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Vitamin B_{12} in diabetes — a new treatment paradigm?

ABSTRACT

Vitamin B_{12} supplementation in specific clinical conditions in diabetic patients has been recommended in the guidelines. These recommendations reflect reports confirming the importance of vitamin B_{12} supplementation in the treatment of diabetic complications, as well as to correct its deficiency during metformin treatment. In the present article, we reviewed the issue of vitamin B_{12} deficiency, the relevant diagnostic approach, and the rationale for vitamin B_{12} supplementation in diabetic patients. (Clin Diabetol 2020; 9; 6: 489–496)

Key words: vitamin B_{12}, diabetes, metformin, diabetic neuropathy

Do we know how important is vitamin B_{12} in humans?

Vitamin B_{12} is absorbed in the terminal part of the ileum. A prerequisite for this process is the presence of a glycoprotein known as the intrinsic factor which is produced by the parietal cells of the stomach. When bound to the intrinsic factor, cobalamin forms a hematopoietic factor which plays a role in cell formation in the hematopoietic system. In addition, it is a necessary factor for erythropoiesis in bone marrow and DNA and RNA synthesis in erythroblasts. Vitamin B_{12} is also involved in purine and pyrimidine metabolism [1, 2].

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The effects of vitamin B12 or cobalamin on the human body are complex. It is directly involved in the conversion of methylmalonyl-coenzyme A to succinyl-coenzyme A (Fig. 1). This reaction creates a substrate for the Krebs cycle, thus providing energy for multiple processes in the human body. Body systems that particularly actively use this process include the nervous, gastrointestinal, immunologic, and hematopoietic systems [3, 4].

Methionine synthesis is another biochemical process vitamin B_{12} is a cofactor of (Fig. 2). An adequate rate of this process is necessary for myelinization (formation of nerve sheaths) which is necessary for maintaining appropriate nerve conduction. In addition, there is an association between methionine synthesis and synthesis of some neurotransmitters (dopamine, noradrenaline, and serotonin) [4, 5].

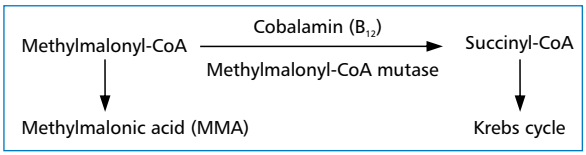

Figure 1. Conversion of methylmalonyl-coenzyme A to succinyl-coenzyme A

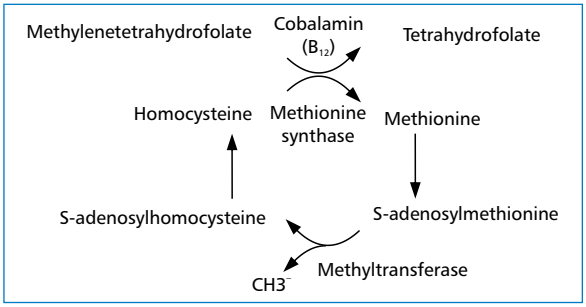

Figure 2. Methionin synthesis

Table 1. Effects of vitamin B_{12} deficiency

Nervous system	Peripheral neuropathy
	Myelopathy
	Optic nerve atrophy
	Spastic paralysis
	Cognitive dysfunction
	Mood disturbances
	Chronic fatigue syndrome
Hematopoietic system	Macrocytosis
	Megaloblastic anaemia
	Leukopenia
	Thrombocytopenia
Gastrointestinal system	Gastrointestinal mucosal atrophy
	Stomatitis
	Change in bowel movement pattern

Due to vitamin B_{12} involvement in the above mentioned processes, its deficiency may have various clinical manifestations (Table 1) [1, 2].

What are the primary sources of vitamin B_{12}?

The process of cobalamin absorption from the food and its utilization in the body is complex and thus may be adversely affected by multiple clinical conditions.

One of the most important determinants of vitamin B_{12} level is its dietary intake. The average vitamin B_{12} dietary content is 3–30 μg, and the daily requirement is only 0.6–1.2 μg. Patients on a diet poor in cobalamin sources (meat, milk, eggs, cheese, fish) are at particular risk of cobalamin deficiency. Body cobalamin stores are large enough to make a deficiency exclusively due to poor dietary intake unlikely, unless an individual is on a restrictive vegetarian or vegan diet. As the latter dietary choices are increasingly popular, this risk should be recognized and patients should be reminded of a potential need for cobalamin supplementation [6].

Vitamin B_{12} deficiency may be due to gastrointestinal disease. Cobalamin absorption in the gastrointestinal tract depends on the presence of several factors that protect the cobalamin moiety and transport it to the target tissues. These include haptocorrin (produced by the salivary glands), intrinsic factor (produced by the gastric parietal cells) and transcobalamin II (present in the ileum where cobalamin is ultimately absorbed to bloodstream). Gastrointestinal pathologies may result in reduced levels of these protective factors, leading to impaired cobalamin absorption and reduction of its serum level [7, 8].

Diseases commonly perceived as inducing vitamin B_{12} deficiency include inflammatory bowel disease (ulcerative colitis and Crohn's disease) and pernicious (Addison-Biermer) anaemia. However, diseases less frequently associated with vitamin B_{12} deficiency, such as celiac disease, chronic pancreatitis and liver disease, may also become major reasons for the need for vitamin B_{12} supplementation [9–11]. Several mechanisms leading to vitamin B_{12} deficiency may operate in chronic alcohol abuse, including chronic gastric and duodenal mucosal inflammation, chronic pancreatitis, and cirrhosis. Finally, small intestine bacterial overgrowth (SIBO) may predispose to impaired absorption of micro- and macronutrients, and another potential cause is the presence of gastrointestinal parasites [3, 4, 12].

Impaired absorption may also have iatrogenic causes, in particular in patients after resection procedures involving those gastrointestinal tract segments which are responsible for vitamin B_{12} absorption, in particular the stomach and the ileum. In the present era of growing popularity of bariatric surgery, it is particularly important to monitor vitamin B_{12} deficiencies and provide adequate supplementation in this patient group [13].

Medications may be a common cause for impaired vitamin B_{12} absorption. These include metformin, proton pump inhibitors, H2 receptor blockers, antibiotics, anticonvulsants, calcium antagonists, and 5-aminosalicylates [14–16]. Paradoxically, the latter stabilize inflammation in inflammatory bowel diseases but may themselves predispose to cobalamin deficiency [17].

What are the causes of vitamin B_{12} deficiency in diabetes?

Diabetic patients are particularly prone to vitamin B_{12} deficiency. The causes of the latter may be somewhat different in patients with type 1 and type 2 diabetes.

In type 1 diabetes, this is mostly associated with an increased risk of concomitant autoimmune disease, such as autoimmune thyroiditis. Individuals with hypothyroidism were shown to have macrocytosis, and often also resultant macrocytic anaemia [18]. It may be a sign of thyroid disease or result from a concomitant autoimmune disease limiting cobalamine absorption. Celiac disease and Addison-Biermer anaemia are also more common in patients with type 1 diabetes [19, 20]. A typical feature of long-standing poorly controlled type 1 diabetes are microangiopathic complications which may result in autonomic neuropathy involving the gastrointestinal system, manifesting with gastroparesis and enteropathy [21].

Patients with type 2 diabetes more often present with macroangiopathic complications resulting in potentially more diffuse perfusion abnormalities [22].

One such manifestation may be atherosclerotic mesenteric artery disease, potentially leading to significantly impaired intestinal absorption due to ischemia, or even intestinal necrosis with more severe ischemic events such as mesenteric artery embolism. Patients with type 2 diabetes are also at a higher risk of inflammatory conditions, resulting in more frequent use of antibiotics [23]. In addition, bariatric procedures are often performed in these patients due to concomitant obesity, depriving them of a large intestinal surface to absorb vitamin B12 [13]. Diabetic patients are often subjected to various dietary interventions. If these are misunderstood or overly restrictive, they may lead to an unbalanced diet with potential deficiencies of multiple micro- and macronutrients.

The effect of medications on cobalamin absorption seems more important in diabetic patients compared to those without diabetes. In addition to metformin, which has been frequently highlighted in this regard in the recent literature, these patients are more commonly treated with calcium antagonists, proton pump inhibitors, and acetylsalicylic acid [14, 15, 24].

Apart from these typical predispositions, it should be always borne in mind during the diagnostic process that vitamin B_{12} deficiency in a diabetic patient may result from clinical conditions independent from diabetes.

What may be the consequences of long-standing vitamin B_{12} deficiency in a diabetic patient?

Vitamin B_{12} deficiency in a diabetic patient may potentially affect both micro- and macroangiopathic changes.

Neuropathy is of major importance among the microangiopathic complications, and it may result from vitamin B_{12} deficiency even without concomitant diabetes. Among various types of neuropathy, thick motor fibres are most sensitive to vitamin B_{12} deficiency, which may manifest with loss of balance or foot deformities. In a diabetic patient, it is difficult to ascertain the primary cause: whether it is uncontrolled diabetes, its long duration, unidentified genetic factors, or vitamin B deficiencies [25]. However, it seems logical that in a patient with diabetic neuropathy, an additional contributing factor may be present. It was shown that cobalamine deficiency accompanying diabetes promotes peripheral neuropathy due to impaired nerve myelinization and may alter its clinical presentation, rendering it more atypical. Other common causes of neuropathy that may coexist with diabetes include alcohol abuse (also via cobalamin-independent mechanisms), use of neurotoxic drugs, and advanced chronic kidney disease (mediated by uremic toxins) [25].

It was initially thought that the major consequence of cobalamin deficiency is damage to thick nerve fibres but a number of recent studies showed an association between vitamin B_{12} deficiency and various components of autonomic nervous system damage. In particular, multiple studies focused on the association between cobalamin deficiency and cardiovascular autonomic dysfunction, including orthostatic hypotension. Hansen et al. [26] showed that vitamin B_{12} deficiency was associated with cardiovascular autonomic dysfunction in patients with type 2 diabetes. Beitzke et al. [27] suggested that orthostatic hypotension in diabetic patients may be caused by vitamin B_{12} deficiency, warranting investigation for the latter. Similar associations were reported for autonomic neuropathy involving the gastrointestinal system (gastroparesis, enteropathy, sialorrhoea), the genitourinary system (neurogenic bladder, erectile dysfunction), and thermoregulation mechanisms (excessive sweating). However, no evidence is available for a direct causal role of vitamin B_{12} deficiency in the development of autonomic neuropathy [28].

Another complication of diabetes is diabetic retinopathy. However, some fundoscopy findings are not specific for diabetic retinopathy. In their study, Satyanayarana et al. [29] suggested that vitamin B_{12} deficiency may be an independent risk factor for the development of diabetic retinopathy. Retinal bleeding identified by ophthalmoscopy may accompany severe anaemia or thrombocytopenia, including due to vitamin B_{12} deficiency. These case reports highlighted the role of hypoxia as a factor that damages the endothelium. Abnormal repair and homeostatic processes are also operating. Retinal lesions seem more frequent in patients with thrombocytopenia accompanying anaemia due to vitamin B_{12} deficiency [30, 31].

In addition to typical retinal pathology, cobalamin deficiency may also result in bilateral optic nerve neuropathy. Clinically, it manifests mostly with centrocecal scotoma and slowly developing optic nerve atrophy. The mechanism of this pathology remains unknown but it seems to be related to the role of vitamin B_{12} as a potent free radical scavenger. Chan et al. [32] found that the antioxidant effect of cobalamin was a protective factor for the optic nerve. These authors showed in vitro and in an animal model that intravitreal cobalamin administration following iatrogenic optic nerve damage reduced oxidative stress and the degree of nerve damage, promoting survival of retinal ganglion cells.

Links between vitamin B_{12} deficiencies and the development of macroangiopathy have been sought for a long time. Such a link may be related to the discussion on the role of homocysteine, particularly in the development of coronary artery disease. Until

recently, homocysteine level measurement was recommended as a cardiovascular risk marker [33, 34]. However, these hopes were not substantiated in later studies. In contrast, Yigit et al. [35] showed a potential association between a MTHFR gene mutation and the presence of diabetic peripheral neuropathy. The genotype distribution and allele frequencies differed significantly between patients with diabetic neuropathy and the control group and correlated with a history of diabetic retinopathy. It was hypothesized that both direct and indirect effects of hyperhomocysteinaemia on endothelial cells led to occlusion of small capillaries which would explain the effect of vitamin B_{12} deficiency on the development of neuropathy.

Does chronic metformin use lead to vitamin B_{12} deficiencies?

In the recent years, numerous reports have indicated that metformin, particularly if used for many years, significantly affects body vitamin B_{12} stores. It was shown in diabetic patients treated with metformin, women with polycystic ovary syndrome receiving metformin treatment, and in healthy women administered metformin in trials evaluating its anticancer effects [36–38]. In 1971, Tomkin et al. [39] were the first to note an association between metformin use and reduced vitamin B_{12} absorption. Randomized clinical trials showed that metformin administration for several months may significantly reduce vitamin B_{12} level [36, 40]. One of the strongest evidence for this association comes from a randomized clinical trial with more than 4 years of follow-up, reported by De Jager et al. [41]. This study showed that vitamin B_{12} level was reduced by as much as 19%. It was the first study to show gradual vitamin B_{12} level reduction in patients receiving metformin, and the first to show the potential of metformin to reduce vitamin B_{12} level to values that usually require pharmacological substitution. The relation reported by De Jager et al. has been supported by more recent metaanalyses and clinical studies [42, 43].

Several theories have been put forward to explain metformin-induced vitamin B_{12} deficiency. One of the earliest proposed explanations was intestinal bacterial overgrowth resulting in binding the intrinsic factor-vitamin B_{12} complex by the bacteria instead of its absorption [44]. Another postulated mechanism was acceleration of intestinal passage by metformin, resulting in reduced vitamin absorption [45]. According to the currently most popular explanation, metformin affects calcium channels in the small intestine which are responsible for absorption of the intrinsic factor-vitamin B_{12} complex [24]. This mechanism is also supported by reversal of defective vitamin B_{12} absorption by oral calcium supplementation. In their study, Bauman et al. [24] divided patients with type 2 diabetes into two groups, one receiving metformin and the other receiving a sulphonylurea. In the metformin group, a significant reduction in vitamin B_{12} and holotranscobalamin level was noted in the first 3 months but such effects were not observed in the sulphonylurea group. At the next step, oral calcium supplementation was initiated in patients receiving metformin. At one month, holotranscobalamin level in the study group increased by as much as 53%, and no intestinal bacterial overgrowth was confirmed [24]. The authors suggested that positively charged metformin moieties target the carbohydrate core of intestinal cell membrane, charging positively the membrane surface itself, and calcium cations are repelled from it as a result.

Does metformin induce neuropathy?

Should we afraid metformin due to vitamin B_{12} deficiencies developing during metformin therapy? It has been a leading anti-diabetic drug for decades, providing multidirectional benefits. Normalization of blood glucose levels associated with long-term improvement of insulin sensitivity protects from the development of diabetic neuropathy. Specific molecular mechanisms of the neuroprotective action of metformin independent from blood glucose control have also been investigated.

On the other hand, development of various forms of diabetes-independent neuropathy due to vitamin B_{12} deficiency may be expected in patients treated with metformin for many years [46]. In a 6-month observational study, Wile and Toth [47] showed a significant effect of metformin use on a reduction of cobalamin level, which was also associated with elevated homocysteine and methylmalonic acid levels. In addition, the severity of peripheral neuropathy was increased compared to the non-metformin treated group. Singh et al. [48] also showed an association between metformin use, vitamin B_{12} deficiency, and the presence of neuropathy. In contrast, Alharbi et al. [49] did not show a significantly higher rate of neuropathy in metformin-treated patients [49]. Older studies also did show a significant association between vitamin B_{12} deficiency and peripheral neuropathy in metformin-treated patients [50].

These apparently discrepant results may result from difficulties with matching the study groups being compared. In observational studies, yielding similar study groups for a comparison is probably impossible to achieve, as comparisons are only performed between metformin-treated versus non-patients, without taking into account other factors inducing neuropathy, which limits the credibility of the study findings. Future studies

with adequate sample sizes and use of more objective tools to evaluate peripheral neuropathy are needed to evaluate the relationship between metformin use and development of peripheral neuropathy in patients with type 2 diabetes [51].

Do other anti-diabetic drugs induce cobalamin deficiency?

A question arises whether other anti-diabetic drugs which also affect the gastrointestinal system function may potentially affect vitamin B_{12} absorption. These include glucagon-like peptide-1 (GLP1) analogues. dipeptidyl peptidase-4 (DDP-4) inhibitors, and alpha-glucosidase inhibitors.

A 2018 study in an animal model showed that administering a conjugate of GLP1 analogue and vitamin B_{12} improved blood glucose control and limited adverse effects associated with the use of GLP1 analogues (vomiting, nausea, fatigue). However, no evidence was provided that use of GLP1 analogues would lead to a reduction of vitamin B_{12} level [52] No data are available in the literature regarding the effect of DPP-4 inhibitors and alpha-glucosidase inhibitors on vitamin B_{12} levels.

Should vitamin B_{12} level be measured, particularly with the presence of other factors predisposing to its deficiency?

Most guidelines recommend monitoring vitamin B_{12} levels if risk factors for its deficiency are present, and the most recent diabetes management guidelines are consistent with this recommendation. The American Diabetes Association (ADA) suggests periodic cobalamin level measurements in patients receiving chronic metformin treatment, but without suggesting specific time intervals for this testing. ADA also recommended targeting individuals with concomitant neuropathy and anaemia [53]. In the most recent Diabetes Poland guidelines, correction of vitamin B_{12} deficiency following its laboratory confirmation was recommended [29].

How reliable are blood vitamin B_{12} level measurements?

In the routine clinical practice, vitamin B_{12} levels are measured either to determine the cause of macrocytic anaemia or due to the presence of clinical manifestations suggesting vitamin B_{12} deficiency. It has been questioned in the literature, however, whether plasma cobalamin levels reflect its clinical effects. Measuring serum cobalamin levels only does not allow an adequate insight into its total body stores, as the metabolic processes that use cobalamin as a cofactor occur at the intracellular level (Figs 1, 2). In addition, the effectiveness of these processes may be evaluated only indirectly, based on plasma levels of transcobalamin, homocysteine, methylmalonic acid (MMA), S-adenosylmethionine (SAM), and S-adenosylhomocysteine (SAH). It seems that vitamin B_{12} deficiency should be considered at two levels: actual (identified by laboratory test) and functional. The latter would be characterized by normal serum vitamin B_{12} levels in the setting of its abnormal intracellular distribution, as reflected by abnormal levels of the above metabolites, the measurements of which are rarely available commercially [3, 54].

Obeid et al. [55] evaluated these relationships in patients with type 2 diabetes and a healthy control group. This study measured vitamin B_{12} and its markers including red blood cell-vitamin B_{12} (B_{12}-RBC), MMA, total transcobalamin (tTC), total homocysteine (tHcy) and methylation markers SAM and SAH. Cobalamin and transcobalamin levels in diabetic patients were similar to those in the healthy control group, while MMA level was higher, and B_{12}-RBC, SAM, and SAH levels were lower. These findings suggest that despite cobalamin levels within the laboratory reference range, its cellular distribution is disturbed. A reverse trend was observed in patients receiving metformin therapy, in whom cobalamin levels were lower compared to the control group. On the other hand, lower MMA levels and normal methylation index suggest normal cobalamin-dependent intracellular processes in these patients. Based on their findings, the authors postulated cellular resistance to vitamin B_{12} in type 2 diabetes [55]. These results also indicate that interpretation of cobalamin level measurements should be cautious, and measuring other parameters listed above might be helpful.

Should cobalamin be supplemented in diabetic patients, particularly those receiving metformin treatment?

Vitamin B_{12} supplementation has been long considered safe due to its hydrophilicity and easy elimination from the body in case of an excess supply. This has been recently questioned, however, by the study findings published by Flores-Guerrero et al. [56] in the Journal of the American Medical Association. These authors showed an association between higher cobalamin level and higher mortality in the general population. Surprisingly, this association became evident with plasma cobalamin levels within the reference range. This Dutch study followed more than 5000 adults for over 8 years. The identified association between cobalamin level and mortality was independent from age, gender, history of malignancies, renal and liver function parameters, concomitant type 2 diabetes, alcohol consumption, and smoking. Obesity, hypertension, dyslipidaemia, and hyperglycaemia were more common in patients with

plasma vitamin B_{12} levels in the upper quartile within the reference range. The exact significance of these correlations and their mechanisms remain unknown and require further analyses. Based on these findings, the authors suggested avoiding vitamin B_{12} supplementation unless its deficiency is documented [56].

How to supplement vitamin B_{12}?

In the past, the main approach to vitamin B_{12} supplementation were regular (usually monthly) intramuscular injections. Low popularity of oral intake was related to the belief that cobalamin absorption disturbances in various conditions lead to low bioavailability of the oral form. However, even with the absence of active cobalamin transport mechanism, it partially crosses the intestinal mucosa by passive diffusion. If an appropriately large vitamin B_{12} dose is administered via this route, achieving an adequate increase in its serum level is possible [3]. Effective sublingual cobalamin administration techniques have also been developed. A vitamin B_{12} preparation administered sublingually as an aerosol has been recently introduced in Poland.

Until recently, the literature reports of effective sublingual supplementation were limited to small studies and case reports. Bensky et al. [57] investigated the efficacy of sublingual supplementation compared to intramuscular cobalamin administration in nearly 4300 Israeli patients with vitamin B_{12} deficiency. Their study indicates that sublingual cobalamin administration effectively increased serum cobalamin level over a short time (the mean duration of follow-up was 7 months in the intramuscular cobalamin group and 9 months in the sublingual cobalamin group). The authors postulated superiority of this form of supplementation due to its convenience, lack of complications related to injections, and independence of the route of administration from the intrinsic factor and the gastrointestinal system status.

Sublingual administration results in cobalamin absorption directly to the bloodstream, avoiding potentially adverse pH of the stomach and bypassing the enterohepatic circulation, which might reduce the amount of actually absorbed active substance of the oral preparation. This route is also beneficial with concomitant dysphagia. It seems, however, that with large vitamin B_{12} deficiencies requiring more rapid correction, the time-honoured intramuscular administration remains the preferred approach as its efficacy is confirmed by years of experience. In the study by Bensky et al. [57], the diagnostic and therapeutic reasoning underlying the choice of a particular route of supplementation in a given patient was not investigated. The intramuscular administration group was smaller and these patients had lower baseline serum cobalamin levels compared to the sublingual supplementation group. Thus, it is difficult to establish whether sublingual supplementation is superior to intramuscular injections [57]. Routes alternative to intramuscular injections are worth considering when an improvement of the patient quality of life is the primary consideration. They are also good alternative if intramuscular administration is contraindicated, e.g., due to coagulopathy (including a iatrogenic one due to commonly used antithrombotic therapies).

Summary

The literature discussion on vitamin B_{12} supplementation in diabetic patients receiving metformin led to an increased interest in the importance of this vitamin in the management of diabetes and its complications. It seems that diabetic patients are at a potentially higher risk of cobalamin deficiency compared to individuals without diabetes. However, available laboratory tests do not allow discerning between blood levels of vitamin B_{12} and its tissue content that drives the clinical manifestations of vitamin B_{12} deficiency.

In addition to the natural disease course leading to micro- and macroangiopathic complications that might contribute to vitamin B_{12} deficiency, diabetic patients receive multi-drug therapy which may also aggravate this problem. In addition to metformin, most commonly used culprit medications include proton pump inhibitors, antibiotics, and non-steroidal anti-inflammatory drugs.

These discussions of the recent decade have been summarized in the diabetic societies' guidelines including those published by ADA and the Diabetes Poland which have indicated the need for an early correction of vitamin B_{12} deficiency.

Conflict of interest

None of the authors have a conflict of interest.

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