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Minireview

Physiology of vitamin B_{12}: a study on its molecular mechanisms using a *Caenorhabditis elegans* model

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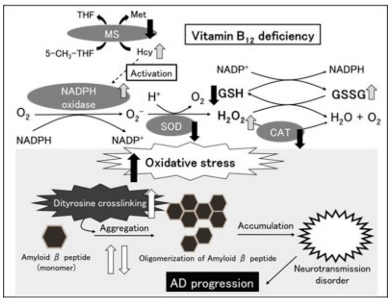
Abstract

Vitamin B_{12} (B_{12}) is a water-soluble substance that is a member of the B-vitamin family. Its recommended daily dose in adult men and women is 2.4 µg, which is the lowest among the 13 vitamins. B_{12} deficiency causes megaloblastic anemia and neurological disorders. It is also associated with cognitive decline, growth retardation, infertility, and other symptoms. Nevertheless, the detailed mechanisms of which remain unclear. *Caenorhabditis elegans* is a small organism, with a length of approximately 1 mm and a lifespan of approximately 3 weeks. It has similar fundamental biological structures, such as the muscles, nervous system, and digestive tract, with mammals. Previous studies have shown that B_{12} is required for the normal development of *C. elegans*, similar to that of mammals. The current study aimed to perform a detailed investigation of the mechanisms underlying the development of B_{12} deficiency using a dietary B_{12}-deficient *C. elegans* model.

Keywords: *Caenorhabditis elegans*, cobalamin, nutrition, oxidative stress, vitamin B_{12}

Graphical abstract



The association between oxidative stress induced by vitamin B_{12} deficiency and Alzheimer's disease progression.

Vitamin B_{12} (Cobalamin)

Among all vitamins, vitamin B_{12} (B_{12}) has the most complex chemical structure and the largest molecular weight at 1355.4. In the body, B_{12} functions as a coenzyme in the form of adenosylcobalamin, where a 5′-deoxyadenosyl group is coordinated as the upper axial ligand, and methylcobalamin, where a methyl group is coordinated (Kräutler 2012). These coenzyme forms function as coenzyme for methylmalonyl-CoA mutase (MCM; EC 5.4.99.2) and methionine synthase (MS; EC 2.1.1.13), contributing to the catabolism of odd-chain fatty acids and branched-chain amino acids (Banerjee 2001) and the metabolism of methionine (Kennedy *et al.* 1990). The primary sources of B_{12} are animal-derived foods, while most plant-based foods contain little to no B_{12}. In foods, B_{12} is tightly bound to proteins, and gastric acid is required to release it for efficient absorption in the body (Žane *et al.* 2023). Therefore, high-risk groups for B_{12} deficiency include strict vegetarians and the elderly with reduced gastric function.

Deficiency of B_{12} leads to decreased activity of MCM and MS, resulting in elevated intracellular levels of methylmalonic acid (MMA) and homocysteine (Hcy) (Andrzej and Kilmer 1993; Toyoshima *et al.* 1995). Notably, increased urinary MMA levels are utilized as a biomarker for the diagnosis of B_{12} deficiency (Clarke *et al.* 2003). B_{12} deficiency is well known to cause megaloblastic anemia and neurological disorders (Voukelatou *et al.* 2016). Additionally, B_{12} deficiency has been reported to contribute to growth retardation, infertility, and cognitive impairment (Green R 2017; Alruwaili *et al.* 2023); however, the precise mechanisms underlying these conditions remain unclear.

Preparation of dietary vitamin B_{12}-deficient *Caenorhabditis elegans*

To model human B_{12} deficiency caused by malnutrition, this study manipulated the B_{12} levels in the diet (*Escherichia coli*) and growth medium of *C. elegans* to generate B_{12}-supplemented worms

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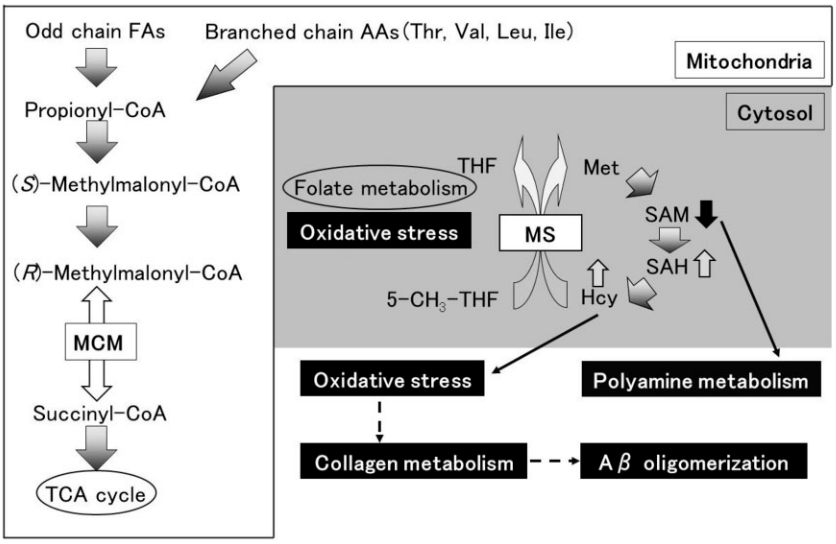

Figure 1. Metabolic pathways involving vitamin B_{12}-dependent enzymes and new findings discovered in vitamin B_{12}-deficient *C. elegans*. Aβ, amyloid-beta; MCM, methylmalonyl-CoA mutase; MS, methionine synthase; SAH, S-adenosylhomocysteine; SAM, S-adenosylmethionine; and THF, tetrahydrofolate.

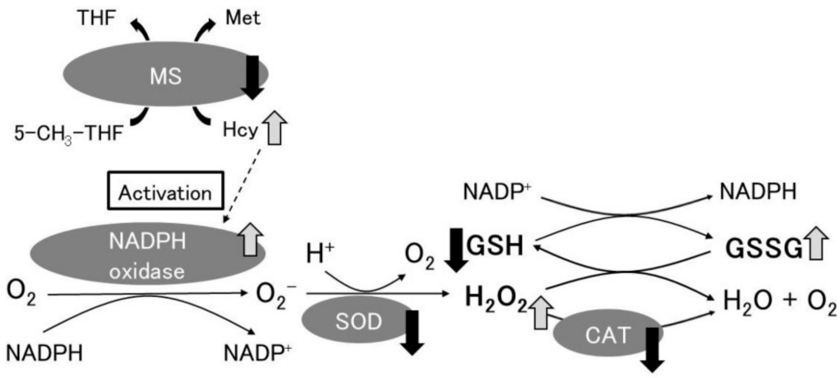

Figure 2. Disruption of intracellular redox regulatory mechanisms induced by vitamin B_{12} deficiency. CAT, catalase; GSH, reduced glutathione; GSSG, oxidized glutathione; MS, methionine synthase; SOD, superoxide dismutase; and THF, tetrahydrofolate.

(control) and dietary B_{12}-deficient worms. Compared with the B_{12}-supplemented worms, the B_{12}-deficient worms exhibited a significant reduction in internal B_{12} levels, to approximately 1/50th, and a significant decrease in the enzymatic activities of the B_{12}-dependent enzymes MCM and MS. These phenomena resulted in a remarkable accumulation of MMA and Hcy in the organism (Bito *et al.* 2013). These abnormal metabolites are used as biomarkers of B_{12} deficiency in clinical practice. Further, the B_{12}-deficient worms had a significant reduction in the *S*-adenosylmethionine (SAM) to *S*-adenosylhomocysteine ratio, a key indicator of methylation reactions in the body (Bito *et al.* 2019). These results are in accordance with the known physiological consequences of B_{12} deficiency in mammals, including humans (Figure 1).

In addition, the B_{12}-deficient worms presented with a reduced egg-laying performance, delayed development, impaired learning and memory function, and shortened lifespan (Bito *et al.* 2013; Bito *et al.* 2017; Bito *et al.* 2019). Taken together, these findings first showed that B_{12} is an essential factor for maintaining normal growth and development in *C. elegans*, similar to mammals. Moreover, *C. elegans* requires other water-soluble B vitamins, B_1, B_2, niacin, pantothenic acid, B_6, biotin, and folate, for normal development (Zečić *et al.* 2019).

Oxidative stress induced by vitamin B_{12} deficiency

Considering the abnormal accumulation of homocysteine, a pro-oxidant, in B_{12}-deficient *C. elegans*, the occurrence of oxidative stress damage was investigated. The levels of oxidative stress markers, including hydrogen peroxide, reactive nitrogen species, lipid peroxides, and carbonylated proteins in B_{12}-deficient worms were significantly elevated compared with those in B_{12}-supplemented worms (Bito *et al.* 2017). Therefore, B_{12} deficiency induces severe oxidative stress damage. In addition, the elevation of these oxidative stress markers was attributed to a significant increase in the enzymatic activities of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase and nitric oxide (NO) synthase (Bito *et al.* 2017), which catalyze the production of superoxide and nitric oxide, respectively (Figure 2).

To assess the effect of B_{12} deficiency on the body's antioxidant system, the levels of vitamin C and reduced glutathione, which are both key antioxidants, were measured. Results showed that the levels of both antioxidants in B_{12}-deficient worms were reduced by half compared with those in B_{12}-supplemented worms (Bito *et al.* 2017). The results showed that in B_{12}-deficient worms, both antioxidants were reduced by half compared to the control. Further,

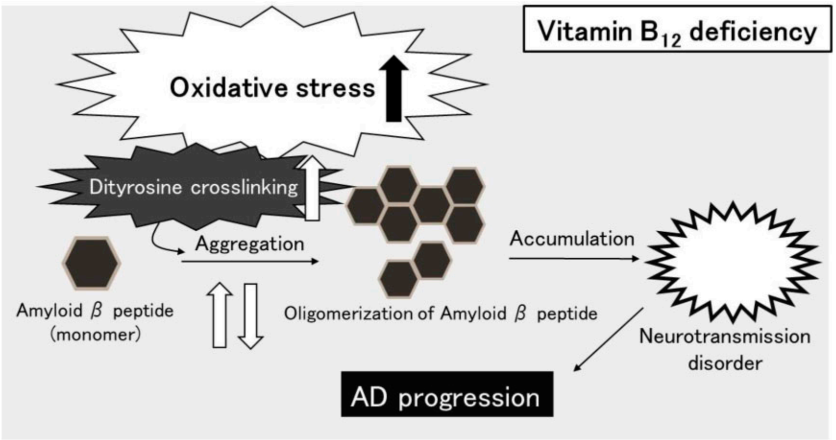

Figure 3. The potential for vitamin B_{12} deficiency to contribute to the progression of Alzheimer's disease.

the activities of the antioxidant enzymes superoxide dismutase and catalase were significantly reduced. Hence, B_{12} deficiency disrupts the body's redox homeostasis, leading to an increase in oxidative stress production and a decrease in oxidative stress elimination.

Metabolic effects related to oxidative stress induced by vitamin B_{12} deficiency

The two B_{12}-dependent enzymes are involved in amino acid metabolism. Therefore, the impact of B_{12} deficiency on amino acid levels was examined. In B_{12}-deficient worms, branched-chain amino acids (threonine, valine, leucine, and isoleucine) accumulated, and significant ornithine accumulation, which is a novel finding, was observed (Bito *et al.* 2019). Ornithine is an amino acid involved in the urea cycle and is generated from arginine by the action of arginase. Although *C. elegans* lacks a urea cycle, it exhibited arginase activity, which was significantly increased by B_{12} deficiency. Ornithine is a precursor of polyamines, which are involved in lifespan regulation. As B_{12} deficiency reduced SAM levels, the synthesis of spermidine decreased (Bito *et al.* 2019). This reduction contributed to the shortened lifespan of B_{12}-deficient worms. In addition, B_{12}-deficient worms had higher arginine levels than the B_{12}-supplemented worms. Since arginine is a substrate for NO synthase, elevated arginine levels might be a contributing factor to the increased production of reactive nitrogen species observed in B_{12} deficiency.

Further, methionine metabolism, which involves B_{12}, is linked to folate metabolism. Worms with excess folate supplementation, similar to B_{12}-deficient worms, exhibit significant homocysteine accumulation and severe oxidative stress damage (Koseki *et al.* 2020).

Association between Vitamin B_{12} deficiency and Alzheimer's disease

Several studies have reported patients with Alzheimer's disease (AD) have lower serum B_{12} levels than healthy individuals (Lauer *et al.* 2022). Moreover, B_{12} deficiency is prevalent among patients with AD (Obeid *et al.* 2024). These findings suggest a potential causal association between B_{12} deficiency and the development of AD. Therefore, in this study, the association between B_{12} deficiency and the onset and progression of AD was investigated by focusing on the aggregation of amyloid-beta (Aβ). The wild-type strain N2 of *C. elegans* does not express Aβ. Hence, we utilized the transgenic strain GMC101, in which human Aβ is expressed and aggregated in the muscle tissue under the control of the muscle-specific *unc-54* promoter, by shifting the cultivation temperature to 25 °C at the L4 larval or adult stage (McColl *et al.* 2012). The current study aimed to elucidate the association between B_{12} deficiency and the onset of AD using this model.

The transgenic *C. elegans* strain GMC101 exhibits paralysis due to the expression and aggregation of Aβ in the muscle tissue following a shift in cultivation temperature from 20 °C to 25 °C. This study first investigated the effects of B_{12} deficiency on the time required for paralysis in GMC101 worms. Moreover, oxidative stress is implicated in the promotion of Aβ aggregation. Thus, ascorbic acid 2-glucoside was administered to B_{12}-deficient GMC101 worms, and the time required for paralysis onset was evaluated.

Compared with the B_{12}-supplemented GMC101 worms, the B_{12}-deficient GMC101 worms exhibited early-onset paralysis after the induction of Aβ expression (Koseki *et al.* 2021). Based on these results, B_{12} deficiency accelerates paralysis onset in GMC101 worms. The polymerization of Aβ is influenced by intermolecular hydrogen bonding and dityrosine cross-linking. Dityrosine immunostaining revealed a significant increase in dityrosine cross-linking in B_{12}-deficient GMC101 compared with the control (Koseki *et al.* 2021). Therefore, B_{12} deficiency may accelerate the polymerization of Aβ by forming dityrosine cross-links, thereby potentially promoting AD progression (Figure 3).

Based on the findings of previous studies, *C. elegans* is a powerful tool for analyzing the mechanisms of B_{12} deficiency from physiology perspective.

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Disclosure statement

No potential conflict of interest was reported by the author.

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