**ABSTRACT**

Consistently all researches have shown that metformin therapy improves glycemic response and lowers HbA1c levels. Besides causing gastrointestinal disorders at higher doses metformin is strongly related to vitamin B12 malabsorption and deficiency. The dose is an independent predictor of deficiency of vitamin B12 and longer duration is associated with higher prevalence. Recommendation by certain authors state that there should be regular screening of Vitamin B12 and supplementation including folate and calcium for patients on long term metformin use, especially the elderly who are at a higher risk of vitamin B12 deficiency. But there is not enough evidence showing adverse effects of vitamin B12 deficiency in patients on metformin drug. Thus there is contradiction, stating supplementation might be of no use if health benefits are not observed. Thus there is need for more research on effects of vitamin B12 deficiency on long term metformin use and effective ways in which this deficiency can be treated. From a public health perspective, it is most relevant to investigate whether metformin use causes nutritional deficiencies and if posing a health risk to the population at exposure by translating evidence based recommendations. The purpose of this article is to review existing data regarding the effects of long term metformin usage as monotherapy or in combination therapy for glycemic control causing vitamin B12 deficiency.

**KEYWORDS:** DIABETES, METFORMIN, VITAMIN B12.

**INTRODUCTION**

Diabetes is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. The chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction, and failure of different organs, especially the eyes, kidneys, nerves, heart, and blood vessels [ADA, 2010]. The number of people with diabetes is increasing due to population growth, aging, urbanization, and increasing prevalence of obesity and physical inactivity [Wild et al., 2004]. In 2005–2008, based on fasting glucose or hemoglobin A1c levels, 35% of U.S. adults aged 20 years or older had prediabetes (50% of adults aged 65 years or older). Applying this percentage to the entire U.S. population in 2010 yields an estimated 79 million American adults aged 20 years or older with prediabetes. About 213,000 people younger than 20 years had diabetes (type 1 or type 2) in the United States in 2010 [CDC, 2011].

Type-2 diabetes carries significant morbidity and is the leading cause of kidney failure, lower-limb amputations, and new cases of adult blindness. Moreover, it is the seventh leading cause of death in the U.S., primarily as a result of cardiovascular morbidity [Brunetti & Kalabakil, 2012]. The epidemic of type 2 diabetes mellitus and the recognition that achieving specific glycemic goals can substantially reduce morbidity have made the effective treatment of hyperglycemia a top priority [Nathan et al., 2009]. Controlled clinical trials involving patients with type 1 diabetes and those with type 2 diabetes have conclusively demonstrated that intensive diabetes therapy aimed at lowering glycemic levels reduces the risk of diabetic retinopathy, nephropathy, and neuropathy [DCCT, 2005]. Pharmacological therapy to prevent type 2 diabetes may be an important therapeutic modality in those patients in whom lifestyle interventions fail, are not sufficiently potent, or are not feasible [Padwal et al., 2005]. Metformin is a commonly prescribed first-line antidiabetic drug, has proven to be safe and efficacious when used as monotherapy or in combination with other oral antidiabetic agents or insulin in patients with type 2 diabetes [DeFronzo et al., 2005]. The use of metformin has extended in the last 2 decades to include diabetes prevention, gestational diabetes mellitus (GDM) and polycystic ovary syndrome (PCOS). There is accumulating evidence from observational studies to suggest a possible role for metformin in cancer prevention and in the management of non-alcoholic fatty liver disease [Fiaid et al., 2013]. Metformin therapy throughout pregnancy in women with PCOS reduces the otherwise high rate of first-trimester spontaneous abortion seen among women not receiving metformin and does not appear to be teratogenic [Glanck et al., 2001]. Also, the initiation of treatment with metformin was associated with a significant reduction in the serum levels of TSH in diabetic patients with primary hypothyroidism [Cappelli et al., 2009].

**MECHANISM OF ACTION OF METFORMIN DRUG:**

Metformin (1, 1-dimethylbiguanide hydrochloride) is a biguanide commonly used in the treatment of type 2 diabetes mellitus. It is frequently referred to as an "insulin sensitizer" because in settings of insulin resistance and hyperinsulinemia, it lowers circulating insulin levels [Zakikhani et al., 2006]. The effect of metformin on glucose is mediated by improving insulin sensitivity in liver, muscle and fat. Conventionally, metformin is known to reduce glucose concentration through reduction in glucose liver output brought about primarily by reducing the rate of gluconeogenesis and to a lesser extent by reducing glycolysis. Metformin also augments peripheral glucose utilization in muscle and fat [Fiaid et al., 2013].

More recent work showed that biguanides impair mitochondrial adenine-5'-triphosphate (ATP) production, which results in the activation of the liver kinase B1 (LKB1)-5’-AMP-activated protein kinase (AMPK) signaling pathway. This pathway is central to the regulation of cellular energy homeostasis, and its activation under conditions of energy stress leads to physiologic down regulation of energy-consuming processes, such as protein synthesis and fatty acid synthesis, to restore ATP levels. The system is involved in appetite control by the central nervous system, and in the special case of hepatocytes, activation of the LKB1-AMPK pathway down regulates gluconeogenesis, which represents the export of energy from hepatocytes to the organism in the form of glucose. This effect in turn reduces blood glucose concentration, which results in a secondary decrease in insulin level [Pollak, 2010]. Metformin activates AMPK in hepatocytes; as a result, acetyl-CoA carboxylase (ACC) activity is reduced, fatty acid oxidation is induced, and expression of lipogenic enzymes is suppressed. Phosphorylation and inactivation of ACC, as a result of AMPK activation, serves to inhibit the proximal and rate-limiting step of lipogenesis. Reduced synthesis of the ACC product, malonyl-CoA, is also predicted to relieve inhibition of CPT-1, resulting in increased fatty acid oxidation [Zhou et al., 2001]. Despite its efficacy, vitamin B12 deficiency is a noted side effect of long term metformin therapy.

**Figure 1:** Metformin’s Action Mechanism
VITAMIN B12:
Vitamin B12 is a water-soluble essential vitamin. A member of vitamin B complex, vitamin B12 is also called cobalamin because it contains the metal cobalt. Vitamin B12 is synthesized by bacteria and is found mainly in meat, egg, and dairy products but lacks a reliable plant source. Vitamin B12 is an essential micronutrient required for optimal hemopoetic, neuro-cognitive and cardiovascular function. It is essential for the formation of red blood cells and maintenance of a healthy nervous system as well as for the rapid synthesis of DNA during cell division. Megaloblastic anemia is the common and serious illness associated with B12 deficiency, but it is believed that a mild decrease in the B12 level is associated with neurologic and psychiatric problems such as ataxia or mood disturbances. A common cause of vitamin B12 deficiency is poor intake or absorption. Vitamin B12 deficiency has been demonstrated to be highly prevalent among patients with type 1 and type 2 diabetes mellitus. [Hanna et al., 2009].

It is known that vitamin B12 deficiency is common and that its prevalence increases with age. Because typical signs and symptoms are frequently absent in early vitamin B12 deficiency, concern should be focused on persons with known risk factors. Lifestyle factors, such as smoking, alcoholism and vegetarian diet, may predispose to vitamin B12 deficiency. Gastrointestinal diseases, gastric acid suppressive drugs and metformin may increase the probability of cobalamin malabsorption [Loika et al., 2007]. It is estimated that 10% to 30% of patients undergoing metformin therapy develops evidence of vitamin B12 deficiency. Another study showed a 22% prevalence of B12 deficiency in type 2 DM on metformin therapy [Kumthekar et al., 2012]. Vitamin B12 malabsorption has been described in diabetics on biguanides [Caspy et al., 1977]. Some evidence supported the hypothesis that metformin induced B12 malabsorption is due to enhanced bacterial overgrowth, especially because diabetic patients are known to exhibit alterations in small bowel motility as well as bacterial overgrowth. Also, vitamin B12 absorption is a calcium-dependent process. 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 acts to displace divalent cations. Thus, biguanides alter membrane potentials and affect divalent cation membrane functions, such as those that are calcium dependent, and may act in general as a calcium channel blocker. Adhesion of many substances to cell surface membranes is affected by calcium. Specifically, the cell surface TcII receptors on all DNA synthesizing cells are calcium dependent, and metformin may interfere with the delivery of vitamin B12 to these cells [Bauman et al., 2000 & Wulfle et al., 2003].

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Some clinical studies have reported that metformin lowered vitamin B12 level. Therefore in this review the association between metformin treatment and vitamin B12 has been assessed [Livet et al., 2014].

### Table: Research Studies on B12 Deficiency due to Metformin Therapy in Management of Diabetes Mellitus

<table>
<thead>
<tr>
<th>STUDY CHARACTERISTICS/ DESIGN</th>
<th>METHODOLOGY</th>
<th>RESULT</th>
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<td>Ko et al., (2014) Aim: Association of Vitamin B12 Deficiency and Metformin Use in Patients with Type 2 Diabetes. (n)-799; Age &lt; 59yr; Men (No %) - 354 (44.3); Diabetic duration (yr)- 11.3±7.9</td>
<td>Vit.B12 and folate levels were quantified by chemiluminescent enzyme immunoassay. Alcohol intake was calculated. Diabetic retinopathy was assessed from retinal photographs at baseline, and the findings were reviewed by a board-certified ophthalmologist.</td>
<td>The prevalence of vitamin B12 deficiency in metformin-treated type 2 diabetes patients was significantly lower than controls, (p-0.002). Mean B12 levels were significantly lower in the S+M group compared with I+M group (17.4% vs. 4.2%, P = 0.001). The study demonstrated that patients with type 2 diabetes who were treated with metformin combined with sulfonylurea require clinical attention for vitamin B12 deficiency. Higher metformin doses and longer treatment durations were independent risk factors.</td>
<td>It was demonstrated that daily metformin dosage and treatment duration were the most consistent risk factors for vitamin B12 deficiency. Higher metformin doses and longer treatment durations were independent risk factors.</td>
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<td>Kang et al., (2014) Aim: To investigate the prevalence of Metformin-Induced Vitamin B12 Deficiency in Sulfonylurea Combination Compared with Insulin Combination in Patients with DM. Cross sectional study; (n)- 394; Duration of study - 2years; Males-179; Females-210;Mean age- 59.4±10.4 years; Duration of diabetes- ≤2 years±0.7</td>
<td>Metformin and sulfonylurea (S+M n = 299) or metformin and insulin (I+M group, n = 95) were consecutively recruited. The vitamin B12 and folate levels were quantified using the chemiluminescent enzyme immunoassay. The medication history was evaluated using a dietary supplement questionnaire.</td>
<td>The mean serum B12 levels were significantly lower in the S+M group compared with I+M group (600.0±266.5 vs.757.7±287.6 pg/mL, P&lt;0.001). The prevalence of vitamin B12 deficiency in the metformin-treated patients was significantly higher in the S+M group compared with I+M group (17.4% vs. 4.2%, P = 0.001). The study demonstrated that patients with type 2 diabetes who were treated with metformin combined with sulfonylurea require clinical attention for vitamin B12 deficiency. Higher metformin doses and regular monitoring of their vitamin B12 levels.</td>
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<td>Ifthik et al., (2013) Aim: To determine prevalence and associations of Vitamin B12 deficiency in patients of type 2 diabetes mellitus treated with metformin. Case control Study; (n)- 219; Duration of study- 3year; Age - 40-70 yr. Males-128; Females-91; Duration of diabetes (mean) - 8.96 to 8.82 years; Patients with vitamin B12 levels of less than 150 pg/ml were less to be B12 deficient. 114 outdoor patients of type 2 diabetes mellitus currently on metformin for at least 6 months were enrolled by consecutive sampling, and 105 age and sex matched patients taken as control. Samples were analyzed the same day for B12 levels &amp; HbA1c. The results were analyzed on SPSS version 16.</td>
<td>Serum B12 levels were low in 31% on metformin as compared to 8.6% among controls, (p=0.002). Mean B12 levels were significantly low in metformin group, 311 pg/ml (±194.4), and p- 0.03. Dose of metformin had inverse correlation with B12 levels and the difference was statistically significant with value &lt; 0.001. The study demonstrated high prevalence of vitamin B12 deficiency in patients treated with metformin with significant effect of dose and duration of metformin use on B12 levels.</td>
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### Reference & Aim

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<td>Sato et al., (2013)</td>
<td>Aim: To study Relationship between metformin use, vitamin B&lt;sub&gt;12&lt;/sub&gt; deficiency, hyperhomocysteinemia and vascular complications in patients with type 2 diabetes.</td>
<td>B&lt;sub&gt;12&lt;/sub&gt; status was analyzed in 62 consecutive metformin-treated patients. The relationship between B&lt;sub&gt;12&lt;/sub&gt; concentration and vascular complications was analyzed in 46 metformin-treated patients. Plasma HC ≥10 μmol/L was regarded as hyperHC.</td>
<td>There were independent correlations between metformin use and B&lt;sub&gt;12&lt;/sub&gt; lowering (P=0.02). B&lt;sub&gt;12&lt;/sub&gt; lowering and elevation of HC (P&lt;0.01).</td>
<td>Metformin induced B&lt;sub&gt;12&lt;/sub&gt; lowering in diabetes was associated with elevation of HC, and hyperHC was independently related to retinopathy. Metformin-induced B&lt;sub&gt;12&lt;/sub&gt; deficiency was correctable with B&lt;sub&gt;12&lt;/sub&gt; supplementation.</td>
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<td>Moore et al., (2013)</td>
<td>Aim: To investigate the association of metformin, serum vitamin B&lt;sub&gt;12&lt;/sub&gt;, calcium supplements, and cognitive impairment in patients with diabetes.</td>
<td>Participants with diabetes had worse cognitive performance. Among participants with diabetes, worse cognitive performance was associated with metformin use (2.23 [1.05–4.75]). After adjusting for age, sex, level of education, history of depression, serum vitamin B&lt;sub&gt;12&lt;/sub&gt;, and metformin use, participants with diabetes who were taking calcium supplements had better cognitive performance (0.41 [0.19–0.92]).</td>
<td>Metformin use was associated with impaired cognitive performance. Vitamin B&lt;sub&gt;12&lt;/sub&gt; and calcium supplements may alleviate metformin-induced vitamin B&lt;sub&gt;12&lt;/sub&gt; deficiency and were associated with better cognitive outcomes.</td>
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<td>Reinstatler et al., (2012)</td>
<td>Aim: To describe the prevalence of biochemical B&lt;sub&gt;12&lt;/sub&gt; deficiency in adults with type 2 DM taking metformin compared with those not taking metformin and those without diabetes, and explore whether this relationship is modified by vitamin B&lt;sub&gt;12&lt;/sub&gt; supplements.</td>
<td>All participants with serum vitamin B&lt;sub&gt;12&lt;/sub&gt; measurements taken within 6 months of cognitive assessment were included. Subgroup analyses were performed for participants who had either type 2 diabetes / IGT. An ordinal logistic regression model was formed with categories of cognitive performance as the response variable and diabetes as a predictor.</td>
<td>Biochemical B&lt;sub&gt;12&lt;/sub&gt; deficiency was present in 5.8% of those with diabetes using metformin compared with 2.4% of those not using metformin (P = 0.002) and 3.3% of those without diabetes (P = 0.0002). Consumption of any supplement containing B&lt;sub&gt;12&lt;/sub&gt; was not associated with a reduction in the prevalence of biochemical B&lt;sub&gt;12&lt;/sub&gt; deficiency among those with diabetes, whereas consumption of any supplements containing B&lt;sub&gt;12&lt;/sub&gt; was associated with a two-thirds reduction among those without diabetes.</td>
<td>Metformin therapy is associated with a higher prevalence of biochemical B&lt;sub&gt;12&lt;/sub&gt; deficiency. The amount available in general multivitamins (6 mg) may not be enough to correct this deficiency among those with diabetes.</td>
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<td>Nervo et al., (2010)</td>
<td>Aim: To evaluate the presence of vitamin B&lt;sub&gt;12&lt;/sub&gt; deficiency and the factors associated with serum vitamin B&lt;sub&gt;12&lt;/sub&gt; levels in a sample of metformin-treated Brazilian diabetic patients.</td>
<td>Participants with diabetes had worse cognitive performance. Among participants with diabetes, worse cognitive performance was associated with metformin use (2.23 [1.05–4.75]). After adjusting for age, sex, level of education, history of depression, serum vitamin B&lt;sub&gt;12&lt;/sub&gt;, and metformin use, participants with diabetes who were taking calcium supplements had better cognitive performance (0.41 [0.19–0.92]).</td>
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<td>De Jager et al., (2010)</td>
<td>Aim: To study the effects of metformin on the incidence of vitamin B&lt;sub&gt;12&lt;/sub&gt; deficiency (&lt;150 pmol/L), low concentrations of vitamin B&lt;sub&gt;12&lt;/sub&gt; (&lt;150 200 pmol/L), and folate and HC concentrations in patients with type 2 diabetes receiving treatment with insulin.</td>
<td>Patients were randomly assigned to receive either 850 mg of metformin three times a day or 850 mg of placebo thrice daily. Percentage change in vitamin B&lt;sub&gt;12&lt;/sub&gt;, folate, and HC concentrations from baseline at 4, 17, 30, 43, and 52 months were analyzed. During visits, physical examination, medical history and laboratory investigations were performed.</td>
<td>Compared with placebo, metformin treatment was associated with a mean decrease in vitamin B&lt;sub&gt;12&lt;/sub&gt; concentration of −19% (P&lt;0.001) and in folate concentration of −5% (P=0.033), and an increase in HC concentration of 5% (P=0.091). The absolute risk of vitamin B&lt;sub&gt;12&lt;/sub&gt; deficiency (&lt;150 pmol/L) was 7.2% in patients with a low vitamin B&lt;sub&gt;12&lt;/sub&gt; concentration and 14.9 pmol/L for patients with a normal vitamin B&lt;sub&gt;12&lt;/sub&gt; concentration (≥220 pmol/L). Long term treatment with metformin increases the risk of Clb deficiency, which results in raised HC concentration. Vitamin B&lt;sub&gt;12&lt;/sub&gt; deficiency is preventable.</td>
<td>High prevalence of Clb deficiency in metformin-treated diabetic patients. Patients in long term treatment with metformin and low vitamin B&lt;sub&gt;12&lt;/sub&gt; intake are probably more prone to this deficiency.</td>
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**Reference & Aim**

1. **Aim:** To study the relationship between metformin use, vitamin B<sub>12</sub> deficiency, hyperhomocysteinemia and vascular complications in patients with type 2 diabetes.

2. **Aim:** To investigate the association of metformin, serum vitamin B<sub>12</sub>, calcium supplements, and cognitive impairment in patients with diabetes.

3. **Aim:** To describe the prevalence of biochemical B<sub>12</sub> deficiency in adults with type 2 DM taking metformin compared with those not taking metformin and those without diabetes, and explore whether this relationship is modified by vitamin B<sub>12</sub> supplements.

4. **Aim:** To evaluate the presence of vitamin B<sub>12</sub> deficiency and the factors associated with serum vitamin B<sub>12</sub> levels in a sample of metformin-treated Brazilian diabetic patients.

5. **Aim:** To study the effects of metformin on the incidence of vitamin B<sub>12</sub> deficiency (<150 pmol/L), low concentrations of vitamin B<sub>12</sub> (<150 200 pmol/L), and folate and HC concentrations in patients with type 2 diabetes receiving treatment with insulin.
**REFERENCES:**


