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Forum

Clinical implications of vitamin B_{12} as redox-active cofactor

Annette K. Offringa,[1] Arno R. Bourgonje,[2] Matthew S. Schrier,[3] Richard C. Deth,[3] and Harry van Goor[4,*]

Vitamin B_{12} is a redox-active compound containing a cobalt atom that cycles between oxidation states. Superoxide scavenging induces its oxidation, disabling activation of the enzymes methionine synthase and methylmalonyl-CoA mutase, disrupting gene expression and energy production. High-dosed vitamin B_{12} may be clinically used to reduce oxidative stress and preserve cofactor functions.

Vitamin B_{12} is a redox-active cofactor

Many studies on vitamin B_{12} (cobalamin, Cbl) focus on its quantitative availability from the diet to fulfill its cofactor roles, while its broader redox-related activities are less often considered. Vitamin B_{12} is a cofactor for two metabolically important enzymes, methionine synthase (MS) and methylmalonyl-CoA mutase (MCM) [1,2]. In both enzymes its cobalt atom cycles between oxidation states, forming reduced Cbl(I) and oxidized Cbl(II) and Cbl(III). Cellular processing of cobalamins to form methylcobalamin (MeCbl) for MS and adenosylcobalamin (AdoCbl) for MCM depends upon adequate levels of the reducing agents NADPH and glutathione (GSH). Thus, cellular reduction–oxidation (redox) status has important implications for vitamin B_{12} function and for the clinical interpretation of vitamin B_{12} deficiency.

Methionine synthase and methylation

As illustrated in Figure 1, MS converts homocysteine (Hcy) to methionine (Met) using methyl groups from 5-methyltetrahydrofolate (Me-THF) and Cbl(I) as electron donors [1]. When oxidation halts enzyme activity by lack of reduced Cbl(I), accumulating Hcy is diverted to synthesis of GSH via the trans-sulfuration pathway, thereby counteracting oxidative stress. Thus, vitamin B_{12} in MS serves as a sensor of redox status that limits Met synthesis during oxidative stress, and, consequentially, hundreds of methylation reactions supported by S-adenosylmethionine (SAM) (e.g., methylation of DNA, RNA, and histones) are also keyed to redox status.

It can thus be appreciated that oxidative-stress-related disorders can limit the effectiveness of Cbl(I) as cofactor for MS, creating a functional vitamin B_{12} deficiency that may cause epigenetic dysregulation of gene expression. A functional shortage of SAM is associated with both hypo- and hypermethylation. For instance, experimental dietary methyl donor deficiency, causing a deprivation of methyl groups for the conversion of Hcy into SAM, is associated with both global hepatic hypomethylation, but, paradoxically, also with cerebral hypermethylation, affecting genes involved in nervous system development and function, inflammation, immune response, and mitochondrial and carbohydrate metabolism [3]. Hypermethylation of DNA is associated with the development of cancer through the inactivation of tumor suppressor genes, but hypomethylation is also associated with cancer through induction of genomic instability [4].

MCM and the Krebs cycle

The second vitamin-B_{12}-dependent enzyme constitutes mitochondrial MCM, in which AdoCbl, formed from Cbl(I), facilitates conversion of methylmalonyl-CoA to succinyl-CoA, which then enters the Krebs cycle to promote ATP production [2] (Figure 2). Thus, oxidative-stress-induced deficiency of reduced Cbl(I) limits the availability of succinyl-CoA for the Krebs cycle and thereby hampers the supply of cellular energy substrates. Succinyl-CoA is involved in the storage of glycogen and synthesis of heme. This mechanism links cellular redox balance to vitamin B_{12} deficiency-associated symptoms such as fatigue, anemia, and neuropathy [5].

Vitamin B_{12} as a superoxide scavenger

Apart from its functions as an enzyme cofactor, vitamin B_{12} also serves as a scavenger of reactive oxygen species (ROS), particularly superoxide ($O_2^{\bullet-}$), yielding oxidized Cbl(III) at a rate comparable with that of superoxide dismutase (SOD1) [6]. This activity of vitamin B_{12} as an $O_2^{\bullet-}$ scavenger helps to offset oxidative stress and occurs at the expense of its cofactor role for MS and MCM.

Biomarkers

There is no gold standard for the diagnosis and treatment of vitamin B_{12} deficiency. Symptoms associated with vitamin B_{12} deficiency do not solely occur at low plasma levels and DNA damage has been observed at vitamin B_{12} levels up to 300 pmol/l, which is generally considered an adequate concentration [7]. Vitamin B_{12} supplements provide various forms bound with cyanide, water (H_2O or OH^-), methyl, or adenosyl groups. Treatment with cyanocobalamin (CNCbl) results in a higher increase of vitamin B_{12} plasma concentrations compared with OHCbl, while the latter is converted to AdoCbl to a greater extent, implying that OHCbl provides a better tissue supply. Notably, plasma and tissue levels of vitamin B_{12} often show a high level of discrepancy, implying that plasma levels do not adequately reflect deficiency or comprise a reliable read-out for assessment of therapeutic effects [8]. Increased Hcy as a marker for vitamin B_{12} insufficiency is not specific, because the enzyme betaine homocysteine methyltransferase also transmethylates Hcy, using betaine instead of vitamin B_{12} as a cofactor. Additionally, Hcy elevation can

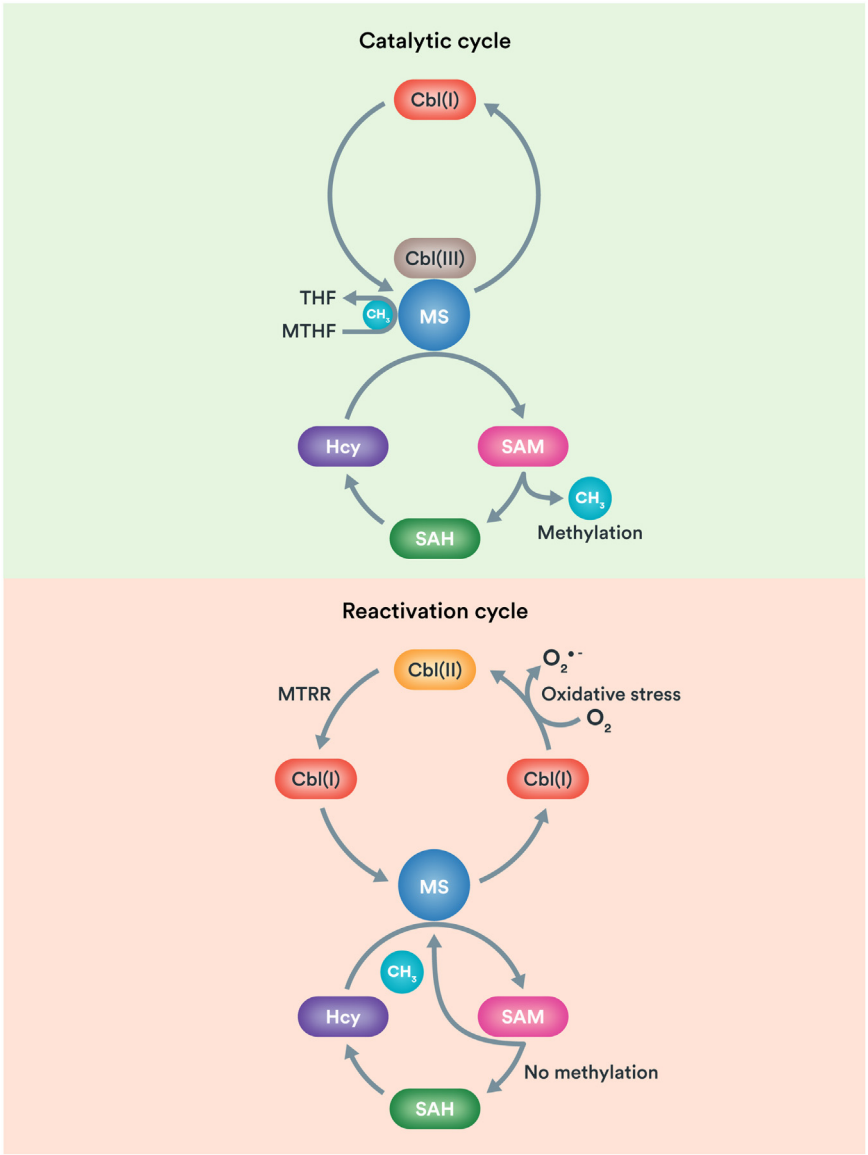

Figure 1. Activation of methionine synthase (MS). MS converts homocysteine (Hcy) into methionine (Met), which is further converted into the biologically active S-adenosyl-L-methionine (SAM). In the catalytic cycle, cobalamin [Cbl(I)] first donates two electrons to the process, supplying the energy to transfer a methyl group from methyltetrahydrofolate (MTHF) to Hcy. In this process, the oxidized methylcobalamin(III) is temporarily formed. When the methyl group is transferred to Met, Cbl(III) receives its two electrons and is reduced back to Cbl(I). When SAM donates the methyl group for the methylation of DNA, RNA and histones, S-adenosyl homocysteine (SAH) is formed, which is converted back to Hcy. Once in ~2000 cycles, Cbl(I) is oxidized to Cbl(II), which is unable to act as a cofactor for MS. In the ensuing reactivation cycle, the enzyme methionine synthase reductase (MTRR) transfers an electron from the vitamin B_2-containing cofactors flavin adenine dinucleotide (FAD) and flavin mononucleotide (FMN). Hereafter, MS cannot accept the two electrons from MTHF, but instead SAM donates two electrons, which does not lead to net SAM formation [1].

reflect decreased activity of cystathionine β synthase; the initial enzyme in the trans-sulfuration pathway. Therefore, Hcy concentrations are an inadequate reflection of physiological vitamin B_{12} levels. When vitamin B_{12} deficiency or insufficiency limits the activation of MCM, its substrate methylmalonyl-CoA accumulates and is deacylated to form methylmalonic acid (MMA) and acetyl-CoA. MMA induces downregulation of the methylmalonyl-CoA-precursor propionyl-CoA, thereby decreasing further formation of MMA while AdoCbl levels remain low (Figure 2). Since defects in MCM can also prevent conversion of methylmalonyl-CoA, increased MMA can be found across all systemic vitamin B_{12} concentrations [2]. A fourth marker consists of transcobalamin II (TC-II)-bound vitamin B_{12} or holoTC, also called active vitamin B_{12}, which may be an indicator for an acute negative balance because TCII is depleted of vitamin B_{12} within days after absorption ceases. However, TCII also acts as an acute-phase reactant, demonstrating elevated concentrations during infection, inflammatory disorders, and cancer [9]. Thus, no meaningful cut-off values of holoTC can be defined to use it as reliable marker for deficiency, perceived normal values may still be too low to supply enough vitamin B_{12} when demand is increased based on disease processes.

Oxidative stress, aging, and Alzheimer's disease

Advancing age is accompanied by a limitation in thiol-reducing capacity and the resultant oxidative stress limits vitamin B_{12} availability, restricting methylation capacity and provision of succinyl-CoA to the Krebs cycle. Remarkably, MS gene expression in human frontal cortex decreases by >400-fold in normal subjects across the lifespan while MeCbl levels decrease by >tenfold [10,11]. Thus, it is not surprising that impaired vitamin B_{12} function contributes to cognitive and neurodegenerative disorders of old age.

Multiple studies indicate impaired MS activity in Alzheimer's disease (AD) pathology. Decreased methylation of protein phosphatase 2A is associated with hyperphosphorylation of tau protein, a hallmark of AD and other neurodegenerative disorders, and MeCbl

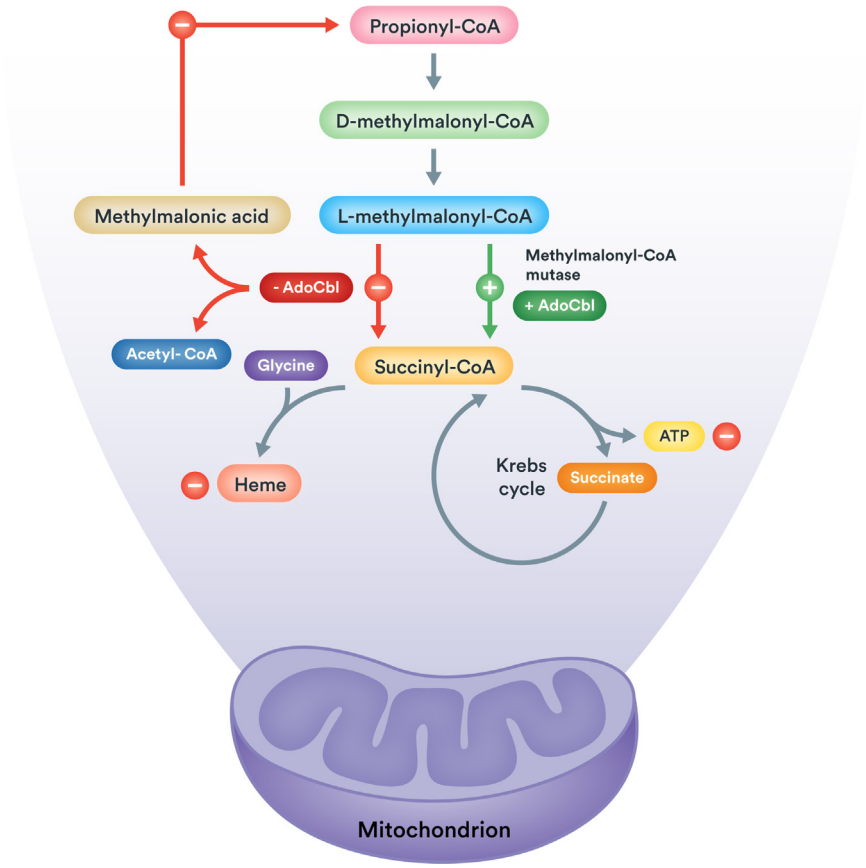

Figure 2. Activation of methylmalonyl-CoA mutase (MCM). MCM uses adenosylcobalamin (AdoCbl), which is formed in mitochondria by the reduction of Cbl(II) to Cbl(I) and the conversion of Cbl(I) to AdoCbl, catalyzed by adenosyltransferase. MCM converts its substrate, L-methylmalonyl-CoA, which is formed from propionyl-CoA and D-methylmalonyl-CoA, into succinyl-CoA. When AdoCbl is deficient, methylmalonic acid (MMA) is formed by a side reaction in which methymalonyl-CoA is deacylated, reducing its concentration. MMA results in a decreased rate of propionyl-CoA conversion in case of vitamin B_{12} deficiency, suggesting that the rate of conversion of propionyl-CoA to L-methylmalonyl-CoA is reduced, limiting the substrate for MCM. This means that, in case of vitamin B_{12} deficiency, MCM activity is downregulated by limited availability of its substrate which results in less production of succinyl-CoA. In the Krebs cycle, succinyl-CoA is converted into succinate, which fuels ATP production. Succinyl-CoA is, amongst others, involved in the formation of heme.

directly binds to tau and interferes with their fibrillization [12]. Also, hypomethylation of the β-site amyloid precursor protein cleaving enzyme-1 (BACE-1) gene increases production of amyloid β [13]. Since oxidative stress has also been demonstrated in AD, we propose that a functional vitamin B_{12} deficiency secondary to a redox-dependent shift in Cbl status may contribute to impaired MS activity. The action of vitamin B_{12} as an $O_2^{\bullet -}$ scavenger opens the possibility to treat numerous oxidative stress-related diseases, such as AD and other neurodegenerative diseases, with supraphysiological doses.

Concluding remarks

Considering vitamin B_{12} as a redox-active compound guides us to re-evaluate related pathologies as reflecting redox-based insufficiency of vitamin B_{12} activity instead of a quantitative deficiency. This perspective also requires a reappraisal of the adequacy of quantitative markers and provides a rationale for future exploration of potential benefits of supraphysiological vitamin B_{12} doses.

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Declaration of interests

There are no interests to declare.

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References

1. Koutmos, M. *et al.* (2009) Insights into the reactivation of cobalamin-dependent methionine synthase. *Proc. Natl. Acad. Sci. U. S. A.* 106, 18527–18532
2. Weidemann, M.J. *et al.* (1970) Gluconeogenesis from propionate in kidney and liver of the vitamin B12-deficient rat. *Biochem. J.* 117, 177–181
3. Pogribny, I.P. *et al.* (2008) Epigenetic alterations in the brains of Fisher 344 rats induced by long-term administration of folate/methyl-deficient diet. *Brain Res.* 1237, 25–34
4. Sheaffer, K.L. *et al.* (2016) DNA hypomethylation contributes to genomic instability and intestinal cancer initiation. *Cancer Prev. Res. (Phila.)* 9, 534–546
5. Huynh, N. *et al.* (2019) Glycogen branching enzyme controls cellular iron homeostasis via Iron Regulatory Protein 1 and mitoNEET. *Nat. Commun.* 10, 5463
6. Chan, M. *et al.* (2018) Cobalamin-associated superoxide scavenging in neuronal cells is a potential mechanism for vitamin B12-deprivation optic neuropathy. *Am. J. Pathol.* 188, 160–172
7. Palmer, A.M. *et al.* (2017) Folate rescues vitamin B12 depletion-induced inhibition of nuclear thymidylate biosynthesis and genome instability. *Proc. Natl. Acad. Sci. U. S. A.* 114, E4095–E4102
8. Greibe, E. *et al.* (2018) The tissue profile of metabolically active coenzyme forms of vitamin B12 differs in vitamin B12-depleted rats treated with hydroxo-B12 or cyano-B12. *Br. J. Nutr.* 120, 49–56
9. Simonsen, K. *et al.* (2014) Vitamin B12 and its binding proteins in hepatocellular carcinoma and chronic liver diseases. *Scand. J. Gastroenterol.* 49, 1096–1102

10. Muratore, C.R. *et al.* (2013) Age-dependent decrease and alternative splicing of methionine synthase mRNA in human cerebral cortex and an accelerated decrease in autism. *PLoS One* 8, e56927
11. Zhang, Y. *et al.* (2016) Decreased brain levels of vitamin B12 in aging, autism and schizophrenia. *PLoS One* 11, e0146797
12. Rafiee, S. *et al.* (2017) Vitamin B12 inhibits tau fibrillization via binding to cysteine residues of tau. *ACS Chem. Neurosci.* 8, 2676–2682
13. Li, P. *et al.* (2019) Epigenetic dysregulation of enhancers in neurons is associated with Alzheimer's disease pathology and cognitive symptoms. *Nat. Commun.* 10, 2246