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Forum

Clinical implications of vitamin B$_{12}$ as redox-active cofactor

Annette K. Offringa, Anmo R. Bourgonje, Matthew S. Schrier, Richard C. Deth, and Harry van Goor

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 cobalamin 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(II), 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$_2^-$•), yielding oxidized Cbl(III) at a rate comparable with that of superoxide dismutase (SOD1) [6]. This activity of vitamin B$_{12}$ as an O$_2^-$• 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$_2$O or O•), 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...
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 deacetylated 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
proposes that a functional vitamin B12 deficiency has also been demonstrated in AD, the β-site amyloid precursor protein cleaving enzyme-1 (BACE-1) gene increases production of amyloid β 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.

**Concluding remarks**

Considering vitamin B12 as a redox-active compound guides us to re-evaluate related pathologies as reflecting redox-based insufficiency of vitamin B12 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 B12 doses.

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**Declaration of interests**

There are no interests to declare.

References


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