores in the liver and these can take up to five years to manifest as a deficiency.[150] The American Diabetes Association's position stand on diabetic neuropathy states that non-diabetic causes should be excluded by considering "undertaking a family and medication history and performing relevant investigations e.g. serum B12, folic acid. . .", but it makes no mention of metformin specifically and provides no recommendations for frequency of screening.[124] The British Society of Haematology published its guidelines on vitamin B12 and folate screening in 2014, at which time they stated that "no definitive advice can be given on the desirable frequency of monitoring" of vitamin B12 in those with T2DM having metformin therapy but recommended that serum B12 be measured "in the presence of strong clinical suspicion of deficiency",[149] which we would opine may be difficult since deficiency is often asymptomatic. For those where metformin is determined as a cause of reduced serum vitamin B12, they recommend oral supplementation of 50 μg for one month and monitoring at 6 months and then yearly intervals, but could not advise on prophylactic administration in those taking metformin. Thus, consensus screening frequencies have not been established, though some recommend initial baseline testing and at subsequent intervals of no more than 1–2 years.[74] Screening and monitoring of vitamin B12 status are not routinely performed in those being administered metformin and the authors propose that it should, particularly since deficiencies are frequently asymptomatic and neuropathy is frequently irreversible. Clinicians should be aware of the potential benefits of screening in those who are older, have been on metformin for >3 years, are going on to high doses of metformin or who have neuropathic symptoms. It should be noted that there is a lack of research assessing the cost–benefit analysis of periodic monitoring of vitamin B12 in those taking metformin, though it is a relatively inexpensive test to undertake.[150]

T2DM and Gastroesophageal Reflux Disease (GORD)

Commonly, the T2DM population has an increased BMI which is well recognised to be associated with symptoms of GORD.[151,152] However, an increased BMI in these patients is not alone as a risk factor in the development of GORD, as peripheral neuropathy has also been identified as a separate independent risk factor for erosive oesophagitis.[153] Amongst these individuals, a greater incidence of erosive esophagitis is observed in those with neuropathy than amongst those without, despite both experiencing similar GORD symptoms. A recent meta-analysis of cross-sectional studies involving 9067 cases and 81,968 controls found a significant association between T2DM and GORD (odds ratio 1.61).[154] Around 60–70% of diabetics have grades of nervous system damage, from mild through to severe forms, which may partly explain why individuals with diabetes experience twice the incidence of low-grade oesophageal dysplasia as non-diabetics.[155] In addition, symptomatic and asymptomatic reflux occurs more commonly in those with T2DM. Moreover, the condition is known to be a risk factor for symptomatic GORD.[156–158] Amongst T2DM patients, 40% are reported to suffer from symptomatic GORD, and of these, 70% are prescribed oral antidiabetic treatments.[157] Therefore, it is possible that millions of patients are managing these comorbidities concomitantly with oral medications such as metformin along with either/or PPIs or H_2RAs. Hence, it is worthwhile considering the likely clinical consequences and drug interactions and that might potentially occur as a result of this common co-prescription scenario.

Histamine H_2 Receptor Antagonist (H_2RA)/ Proton Pump Inhibitor (PPI) –Induced Vitamin B_{12} Depletion

Literature suggests that drugs which suppress stomach acid production such as PPIs[159–163] and H2RAs[164–167] interfere with vitamin B_{12} absorption, by reducing dietary B12 release from food proteins. A 53–89% reduction in protein-bound B_{12} absorption was noted following H2RA treatment,[159,162] with ranitidine specifically reported to induce decreases in B_{12} status.[168–171] Similarly, there have been other reports of an inverse correlation between vitamin B_{12} levels and duration of PPI therapy.[172–175] The enzyme cytochrome P450 2C19 (CYP2C19), catalyses the metabolism of PPIs and a polymorphism of this enzyme has been demonstrated to influence levels of vitamin B_{12} in patients using these medications.[173] Hence, those who poorly metabolize PPIs are likely to exhibit increased suppression of acid production and thus more interference with absorption of vitamin B_{12}. Alternatively, patients who have enhanced PPI metabolism are likely to experience diminished acid suppression with a lower interference with absorption of B_{12}.

A number of studies have ascribed this effect to a combination of reduced secretion of IF, gastric acid and pepsin.[174,175] In cases of vitamin B_{12} depletion induced by H2RA medications, levels of the vitamin have improved as a result of oral vitamin B_{12} supplementation (as opposed to food source vitamin B_{12} ingestion) as well as discontinuation of H2RA therapy.[162,166,168,176] In the case of PPI-induced B_{12} depletion, vitamin B_{12} supplementation, potentially above recommended daily allowance levels has been demonstrated to be effective.[165,167] A 2015 meta-analysis and systematic review examined the long term (≥10 months) association between the development of vitamin B_{12} depletion and use of PPIs and H2RA medications.[177] Five studies met the criteria for inclusion – four were case controlled, evaluating over 23,000 subjects and the other an observational study. However, they all differed in the methodology and criteria used to identify vitamin B_{12} deficiency as well as in the definition used.[159–161,163,178] In these four studies, a significantly increased risk of developing vitamin B_{12} depletion as a result use of acid-lowering medication was identified and data from the two studies evaluating duration of treatment supported the general assumption that long-term use of these drugs is associated with an elevated risk of deficiency of this vitamin, but the results were not conclusive.

Apart from the effects of these medications on vitamin B12 status, drugs that inhibit acid secretion have been observed to have negative impacts on the status of certain other micronutrients and the microbiome. PPIs specifically have been shown to cause irregularities in magnesium status, small intestinal bacterial overgrowth and enteric infections including clostridium difficile, resulting in a 20% difference in bacterial taxa compared to non-users of these drugs which favours streptococcal abundance irrespective of *H.pylori* status.[179–182]

Clinical Issues Around Metformin Use and Vitamin B_{12} Status: Polypharmacy and Treatment Strategies

In light of the increasing prevalence of obesity, T2DM and GORD, there exists a potential for greater concomitant use of acid-suppressing medications alongside antidiabetic treatments. Since monotherapy with metformin, PPIs, or H2RAs, has been demonstrated to significantly deplete

vitamin B_{12} independently, co-prescription of these medications might induce an additive effect. This has been observed in clinical practice where 34% of patients co-prescribed metformin alongside PPIs were vitamin B_{12} deficient, significantly greater than those on metformin (22%) or PPIs (26%) monotherapy, suggesting an additive effect.[183] It is therefore important to recognise the potential likelihood of neuropathy induced by vitamin B_{12}, occurring or being exacerbated as a result of the co-prescription of medications commonly used to treat these co-morbidities, particularly since they are typically long-term drug treatments. Awareness of this issue by medical professionals, specifically pharmacists, is particularly important because of the common frequency of exposure of the diabetic population to both metformin and either PPIs or H_2RAs, especially since the two latter medications are readily available as over-the-counter versions. Given that T2D patients have up to 60–70% likelihood of experiencing diabetic neuropathy, what might sometimes be considered to be "diabetic" neuropathy may, at least in part, be a neuropathy induced by vitamin B_{12} deficiency as a consequence of concomitant use of metformin alongside PPI/H_2RAs.

An evaluation of the issues associated with the use of metformin and vitamin B_{12} status that are clinically relevant and significant to most healthcare practitioners appeared in an editorial in The New Zealand Medical Journal entitled – "A safe and effective drug?".[176] It noted that whilst formularies identify vitamin B_{12} deficiency as a possible side effect of metformin, there is a lack of guidance relating to the investigation of the risk, despite there being a deficiency prevalence of 17% in the study cited in the editorial.[68] It further suggests that unlike GI side effects of metformin which can be readily identified, early vitamin B_{12} deficiency is asymptomatic and hence unlikely to be diagnosed. However, when it does become manifest, it presents as a peripheral neuropathy which is also a complication of diabetes making it less likely to be diagnosed by symptoms alone. It concludes that whilst the neurological effects of this deficiency are permanent, its presence at an early stage can be detected with readily and cheaply available diagnostic tools, and reversed with effective supplementation.

Common patient information leaflets underplay the incidence of metformin-induced vitamin B12 deficiency, stating that the risk of this "rare side effect" is 1:10,000.[184] A UK community practice study reported that only 36% of patients using metformin had their vitamin B12 levels checked and had this assessment added to the yearly recall, despite the test being relatively inexpensive and adding little extra burden on patient or appointment availability.[185] Authors have reported that decreased serum levels of vitamin B_{12} levels in T2DM patients treated with metformin can be corrected by either sublingual methylcobalamin supplements or hydroxocobalamin injections.[82,132,186] Indeed, the British Association for Haematology recommends that in cases of Vitamin B12 deficiency, intramuscular injections of 1000 μg should be administered three times per week for two weeks in individuals without neurologic deficits and every other day for three weeks in those with neurological deficits. However, since major drawbacks of intramuscular injections are the general need for medical personnel to perform the intervention and the fact that they are sometimes painful,[187] oral supplementation appears preferable in the absence of severe deficiency, except when poor absorption is expected. The British Association for Haematology supports oral supplementation in those without severe deficiencies, malabsorption syndromes or compliance issues. Not every study has demonstrated that oral B12 supplementation reduces the risk of deficiency in metformin users, which the researchers indicated may be explained because the dose of 0.6 μg typically found in multivitamin supplements is inadequate for those on metformin treatment in terms of prophylaxis,[56] whereas other studies have found that multivitamin use was associated with reduced risk of deficiency.[134,188] Other researchers propose strategies to correct vitamin B12 deficiency which are similar to the British Association for Haematology's position statement at 1000 μg daily doses orally or via intramuscular injections for a week followed by once per week for four weeks to correct this deficiency.[189] In contrast, from a prophylactic approach, another research group proposed what they deemed a cost-effective approach of yearly injections of 1000 μg of vitamin B12 to every patient on metformin to reduce the risk of deficiency and also stated that this could remove the requirement for annual screening, though this has not been empirically tested. Additionally, Swedish researchers recommend that to optimally benefit from medication with metformin, morbidly obese patients should undergo routine assessments of both vitamin B_{12} and magnesium levels, with relevant supplementation considered where necessary to maintain optimal status.[190] As detailed earlier in this review, the recommendation that calcium supplementation could potentially

prevent metformin-induced deficiency of vitamin B_{12} is premature due to the existing paucity of studies.[59]

Conclusion

That metformin is generally a safe, effective and inexpensive first-line therapy in the treatment of T2DM is unquestionable. In addition, recent research indicates that it may partially mediate its antidiabetic properties through alteration to the gut microbiota. However, this review provides evidence that its use can impact upon the status of vitamins B_1, B_{12}, D, folic acid and magnesium as well as causing disruption to the microbiome, all of which may to some extent predicate the desired outcomes with this medication. Given that the status of these micronutrients in patients with T2DM is already likely to be suboptimal and that these deficiencies are variously associated with diabetic complications such as compromised endothelial, microvascular, vascular and neurological function,[38,43,46–49,80,85,95–97] it may be appropriate to consider supplementation where appropriate to mitigate these negative impacts of metformin. Since elderly T2DM patients are likely to experience greater prolonged exposure to both metformin and polypharmacy involving other drugs that are known to negatively impact upon micronutrient status makes the monitoring and management of this group particularly important. In many cases, this could be easily prevented by a simple supplementation approach, particularly as vitamin B12 has low toxicity. A greater number of randomized controlled trials as opposed to cross-sectional studies are required to conclusively identify the extent to which H_2RAs, PPIs and metformin independently and in combination contribute to patients' increased risk of vitamin B_{12} deficiency and potentially consequent neuropathy. Until then, it would be reasonable for physicians prescribing metformin singly or in combination with these drugs to monitor the patient's vitamin B_{12} status and to prescribe vitamin B_{12} supplements if blood biomarkers or clinical symptoms are consistent with a low vitamin B_{12} status.[191]

Ultimately, the cost of drug treatment-even with the most apparently safe and effective of drugs, such as metformin-is not simply the price of that treatment. Awareness of, monitoring and practical management of the possible, often underappreciated, side effects are necessary to mitigate patient harm and deliver optimal management of the condition.

Disclosure

The authors report no conflicts of interest in this work.

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