Review Article

# A Review on Effects of Metformin on Vitamin B12 Status

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## ABSTRACT

Metformin mostly prescribed first line therapy in diabetes mellitus type 2 patients because it decreases morbidity and mortality. Vitamin B12 is an essential micronutrient required for optimal hemopoetic, cardiovascular and neuro-cognitive function. Biochemical and clinical vitamin B12 deficiency has been demonstrated to be highly prevalent among diabetes mellitus type 2 patients on metformin. It presents with diverse clinical manifestations ranging from impaired memory, dementia, delirium, peripheral neuropathy, sub acute combined degeneration of the spinal cord, megaloblastic anemia and pancytopenia.
This review article offers a current perspective on vitamin B12 deficiency due to metformin therapy vitamin B12 supplementation in diabetes mellitus type 2 patients.

**Keywords**: Metformin, vitamin B12 defeciency, diabetes mellitus type 2, neuro-cognitive function.

## INTRODUCTION

Metformin is considered a cornerstone in the treatment of diabetes and is the most frequently prescribed first line therapy for individuals with type 2 diabetes. In addition, it is one of a few antihyperglycaemic agents associated with improvements in cardiovascular morbidity and mortality, which is a major cause of death in patients with type 2 diabetes.

Metformin does, however, induce vitamin B-12 malabsorption, which may increase the risk of developing vitamin B-12 deficiency-a clinically important and treatable condition. In addition, metformin treatment has been reported to be associated with decreased folate concentration, although the mechanism of this effect has not been elucidated. Finally, decreases in both folate and vitamin B-12 concentrations might, in turn, result in an increase in homocysteine concentration, an independent risk factor for cardiovascular disease, especially among individuals with type 2 diabetes.[1]

The United Kingdom Prospective Diabetes Study demonstrated that the biguanide metformin is as effective as sulfonylurea for glycaemic control and has

additional cardiovascular protective effects. The American Diabetes Association suggests using metformin as the first-line medical therapy for type-2 diabetes. When used alone, metformin rarely causes hypoglycaemia in older people. It increases insulin sensitivity and improves weight loss and the lipid profile. Its side effects include lactic acidosis in patients who experience heart failure, renal failure, and among alcoholic patients, as well as vitamin B12 deficiency.[2]

## Long term effects of metformin on vitamin B 12 status

Metformin is a first-line medication used in the treatment of type 2 diabetes but has also been shown in multiple studies to reduce serum B12 levels in 10–30% of patients[20]

All current evidence on vitamin B-12 deficiency in metformin treatment comes from short term studies. No long term, placebo controlled data on the effects of metformin on concentrations of vitamin B-12 in patients with type 2 diabetes have been reported. In addition, placebo controlled data on the effects of metformin on homocysteine concentrations in type 2 diabetes are sparse, and again no long term data are available.[1]

Vitamin B12 or cobalamin is a water soluble vitamin that plays a very fundamental role in DNA synthesis, optimal haemopoesis and neurological function. The clinical picture of vitamin B12 deficiency hence, is predominantly of features of haematological and neurocognitive dysfunction.[3]

Decrease in vitamin B12 absorption and levels following metformin use typically starts as early as the 4th month. The proposed mechanisms to explain metformin induced vitamin B12 deficiency among patients with T2DM include: alterations in small bowel motility which stimulates bacterial overgrowth and consequential vitamin B12 deficiency, competitive inhibition or inactivation of vitamin B12 absorption, alterations in intrinsic factor (IF) levels and interaction with the cubulin endocytic receptor. Metformin has also been shown to inhibit the calcium dependent absorption of the vitamin B12-IF complex at the terminal ileum.[3]

The hydrophobic tail of biguanides such as metformin, extends into the hydrocarbon core of membranes. The protonated biguanide group gives a positive charge to the surface of the membrane, which displaces divalent cations. Thus, the biguanides alter membrane potentials and affect their calcium-dependent functions. Metformin also has an effect on the cubilin, which may affect B12-instrinsic factor complex absorption and result in the deficiency.[2]

Vitamin B12 deficiency may have serious consequences such as megaloblastic anaemia, myelopathy and neuropathy, and subnormal cobalamin concentrations have been associated with dementia. Megaloblastic anaemia due to metformin-associated vitamin B12 deficiency has been reported,but it can be treated successfully with cyanocobalamin. Symptoms of B12-related neuropathy can be misinterpreted as diabetes neuropathy.[4]

Metabolically significant vitamin B12 deficiency hence will result in disruption of the methylation process and accumulation of intracellular and serum homocysteine. Hyperhomocysteinemia has been shown to have potentially toxic effects on neurones and the vascular endothelium. This reaction is also essential in the conversion of dietary folate (methyl-tetrahydrofolate) to its active metabolic form, tetrahydrofolate. In another essential enzymatic pathway, vitamin B12 as a co-factor mediates the conversion of methylmalonyl coenzyme A (CoA) to

succinyl-CoA. In the presence of vitamin B12 deficiency, this conversion pathway is diminished and an increase in the serum methylmalonic acid (MMA) ensues. This is followed by defective fatty acid synthesis of the neuronal membranes.[5] Vitamin B12 is also essential in the synthesis of monoamines or neurotransmitters like serotonin and dopamine.[6] Vitamin B12 deficiency impairs this synthesis.

Vitamin B12 (Cobalamin (Cbl)) deficiency may co-occur with diabetes.Although it is most classically associated with sub-acute combined degeneration, an exclusive peripheral neuropathy presentation can occur.[7]

Increased risk of vitamin B 12 deficiency associated with current dose and duration of metformin use, despite adjustment for many potential confounders. The risk factors identified have implications for planning screening or prevention strategies in metformin-treated patients.[17]

## Vitamin B12 deficiency among patients with type 2 diabetes mellitus: A comparative review

Several studies and case reports have documented an increased frequency of vitamin B12 deficiency among type 2 DM patients.

Metformin has been associated with a lowering of vitamin B12 levels for over 40 years. An increasing number of studies published worldwide highlighted this correlation.[7] It is well known that the risks of both type 2 diabetes and B12 deficiency increase with age. National data estimate a 21.2% prevalence of diagnosed diabetes among adults ≥65 years of age and a 6 and 20% prevalence of biochemical B12 deficiency (serum B12,148 pmol/L) and borderline deficiency (serum B12 $148–221 pmol/L) among adults ≥60 years of age.[8]

Vitamin B12 deficiency affects approximately 20% of elderly people, although the prevalence varies greatly depending on population studied and B12 cut-off used. Several studies have screened outpatients taking biguanides for B12 deficiency. Thirty per cent of 46 patients undergoing biguanide therapy developed B12 malabsorption, which resolved in half on stopping the drug. In 71 metformin patients, 21 had low B12 absorption, and four had low B12 levels. Fifty-four of 600 patients on long term biguanides had B12-related megaloblastic anaemia.[11]

A recent, randomized control trial designed to examine the temporal relationship between metformin and serum B12 found a 19% reduction in serum B12 levels compared with placebo after 4 years. Several other randomized control trials and cross-sectional surveys reported reductions in B12 ranging from 9 to 52%.[8]

An observational, cross-sectional cohort study conducted in 2003 showed that patients with type 2 diabetes on long-term metformin treatment exhibit lower levels of serum cobalamin and holotranscobalamin and higher HCy than patients not exposed to metformin. This means that metformin therapy carries a potential risk for development of vitamin B12 deficiency. The study highlights the necessity of checking B12 status during metformin treatment in order to avoid this potential adverse drug reaction and preserve the beneficial effects of metformin.[4]

A National Health and Nutrition Examination survey (NHANES) conducted during 1999-2006 showed that metformin therapy is associated with a higher prevalence of biochemical B12 deficiency. The survey revealed biochemical Cbl deficiency (defined as serum Cbl concentration ≤148 pmol/L ) presenting in 5.8% of those using metformin .The amount of B12 recommended by the Institute of Medicine (IOM) (2.4 mg/day) and the amount available in general multivitamins (6

mg) may not be enough to correct this deficiency among those with diabetes.[8]

An observational study conducted during 2006-07, showed that metformin therapy is associated with lower vitamin B12 status but there does not appear to be any significant effect on peripheral neuropathy in those receiving metformin.[7]

The HOME (the Outcome of its Metabolic Effects) trial was conducted in the outpatient clinics of three non-academic hospitals in the Netherlands showed that long term treatment with metformin increases the risk of vitamin B-12 deficiency, which results in raised homocysteine concentrations. Vitamin B-12 deficiency is preventable; therefore, findings suggested that regular measurement of vitamin B-12 concentrations during long term metformin treatment should be strongly considered.[1]

An observational, cross-sectional study conducted during from September 2005 to June 2006, compared the prevalence of vitamin B12 deficiency in DM patients aged 61 to 93 years who were treated with and without metformin showed that metformin use was significantly associated with vitamin B12 deficiency.[12]

A cross-sectional study conducted in 2006 found a 22% prevalence of metabolically confirmed B12 deficiency in the primary care type 2 diabetic population. Although further research needs to be performed to determine the clinical implications of our findings, B12 deficiency should be considered in type 2 diabetic patients, especially those taking metformin. Furthermore, a daily multivitamin may protect against B12 deficiency.[9]

A Cross-sectional study conducted during July 3 to August 13, 2009, in metformin-treated Brazilian diabetic patients showed a high prevalence of vitamin B12 deficiency in metformin-treated diabetic patients. Older patients, patients in long term treatment with metformin and low vitamin B12 intake are probably more prone to this deficiency.[10]

Two cases illustrate the problem of vitamin B 12 deficiency, these two patients had B12 deficiency associated with metformin therapy.[11]

A study on 280 patients on high dose metformin for more than four years. Only 70(25%)had vitamin B12 levels checked. All of these 70 cases had peripheral neuropathy. Vitamin B12 deficiency (<150pg/ml) was recorded in 23 cases, where vitamin B12 level deficient were replacement with vitamin B12 supplement in only 2 cases and improvement in neuropathic symptoms were documented.[15]

A multicenter trail of 390 patients with DM receiving insulin therapy who were randomized to receive metformin or placebo assessed for risk of decrease in vitamin B 12 levels over 4 years. Compared with placebo, patients taking, metformin had an increased risk of vitamin B12 deficiency and low vitamin B 12. The effects increased with duration of therapy.[16]

In patients with type 2 DM 16 weeks of treatment with metformin reduces levels of folate and vitamin B 12 which results in modest increased in homocysteine.[18]

A recent cohort study conducted at the University Hospital of Strasbourg, France, examined patients with a diagnosis of metformin-associated cobalamin deficiency and concluded that metformin causes at least 6 percent of the incidence of vitamin $B_{12}$ deficiency and that resulting hematologic abnormalities and peripheral neuropathy are quite common.[19]

Proton pump inhibitors and metformin alone were not associated with a significantdifference in vitamin B12 deficiency, but the combination was associated with a significant increase in vitamin B12 deficiency. More studies are needed to elucidate the exact mechanisms by

which proton pump inhibitors andmetformin affect vitamin B12 levels and relate these changes to clinical findings.[20]

Intrinsic factor (IF), also produced by gastric parietal cells, is required for B12 absorption from the GI tract. Metformin treatment can result in B12 deficiency mediated by depression of IF secretion. GI symptoms can be a limiting factor in optimizing metformin therapy, and the underlying cause remains unclear. Many a time, these patients are prescribed H2RAs/PPIs to help the GI side effects and gradually optimise the dose of metformin. H2RAs/PPIsmay therefore impair the absorption of protein-bound dietary B12 and could contribute to the development of B12 deficiency with prolonged use. Patients taking these medications for extended periods of time should be monitored for B12 status. The role of H2RA/PPI affecting the absorption of B12, especially in diabetic patients on metformin therapy with GI side-effects is less commonly perceived in routine daily clinical practice and also needs to be appreciated and recognised even further.[24]

## When to suspect vitamin B 12 deficiency

Vitamin B12 deficiency should be suspected in all patients with unexplained anemia, unexplained neuropsychiatric symptoms, and/or gastrointestinal manifestations, including sore tongue, anorexia, and diarrhea. Special attention should be paid to patients at risk of developing vitamin B12 deficiency. This includes mainly elderly people because of their high prevalence of atrophic gastritis, vegetarians and vegans, and patients with intestinal diseases. Other groups may be considered at risk, including patients with autoimmune disorders such as Graves' disease, thyroiditis, and vitiligo as well as patients receiving proton pump inhibitors, histamine receptor antagonists, or biguanides for prolonged periods.[22]

## Screening approach for vitamin B12 deficiency

Vitamin B12 depletion has been described in four stages ranging from mild depletion to clinical deficiency. Stage 1 is early vitamin B12 depletion characterized by a decrease in holoTC concentration. Stage 2 is cellular depletion characterized by a further decrease in holoTC.

Serum total B12 takes longer to respond to changes in status and can remain within the normal range in stages 1 and 2. Stage 3 is characterized by metabolic evidence of deficiency, with elevated concentrations of both plasma homocysteine and serum MMA, low holoTC and normal or below normal serum total B12 concentrations. Stage 4 is clinical deficiency where both serum total B12 and holoTC are low, the metabolic indicators are elevated and there are clinical signs of deficiency including haematological and neurological manifestations.

Haematological features, which occur as a result of impaired DNA synthesis, include megaloblastic anaemia characterized by megaloblastic (large immature blood cells with low concentrations of haemoglobin) blood cells, hypersegmentation of neutrophils, leukopaenia and thrombocytopaenia. Neurological symptoms are diverse and irreversible if left untreated.

The most severe form manifests as a sub-acute combined degeneration of the spinal cord and is characterized by degeneration of the posterior and lateral columns of the spinal cord.[25]

Accumulating evidence suggests that Cbl associated metabolites methylmalonic acid and homocysteine are more sensitive and specific indicators of early symptomatic Cbl deficiency than serum Cbl itself.[13]

Measurement of serum MMA or homocysteine concentrations and holo-transcobalamin II is a more sensitive and specific approach for screening especially among type 2 diabetic patients with borderline serum vitamin B12 concentrations of 200- 400 pg/ml. Normal serum tHcy concentration ranges between 5 and 15 µmol/L. Normal serum MMA concentration is considered the value less than 0.28µmol/L.

In the case of direct measures of vitamin B-12 [plasma or serum vitamin B-12 or holotranscobalamin (holoTC)], low vitamin B-12 status is indicated by being below the lower limit of the reference range (for vitamin B-12, 200 pg/mL or ,148 pmol/L; for holoTC, 35 pmol/L), whereas for indirect measures of metabolites (methylmalonic acid or homocysteine),low vitamin B-12 status would be indicated by a level above the upper limit of the reference range (for methylmalonic acid, .260 nmol/L; for homocysteine, .12 lmol/L). The distinction between low vitamin B-12 status and outright vitamin B-12 deficiency is not directly attributable to the actual measured concentrations of circulating vitamin B-12 or associated metabolites but is made on the basis of on the presence or absence of morbidity that is attributable to the vitamin B-12 deficiency state.[23]

### Vitamin B 12 supplementation among DM patients on metformin

Currently, there are no guidelines for the supplementation and appropriate dose of vitamin B12 for DM patients on metformin. It must be remembered that Cbl deficiency induced neuropathy precedes the appearance of megaloblastic anaemia and while anaemia of Cbl deficiency is reversible, the progression of neuropathy is only arrested not reversed with initiation of Cbl therapy of diabetic neuropathy resembles metformin induced neuropathy will add to confusion. So an alternative and a more practical and cost-effective method to avoid Cbl deficiency, not evaluated yet would be annual 1000µg Cbl injection that would provide more than annual Cbl need for every patient on long term metformin therapy.[14]

A recently published follow up study from the United States of America showed that administration of oral vitamin B12 among type 2 DM patients on long term use of metformin was ineffective in correcting biochemical vitamin B12 deficiency.[8]

Oral administration of high dose vitamin B 12(1-2 mg daily) in as effective as intramuscular administration in correcting the deficiency, regardless of etiology. Because crystalline formulation are better absorbed than naturally occurring vitamin B 12 . Although it is not known if prophylactic vitamin B 12 supplement prevents deficiency. It seems prudent to monitor vitamin B 12 levels periodically in patients on metformin therapy.[16]

## CONCLUSION

Vitamin B12 deficiency is more common among patients with type 2 DM. There is a need for more accurate differential diagnosis to distinguish between diabetic neuropathy and metformin induced neuropathy. Patients receiving long term metformin therapy should considerably require appropriate screening strategies to prevent Cbl deficiency. Patients on metformin therapy should routinely check for holotranscobalamin,methlymalonic acid and homocysteine levels. Long term metformin therapy leads to increase homocysteine levels. High homocysteine levels have been associated with increased risk of coronary artery disease, high blood pressure, hip and other bone fractures, rheumatoid arthritis, diabetes, and other serious chronic diseases. Although these are all very dangerous diseases, to the degree that they are caused or aggravated by elevated homocysteine levels,

they can be remarkably easy to treat by consuming adequate amounts of foods and especially supplements containing folic acid (folate), vitamin B12 (cobalamin), vitamin B6 (pyroxidine), and betaine (trimethylglycine), all of which combine to minimize homocysteine levels. Elderly patients on metformin therapy are at high risk of developing vitamin B12 deficiency and requries nutritional supplement. There is further need for studies to determine the optimal vitamin B12 supplementation and the dose and frequency of supplementation among patients with DM.

## Competing interests

The authors declare no competing interests.

## Authors' contributions

Both authors equally contributed to the development of the concept and manuscript, critically read and approved the final manuscript.

## ABBREVATIONS

DM: Diabetes Mellitus.
Cbl: Cobalamin.
tHyc: Trans homocysteine.
MMA: Methymalonic Acid.
HOME: The Outcome of its Metabolic Effects.
NHANES: National Health and Nutritional Examination Survey.

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**Table 1.** Major clinical manifestations of cobalamin Deficiency[21]

| System | Manifestation |
|---|---|
| Hematology | Macrocytosis; hypersegmentation of the neutrophils; are generative macrocytary anemia; medullary megaloblastosis ("blue spinal cord") |
| | Isolated thrombocytopenia and neutropenia; pancytopenia |
| | Hemolytic anemia; thrombotic microangiopathy (presence of schistocytes) |
| Neuropsychiatric | Combined sclerosis of the spinal cord |
| | Polyneurites (especially sensitive ones); ataxia; Babinski's Phenomenon |
| | Cerebellar syndromes affecting the cranial nerves, including optic neuritis, optic atrophy, urinary or fecal incontinence |
| | Changes in the higher functions, even dementia, stroke and Atherosclerosis (hyperhomocysteinemia); Parkinsonian syndromes; Depression |
| Digestive | Hunter's glossitis; jaundice; lactate dehydrogenase and bilirubin elevation ("intramedullary destruction") |
| | Resistant and recurring mucocutaneous ulcers |
| | Abdominal pain; dyspepsia; nausea; vomiting; diarrhea; disturbances in intestinal functioning |
| Gynecological | Atrophy of the vaginal mucosa and chronic vaginal and urinary infections (especially mycosis); hypofertility and repeated miscarriages |
| Other | Venous thromboembolic disease; angina (hyperhomocysteinemia) |