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*REVIEW ARTICLES*

# Cobalamins and Methylcobalamin: Coenzyme of Vitamin B12

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***Cobalamins and methylcobalamin: Coenzyme of vitamin B12***

***SUMMARY***

*Vitamin B12, also called cobalamin, is one of the B vitamins. Cobalamin may refer to several chemical forms of vitamin B12, depending on the ligand of the cobalt ion. These are cyanocobalamin, hydroxycobalamin, methylcobalamin and adenosylcobalamin. For many years preparations of vitamin B12 (cobalamin) have been finding use in medicine. All of the forms of cobalamin are present in substantial amounts in the human and animal organism. Cobalamin plays a specific role in amino acid metabolism, i.e. in the methionine synthase reaction. Crystalline cobalamins are administered parenterally (intramuscularly) or orally (except hydroxycobalamin) for treating deficiency states. The absorption of physiological doses of cobalamin is limited to approximately 1.5 – 2 µg/dose. Methylcobalamin is one of the two coenzyme forms of cobalamin. It is the metabolically active form required for cobalamin-dependent enzyme function. Intramuscular administration is widely accepted as a treatment method. Oral cobalamin supplementation is also used but it is considered to be less reliable. Therefore, a nanoemulsion formulation was developed to overcome this problem by the authors. In this review, especially the general information about cobalamin and methylcobalamin will be shared. Besides, information about the developed nanoemulsion will be given.*

***Key Words:*** *Cobalamin, methylcobalamin, vitamin B12.*

***Kobalaminler ve metilkobalamin: Vitamin B12 koenzimi***

***ÖZET***

*Kobalamin olarak da adlandırılan vitamin B12, B vitaminlerinden biridir. Kobalamin, kobalt iyonunun bağlanışına bağlı olarak vitamin B12'nin çeşitli kimyasal formları şeklinde adlandırılabilir. Bunlar siyanokobalamin, hidroksikobalamin, metilkobalamin ve adenozilkobalamindir. Birçok yıldır vitamin B12 (kobalamin) preparatları sağlık bilimlerinde kullanılmaktadır. Kobalaminin tüm formları insanlarda ve hayvan organizmalarında önemli miktarlarda bulunmaktadır. Kobalamin, aminoasit metabolizmasında, örneğin metiyonin sentaz reaksiyonunda, önemli bir rol oynamaktadır. Kristalize kobalaminler eksiklik durumunun tedavisinde parenteral (intramüsküler) ya da oral olarak (hidroksikobalamin hariç) uygulanırlar. Kobalaminin fizyolojik dozlarının emilimi yaklaşık olarak 1.5 – 2 µg/dozdur. Metilkobalamin, kobalaminin iki koenzim formundan biridir. Kobalamine bağlı enzim fonksiyonları için metabolik olarak aftif formudur. Tedavi yöntemi olarak çoğunlukla intramüsküler uygulama kabul edilir. Oral kobalamin tedavisi de kullanılmakla birlikte, bu tedavinin daha az güvenilir olduğu düşünülmektedir. Bu nedenle, bu problemin üstesinden gelmek için yazarlar tarafından bir nanoemülsiyon formülasyonu geliştirilmiştir. Bu derlemede, özellikle kobalamin ve metilkobalamin hakkındaki genel bilgiler paylaşılacaktır. Bunun yanı sıra, geliştirilmiş olan nanoemülsiyon hakkındaki bilgiler de verilecektir.*

***Anahtar kelimeler:*** *Kobalamin, metilkobalamin, vitamin B12.*

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## INTRODUCTION

### Cobalamins

Vitamin B12 is the generic name given to a group of related compounds containing cobalt as the central ion in a corrin ring (Figure 1). This group of biologically active cobalt containing corrinoids is also described as 'cobalamins'. The cobalt ion can be coordinated to a methyl-, 5'-deoxyadenosyl-, hydroxy- or cyano- group (Wolters *et al.*, 2004). Hydroxycobalamin and cyanocobalamin used in food supplements are transformed in the human body by coordinating with other ligands into methylcobalamin and 5'-deoxyadenosylcobalamin. The methylcobalamin and 5'-deoxyadenosylcobalamin are actively involved in endogenous metabolism (Hillman, 1985; Kelly, 1997).

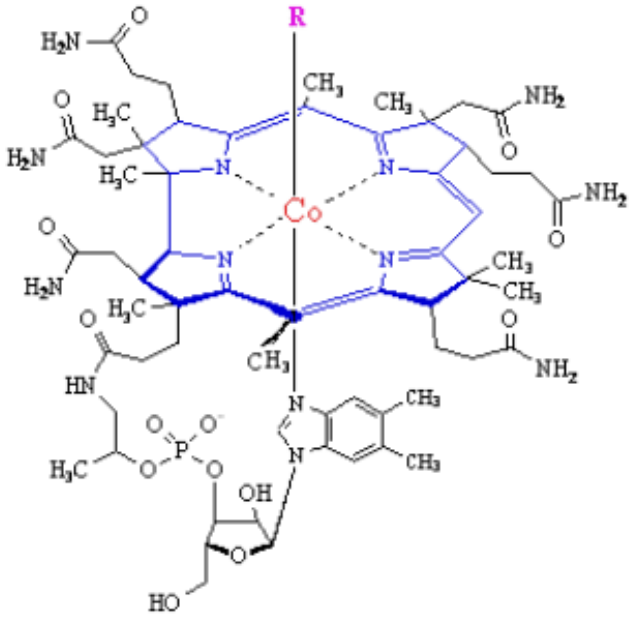


**Figure 1.** Chemical structure of cobalamin (Aguilar *et al.*, 2008).

Cobalamin is a large water-soluble molecule (MW: 1355 g/mol) containing a cobalt atom. It is present in all foods of animal origin but not in vegetables (Lancaster, 1980). In foods of animal, hydroxy-, methyl- and 5'-deoxyadenosyl cobalamin are the main cobalamins present (Aguilar, 2008).

Cobalamin plays a specific role in amino acid metabolism, i.e. in the methionine synthase reaction, and in the rearrangement of methylmalonyl CoA into succinyl CoA. In humans these cobalamin dependent reactions have been identified with methionine synthase functioning with methylcobalamin and the methylmalonyl coenzymeA mutase reaction with 5'-deoxyadenosylcobalamin as the active coenzyme (Aguilar, 2008). It is considered to function as an essential factor in DNA synthesis for chromosomal replication and division. As cobalamin regulates the production of tetrahydrofolate, a key substrate for the subsequent synthesis of deoxythymidine monophosphate, its deficiency inhibits DNA synthesis, leading to the subsequent retardation of cellular proliferation and differentiation (Kaushansky and Kipps, 2011).

Intestinal uptake of cobalamin occurs in the terminal ileum (Ermens *et al.*, 2003). Cobalamin is absorbed and transported across cellular plasma membranes by two mechanisms, including (Castle and Hale, 1998; Andre's *et al.*, 2004):

1) Active transport. Endocytosis of dietary cobalamin bound to gastric intrinsic factor (IF) by ileal enterocytes, which is a receptor-mediated process with a specific IF-cobalamin receptor.

2) Passive diffusion. Significant amounts of the vitamin can be absorbed with this process when large quantities of cobalamin are ingested. The rate of absorption by the passive process has been reported to be 1% of the ingested amount of cobalamin (Scott, 1997; Baik and Russell, 1999).

It is considered that, dietary cobalamin is absorbed

by the active transport mechanism. The commonest disorder causing cobalamin deficiency in Europe and North America is pernicious anemia in which IF is deficient as a consequence of atrophy of the fundal mucosa. In such patients, oral cobalamin replacement therapy is ineffective (Lancaster, 1980).

It was concluded that the physiological dose of cobalamin, that healthy individuals need, is approximately 1.5 – 2 μg/dose. Regardless of dose, approximately 1.2% of cobalamin is absorbed by passive diffusion and consequently this process becomes quantitatively important at high levels of exposure (Aguilar *et al.*, 2008). Current intake recommendations are 2.4 μg/day for adults, slightly more during pregnancy (2.6 μg/day) and lactation (2.8 μg/day) (Sloan, 2008).

All the cobalamins have the same pattern of adverse reactions. The adverse effects of high doses of cobalamins include urticaria, eczematous and exanthematous skin lesions, and anaphylactic reactions, but it is not clear whether the reactions are caused by the drug itself, a preservative, or possibly by contaminants. High oral or parenteral doses of vitamin B6 and especially hydroxycobalamin are also on rare occasions suspected to induce acne which is, however, always benign (Dupre *et al.*, 1975). Several cases of cobalamin-induced folliculitis and acneiform eruptions have been described, in one case in connection with a patient receiving total parenteral nutrition (Heyworth-Smith and Hogan, 2002). Adverse effects associated with the parenteral administration of hydroxycobalamin include mild diarrhea, urticaria, skin rash, and anaphylactic reactions. Cross-sensitivity of hydroxycobalamin and cyanocobalamin has been reported (Hathcock and Troendle, 1991).

Cobalamin is stored in the liver, 3 – 5 mg being present in normal subjects. As the daily requirement is very small (2 – 3 μg/day), symptoms of cobalamin deficiency do not develop for 3 – 10 years after the development of cobalamin malabsorption. Negligible amounts of dietary cobalamin are excreted unchanged in the urine, except when large amounts are administered parenterally (Lancaster, 1980).

The main causes of cobalamin deficiency are pernicious anemia (20–30%) and food-cobalamin malabsorption (50–70%) in elderly (Andre´s *et al.*, 2000). Food-cobalamin malabsorption syndrome, which has only recently been identified, is a disorder characterized by the inability to release cobalamin from food or its binding proteins (Dawson *et al.*, 1988; Carmel, 1995). The partial nature of this form of malabsorption might produce a more slowly progressive depletion of cobalamin than does the more complete malabsorption engendered by disruption of intrinsic-factor-mediated absorption (Andre´s *et al.*, 2005). This syndrome is usually caused by atrophic gastritis, related or unrelated to *Helicobacter pylori* infection, and long-term ingestion of antacids (Bellou *et al.*, 1996) and biguanides (Sorich *et al.*, 2008). In food-cobalamin malabsorption, the absorption of 'unbound' cobalamin (free crystalline) is normal (Carmel, 1995). Second, between 1 and 5% of free cobalamin (or crystalline cobalamin) is absorbed along the entire intestine by passive diffusion. This absorption explains the mechanism underlying oral treatment of cobalamin deficiencies (Hathcock and Troendle, 1991; Lane and Rojas-Fernandez, 2002; Andre's *et al.*, 2004).

The representative symptoms of cobalamin deficiency are megaloblastic anemia and peripheral neuropathy, reflecting the fact that the hematopoietic and nervous systems are readily affected (Goto *et al.*, 2015).

**Classical Treatment of Cobalamin Deficiency**

The classical treatment for cobalamin deficiency, particularly when the cause is not dietary deficiency, is parenteral administration because of the unpredictable efficacy of oral treatment – in most countries intramuscular injection – of this vitamin (in the form of cyanocobalamin and, more rarely, hydroxy or methylcobalamin) (Lane and Rojas-Fernandez, 2002; Andre´s *et al.*, 2004; Hvas and Nexo, 2006; Andre´s *et al.*, 2007 ).

The medical treatment of cobalamin deficiency involves regular, usually monthly, intramuscular (im) cobalamin injections for life. However, traditions concerning both dose and schedule of administration vary considerably (Andre´s *et al.*, 2005). In France, the treatment involves the administration of 1000 μg of cyanocobalamin per day for 1 week, followed by 1000 μg/week for 1 month, followed by 1000 μg/month, normally for the rest of the patient´s life (Andre´s *et al.*, 2004). In USA and UK, dosages ranging from 100 to 1000 μg/month (or every 2–3 months when hydroxycobalamin is given) are used during the rest of the patient´s life (Andre´s *et al.*, 2007).

**Oral Therapy**

As cobalamin is absorbed by IF-independent passive diffusion, daily high dose (pharmacological dose) oral cyanocobalamin can induce and maintain remissions in

patients with megaloblastic anemia (Elia, 1998; Lane and Rojas-Fernandez, 2002). In cases of cobalamin deficiency other than those caused by nutritional deficiency, alternative routes of cobalamin administration have been used in a curative perspective: oral (Lane and Rojas-Fernandez, 2002; Solomon, 2007) and nasal (Slot *et al.*, 1997; Vidal-Alaball *et al.*, 2005). These other routes of administration have been proposed to prevent discomfort, inconvenience and cost of monthly injections (Andre´s *et al.*, 2007). A recent review of Lane (Lane and Rojas-Fernandez, 2002) has reported preliminary data of the usefulness of oral cobalamin treatment. It is notable that to date, oral cobalamin curative treatment accounts for more than 70% of the total cobalamin prescribed in Sweden in 2000 (Van Asselt *et al.*, 1998). Historically, the Swedish team was the first to routinely propose oral cobalamin therapy to cure cobalamin deficiency (Hathcock and Troendle, 1991).

**Methylcobalamin**

The chemical name for methylcobalamin is Co$\alpha$-[$\alpha$-(5,6-dimethylbenz-1H-imidazolyl)]-Co$\beta$-methylcobamide. Synonyms and trade names are mecobalamin, methylcobalamin, cobaltmethylcobalamin, Algobaz and Cobamet. Its molecular weight is 1344.4 g/mol (Aguilar *et al.*, 2008). Methylcobalamin is one of the two coenzyme forms of cobalamin (the other being adenosylcobalamin). They are the metabolically active forms required for cobalamin-dependent enzyme function. Evidence indicates these coenzyme forms of cobalamin, in addition to having a theoretical advantage over other forms of cobalamin, actually do have metabolic and therapeutic applications not shared by the other forms of cobalamin. Methylcobalamin is a cofactor in the enzyme methionine synthase which functions to transfer methyl groups for the regeneration of methionine from homocysteine (Bachmann, 2016).

Methylcobalamin has been available in Japan since the mid-1970s, and is used in peripheral neuropathies, neuritides, polyneuritides, and a number of movement disorders (Tsukerman *et al.*, 1991). In France, methylcobalamin is used for treating pain syndromes of neurological origin. Positive results have been obtained using methylcobalamin to treat experimental diabetes, toxic hepatitis and hyper chromic anemia in rats and rabbits (Tsukerman *et al.*, 1991).

A therapeutic dose for conditions requiring methylcobalamin would be a minimum of 1500 µg and a maximum of 6000 µg per day. No significant therapeutic advantage appears to occur from dosages exceeding this maximum dose; however, it is likely that beneficial physiological effects occur at dosages as low as 100 µg per day, especially if this dose is given repetitively over time (Bachmann, 2016). Clinical studies have reported no adverse effects following administration of up to 6.0 mg/day of methylcobalamin for several weeks and up to 1.0 mg/day cyanocobalamin for several years (Aguilar *et al.*, 2008). Methylcobalamin have been administered orally, intramuscularly, and intravenously; however, positive clinical results have been reported irrespective of the method of administration. It is not clear whether any therapeutic advantage is gained from non-oral methods of administration (Bachmann, 2016).

Methylcobalamin have usually been administered in divided doses three times daily. These supplements have excellent tolerability and no known toxicity. No rationale exists to suspect methylcobalamin would not also be safe during pregnancy (Bachmann, 2016).

The quantity of methylcobalamin to be added to food supplements will be determined by individual formulators but is normally the quantity necessary to supply adults up to 500 µg cobalamin/day (Aguilar *et al.*, 2008). Methylcobalamin is equivalent physiologically to cobalamin, and can be used to prevent or treat pathology arising from a lack of cobalamin (cobalamin deficiency), such as pernicious anemia.

Most of the cobalamin in blood and breast milk is methylcobalamin (Sloan, 2008). Evidence indicates cobalamin from methylcobalamin is utilized more efficiently than cyanocobalamin to increase the levels of coenzyme forms of cobalamin. Although free methylcobalamin is not very stable in the gastrointestinal tract, and considerable loss of the methyl group can take place under experimental conditions, in physiological situations if probably partially protects methylcobalamin from degradation. Paper chromatography of digested ileal mucosa has demonstrated unchanged absorption of methylcobalamin following oral administration. The quantity of cobalamin detected following a small oral dose of methylcobalamin is similar to the amount following administration of cyanocobalamin; but, significantly more cobalamin accumulates in liver tissue following administration of methylcobalamin. Human urinary excretion of methylcobalamin is about one-third that of a similar dose of cyanocobalamin, indicating substantially greater tissue retention (Bachmann, 2016).

Although it has been suggested that oral cobalamin treatment may be effective for food-cobalamin malabsorption, most of the internists believe that there were

no effective oral cobalamin preparations (Lederle, 1991; Herbert, 1996; Chalmers *et al*., 2000). A retrospective study was performed to reveal the lack of oral treatment in B12 deficiency by the authors of this review. The results of retrospective study showed that parenteral treatment was preferred for the patients who have vitamin B12 deficiency (Akkuş Arslan *et al*., 2016) (Table 1).

To overcome this problem, an oral nanoemulsion formulation of methylcobalamin was developed by the authors Akkuş Arslan, Arslan and Tırnaksız (Akkuş Arslan *et al*., 2016). The aims of the study were to investigate if the developed nanoemulsion is effective as much as parenteral form and to compare bioavailability of developed nanoemulsion with commercial tablet and intramuscular forms of vitamin B12. The serum analysis results were given in Table 2.

**Table 1.** Investigation results of retrospective study

| **Location** | Ankara Educational Research Hospital, Department of Family Medicine, Policlinics of Bahçelievler-Ulus-Yenimahalle-Hüseyingazi | |
|---|---|---|
| **Year of research** | 2012 and 2013 | 2014 |
| **% im treatment** | 100 | 100 |
| **% oral treatment** | 0 | 0 |
| **Number of patients** | 592 | 393 |

**Table 2.** The results of analysis of the serum samples

| **Dose (µg/kg)** | **$t_{max}$ (hour)** | **Administration way** | **Formulation type** | **Form of B12** | **Mean serum concentration* at $t_{max}$ (pg/mL) (n=3, ˋX±SS)** |
|---|---|---|---|---|---|
| 15 | 2 | Oral | Nanoemulsion | Methylcobalamin | 4250 ± 2 |
| | | Intramuscular | Parenteral | Cyanocobalamin | 4570 ± 5 |
| | | Oral | Tablet | Cyanocobalamin | 3950 ± 3 |

* The serums were analyzed by using ‘rat, vitamin B12, ELISA, Cusabio kits´ using 1/100 dilution.

According to the results; the use of active form of vitamin B12 (methylcobalamin) dramatically affected the serum levels, the nanoemulsion found more bioavailable in comparison with commercial tablet form, the bioavailability of developed nanoemulsion found closer to commercial parenteral form and a non-invasive and effective formulation could able to be developed with the study.

## CONCLUSION

Cobalamin is an essential vitamin that has to be used in the patients who have cobalamin deficiency and for the individuals who use the cobalamin for daily requirement. Methylcobalamin is one of the two coenzyme forms of cobalamin utilized in the cobalamin-dependent enzymes in humans. It is a cofactor in the enzyme methionine synthase which functions to transfer methyl groups for the regeneration of methionine from homocysteine. It offers a theoretical advantage in cobalamin supplementation. Methylcobalamin is retained in the body better and increase tissue concentrations of cobalamin better than cyanocobalamin. Additionally, it demonstrates a range of activity and clinical results not shown by the other supplemental forms of cobalamin. The metabolic fate and biological distribution of methylcobalamin and 5´-deoxyadenosylcobalamin are expected to be similar to that of other sources of cobalamin in the diet. The use of 5´-deoxyadenosylcobalamin and methylcobalamin as a source of cobalamin in food supplements for the general population at the proposed uses and use levels is not of safety concern.

Most of the medical doctors prefer parenteral treatment for the patients who have cobalamin deficiency, because they do not believe in the efficiency of oral treatment. But oral cobalamin formulations were told to be effective and the results of our study confirm that an effective oral cobalamin nanoemulsion system could be developed.

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