

Review

Vitamin B₁₂ in Health and Disease

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Abstract: Vitamin B₁₂ is essential for DNA synthesis and for cellular energy production. This review aims to outline the metabolism of vitamin B₁₂, and to evaluate the causes and consequences of sub-clinical vitamin B₁₂ deficiency. Vitamin B₁₂ deficiency is common, mainly due to limited dietary intake of animal foods or malabsorption of the vitamin. Vegetarians are at risk of vitamin B₁₂ deficiency as are other groups with low intakes of animal foods or those with restrictive dietary patterns. Malabsorption of vitamin B₁₂ is most commonly seen in the elderly, secondary to gastric achlorhydria. The symptoms of sub-clinical deficiency are subtle and often not recognized. The long-term consequences of sub-clinical deficiency are not fully known but may include adverse effects on pregnancy outcomes, vascular, cognitive, bone and eye health.

Keywords: vitamin B₁₂; physiology; nutrition; adults; chronic disease

Vitamin B₁₂ deficiency was first described in 1849, and was considered to have a fatal outcome until 1926 when a diet of liver, high in vitamin B₁₂, was shown to slow the disease process. Much is now known about the biochemistry and metabolism of vitamin B₁₂, however, the diagnosis of its deficiency has become more complicated with the classification of a “sub-clinical” deficiency category, characterized by serum vitamin B₁₂ concentrations that were once considered to be adequate. Vitamin B₁₂ deficiency was previously thought to take many years to develop, and only in strict vegetarians or those with pernicious anaemia. More recent research has suggested that there are disease implications associated with sub-clinical B₁₂ deficiency, which develop most commonly due to malabsorption or dietary inadequacy. The rates of sub-clinical deficiency of vitamin B₁₂ are high in

developing countries, in the elderly, and in vegetarian populations. The long term consequences are not fully known but may include adverse effects on pregnancy outcomes and aspects of ageing.

1. Vitamin B₁₂ Function

Vitamin B₁₂ also known as cobalamin, comprises a number of forms including cyano-, methyl-, deoxyadenosyl- and hydroxy-cobalamin. The cyano form, which is used in supplements, is found in trace amounts in food [1]. The other forms of cobalamin can be converted to the methyl- or 5-deoxyadenosyl forms that are required as co factors for methionine synthase and L-methyl-malonyl-CoA mutase.

Methionine synthase is essential for the synthesis of purines and pyrimidines. The reaction depends on methyl cobalamin as a co-factor and is also dependent on folate, in which the methyl group of methyltetrahydrofolate is transferred to homocysteine to form methionine and tetrahydrofolate. A deficiency of vitamin B₁₂ and the interruption of this reaction leads to the development of megaloblastic anaemia. Folate deficiency independent of vitamin B₁₂ also causes megaloblastic anaemia [2]. Methylmalonyl CoA mutase converts methylmalonyl CoA to succinyl CoA, with 5-deoxyadenosyl cobalamin required as a cofactor. It is a defect in this reaction, and the subsequent accumulation of methylmalonyl CoA that is thought to be responsible for the neurological effects in vitamin B₁₂ deficiency [2].

Serum vitamin B₁₂ is bound to proteins known as transcobalamins (TC). The majority of the vitamin, approximately 80%, is transported on the inactive TCI (also called haptocorrin). The active transport protein for vitamin B₁₂ is transcobalamin II (TCII), which carries about 20% of the vitamin in the circulation [3]. Holo-transcobalamin (holo-TC) is TCII with attached cobalamin, which delivers vitamin B₁₂ to cells. A low serum vitamin B₁₂ concentration can be associated with a deficiency of TCI, while TCII levels and so vitamin B₁₂ status remain adequate [4].

2. Biochemical Assessment of Vitamin B₁₂ Status

Traditionally vitamin B₁₂ status is assessed by its concentrations in serum, however, concerns have been raised about the use of serum vitamin B₁₂ measurements alone. Although low serum vitamin B₁₂ concentrations are a sensitive indicator of vitamin B₁₂ deficiency and high vitamin B₁₂ concentrations generally indicate sufficiency, the interpretation of the intermediate range of vitamin B₁₂ concentrations is unclear [4].

Methylmalonic acid (MMA) and homocysteine (tHcy) are recognized indicators of vitamin B₁₂ status. Their measurement has highlighted the existence of sub-clinical deficiency, the consequences of which are still being elucidated. MMA is considered to be the specific indicator of cobalamin metabolism, and tHcy is raised in vitamin B₁₂ deficiency along with deficiencies of folate and vitamin B₆. These biomarkers can be confounded by physiological or environmental conditions. Plasma tHcy concentrations are elevated also with renal impairment, polymorphisms in methylenetetrahydrofolate reductase (MTHFR), or the use of some medication. Plasma MMA concentrations are elevated also in renal insufficiency, common in older people [4,5].

It has been recommended by some authors [6,7] that measuring serum vitamin B₁₂ concentrations and following up low values with MMA measurements is an appropriate strategy for the assessment of

vitamin B₁₂ status. However, the threshold of vitamin B₁₂ at which further testing should occur is controversial. A study of serum vitamin B₁₂, MMA and tHcy concentrations indicates that if a lower limit of normal (200 ng/L or 147 pmol/L) is used, patients with increased MMA would be missed, however, if higher values (500 ng/L or 370 pmol/L) are used, most patients would need follow-up MMA tests which may be within the normal range [8]. Carmel recommends a composite criteria based on serum vitamin B₁₂ < 148 pmol/L, or 148–258 pmol/L and MMA > 0.30 μmol/L, or tHcy > 13 nmol/L (females) and >15 nmol/L (males) be used to define inadequate vitamin B₁₂ status [9].

Studies that have assessed the use of holo-TC as a marker of vitamin B₁₂ status show similarity in specificity and sensitivity to serum vitamin B₁₂ concentrations. However, when used in combination with vitamin B₁₂ the predictive value for determining vitamin B₁₂ deficiency is improved [10].

3. Absorption

Vitamin B₁₂ is bound to protein in food and is available for absorption after it has been cleaved from protein by the hydrochloric acid produced by the gastric mucosa. The released cobalamin then attaches to R protein and passes into the duodenum where the R protein is removed and free cobalamin binds to Intrinsic Factor (IF). The IF-cobalamin complex is absorbed by the distal ileum and requires calcium [2]. Vitamin B₁₂ enters the circulation about 3–4 hours later bound to TC.

Vitamin B₁₂ is secreted in bile and reabsorbed via the enterohepatic circulation by ileal receptors which require IF, thus the development of vitamin B₁₂ deficiency is likely to be more rapid in patients with pernicious anaemia as IF is lacking [3]. Vitamin B₁₂ is excreted via the faeces, which is composed of unabsorbed biliary vitamin B₁₂, gastrointestinal cells and secretions, and vitamin B₁₂ synthesised by bacteria in the colon. It is estimated that daily vitamin B₁₂ losses are in proportion to body stores with approximately 0.1% excreted per day [11]. Excessive vitamin B₁₂ in the circulation, e.g., such as after injections, usually exceeds the binding capacity of TC and is excreted in the urine [3].

Historically, vitamin B₁₂ absorption has been measured by a number of methods including whole body counting of radiolabeled vitamin B₁₂, metabolic balance studies [1] or controlled feeding studies in vitamin B₁₂-depleted individuals [12].

It is known that the total amount of vitamin B₁₂ that is absorbed increases with vitamin B₁₂ intake but that the percentage absorption decreases with increasing doses [13]. One study using crystalline vitamin B₁₂ supplements reported that 50% was retained at a 1 μg dose, 20% at a 5 μg dose and 5% at a 25 μg dose, suggesting saturation of the absorption mechanisms [14]. The absorption capacity is thought to recover to baseline levels within 4–6 hours allowing for efficient absorption of the next dose [11]. Approximately 1% of large doses of crystalline vitamin B₁₂ found in some supplements (1,000 μg), are absorbed through a mass action process, even in the absence of IF [15], indicating crystalline vitamin B₁₂ in high doses and food vitamin B₁₂ are absorbed by different mechanisms.

The Schilling test was the classical procedure for assessing the absorption of vitamin B₁₂ but is now rarely used. As there has been no replacement a number of individual tests must be used to diagnose the cause of vitamin B₁₂ deficiency. Tests that diagnose atrophic gastritis, a common cause of vitamin B₁₂ malabsorption, include gastroscopy or serum gastrin and pepsinogen levels. Specific tests for pernicious anaemia include IF antibodies and serum gastrin estimation. MMA and tHcy are better markers of vitamin B₁₂ status, although they are not appropriate for testing absorption [16]. An

overview of the medical management of vitamin B₁₂ deficiency can be found in a recent article by Ralph Carmel [17].

4. Food Sources and Bioavailability of Vitamin B₁₂

Vitamin B₁₂ is synthesised by certain bacteria in the gastrointestinal tract of animals and is then absorbed by the host animal. Vitamin B₁₂ is concentrated in animal tissues, hence, vitamin B₁₂ is found only in foods of animal origin [11]. Foods that are high in vitamin B₁₂ (µg/100g) include: liver (26–58), beef and lamb (1–3), chicken (trace-1), eggs (1–2.5) and dairy foods (0.3–2.4).

There are **no naturally occurring bioactive forms of vitamin B₁₂ from plant sources**. Some plant foods contain added vitamin B₁₂ and others e.g., seaweed and mushrooms contain vitamin B₁₂ analogues that are inactive in humans, although **2 studies suggest certain types of Japanese seaweed (nori) have prevented vitamin B₁₂ deficiency in vegans [18]**. Some foods that are contaminated or fermented by bacteria e.g., tempeh and Thai fish sauce, have been reported to contain vitamin B₁₂ [18], although these may have low affinity with IF and may be poorly absorbed [19].

A number of methods have been used to determine the vitamin B₁₂ content of foods. Microbiological assays using vitamin B₁₂ requiring bacteria were used, however, they are no longer the reference method as measurement uncertainty is high. Radio isotope dilution assays with labeled vitamin B₁₂ and hog IF are used [20]. Further advances are expected with the development of more specific monoclonal antibodies tests using specific binding proteins [21].

The bioavailability of vitamin B₁₂ in humans is dependent on an individual's gastrointestinal absorption capacity. As outlined previously, vitamin B₁₂ absorption is complex and there are adverse changes with age. In view of the technical challenges and biological factors, there is little data on the bioavailability of dietary vitamin B₁₂ in humans. It is thought that 1.5–2.0 µg of synthetic vitamin B₁₂ saturates the IF-cobalamin ileal receptors, but other studies have shown higher absorption rates [1,11]. In normal humans the absorption of vitamin B₁₂ from foods has been shown to vary depending on the quantity and type of protein consumed [19]. Vitamin B₁₂ from foods appear to have different absorption rates with better absorption from chicken and beef as compared to eggs. Studies assessing absorption of food bound vitamin B₁₂ from whole foods are described in Table 1.

5. Vitamin B₁₂ Requirement

The Recommended Dietary Intake (RDI) is set to prevent megaloblastic anaemia and maintain adequate serum vitamin B₁₂ concentrations. **It is assumed that 50% of dietary vitamin B₁₂ is absorbed**. The RDI and estimated average requirement (EAR) do not vary once adulthood is reached. However, the US and Australian Nutrient Reference Values suggest that older adults with atrophic gastritis may require higher intakes of vitamin B₁₂-rich foods, vitamin B₁₂ fortified foods or supplements [3,22]. **The US Institute of Medicine has recommended that adults over 51 years consume most of their vitamin B₁₂ from fortified foods or from supplements, again recognising the high rates of malabsorption due to gastritis that occurs with age. Vitamin B₁₂ stores last several years and the development of deficiency is slow, however the combination of malabsorption and inadequate dietary intake will hasten deficiency [3].**

Table 1. Bioavailability of vitamin B₁₂ from whole foods.

Food Type	Subjects	Vitamin B ₁₂ content	% Absorption, mean (range)	Analysis method	Reference
Mutton	3 healthy young subjects	0.9 µg in 100g portion	65 (56–77)	Radiolabelled vitamin B ₁₂ , whole body counting	[11]
	2 healthy young subjects	3.03 µg in 200g portion	83		
	2 healthy young subjects	5.11 µg in 300g portion	53		
Liver pate	6 healthy subjects		9.1 (5.1–19.5)	Radiolabelled vitamin B ₁₂ , whole body counting	[11]
	4 older subjects	38 µg per serve	4.5 (2.4–6.0)		
	5 subjects with pernicious anaemia		1.8 (0–3.7)		
Chicken	3 healthy subjects	0.4–0.6 µg in 100g portion	65 (58–74)	Radiolabelled vitamin B ₁₂ , faecal excretion studies	[23]
		0.8–1.3 µg in 200g portion	63 (48–76)		
		1.3–1.9 µg in 300g portion	61 (49–75)		
Fish	3 healthy subjects	2.1 µg in 50g portion	42	Radiolabelled vitamin B ₁₂ , faecal and urinary excretion studies	[24]
		3.1 µg in 100g portion	38		
		9.2 µg in 200g portion	42		
		13.3 µg in 300g portion	30		
Eggs: boiled, scrambled, fried	18 healthy subjects	0.9–1.4 µg in 100g portion	3.7–9.2	Radiolabelled vitamin B ₁₂ , faecal and urinary excretion	[25]

6. Vitamin B₁₂ Deficiency

Deficiency is usually caused by the malabsorption of vitamin B₁₂ although dietary inadequacy is common in the elderly, vegans or ovo-lacto vegetarians with poor diets. Causes can also relate to inadequate IF production, atrophic gastritis, interference with the ileal uptake of vitamin B₁₂ due to disease, resection or interference by bacterial overgrowth, drug-nutrient interactions as well as some less common genetic defects [3].

Vegans who consume no foods of animal origin can meet their vitamin B₁₂ requirement from fortified foods or supplements. Ovo-lacto vegetarians with only a small intake of dairy foods or eggs, may require supplemental vitamin B₁₂. Pregnant and/or lactating women following vegetarian or vegan diets are at high risk of deficiency due to the increased metabolic demand for vitamin B₁₂ and require adequate intake of vitamin B₁₂-containing foods or supplements.

The elderly are at risk of undernutrition in general, predominately due to reduced intake related to illness but also due to physical capacity e.g., difficulties with food preparation, and psychological factors e.g., depression. Protein bound malabsorption is thought to be the most common cause of sub-clinical vitamin B₁₂ deficiency in the elderly and is commonly associated with some degree of atrophic gastritis. Gastritis or inflammation of the gastric mucosa increases with age and results in a reduction, or in some cases, complete loss of the acid required to cleave vitamin B₁₂ from protein. Synthetic vitamin B₁₂ remains available for absorption as it is not protein bound [3,22].

Pernicious anemia is the end stage of an auto-immune gastritis and results in the loss of synthesis of IF. It is this loss of IF that causes vitamin B₁₂ deficiency and if untreated, megaloblastic anaemia and neurological complications develop. Pernicious anaemia is treated with vitamin B₁₂ injections, or large doses of oral vitamin B₁₂. Vitamin B₁₂ deficiency will also develop after gastric antrum resection as this is the site of secretion of IF and acid [3].

Reduced ileal uptake of vitamin B₁₂ can be caused by competition for vitamin B₁₂ in patients with bacterial overgrowth or parasitic infection. Resection or diseases of the ileum such as Crohn's Disease or other chronic bowel inflammatory conditions also cause malabsorption of vitamin B₁₂ [3].

The potential masking of vitamin B₁₂ deficiency by folate fortification of the food supply has also raised some safety concerns. When vitamin B₁₂ concentrations are low, high doses of folate (supplements or food fortification) allow DNA synthesis to continue, prevent megaloblastic anaemia and potentially "mask" vitamin B₁₂ deficiency, potentially allowing homocysteine and MMA concentrations to rise and neurological damage to progress. Neurological damage in the absence of anaemia has been reported in 20-30% of cases of vitamin B₁₂ deficiency [4]. In view of this and the effect of vitamin B₁₂ deficiency on pregnancy outcomes [26,27], there is discussion of the need to fortify flour with vitamin B₁₂. Vitamin B₁₂ fortification of flour is most likely to benefit those with poor dietary intake of vitamin B₁₂ and the elderly with food bound malabsorption, but would be inadequate for those with pernicious anaemia, which affects 2–4% of the US population depending on ethnicity [28]. Patients with pernicious anaemia require larger oral supplements (e.g., 500–1,000 µg/d) or intramuscular injections. In developing countries, fortification could potentially have a more significant impact as the population's intake is often low. However, as yet there not enough intervention trials on the effect of different fortification levels of flour in different populations [29].

7. Drug-nutrient Interactions

Some medications are thought to interfere with the absorption or metabolism of vitamin B₁₂. These include proton pump inhibitor (PPI) medications, metformin, nitrous oxide anaesthesia, some epileptic medications and colchicine.

PPI medications are commonly used in the elderly for the treatment of gastro-oesophageal reflux disease. PPI medications act by reducing the secretion of gastric acid and pepsin, theoretically leading to a decrease in the absorption of protein-bound vitamin B₁₂. However, the current literature on PPI usage and vitamin B₁₂ status is inconsistent [30-33]. The monitoring of vitamin B₁₂ concentrations is recommended for patients undergoing prolonged PPI treatment, in recognition that the bioavailability of food-bound vitamin B₁₂ may be compromised [3].

Metformin is a biguanide used for the treatment of non-insulin dependent diabetes and some patients taking this medication develop megaloblastic anaemia [34,35]. This may relate to intestinal

mobility changes or bacterial overgrowth competing for vitamin B₁₂ in the gastrointestinal tract. It has also been shown that calcium improves the uptake of vitamin B₁₂ in metformin users [35].

Nitrous oxide anesthesia inhibits methionine synthase and L-methylmalonyl-CoA mutase and produces deficiency symptoms despite concentrations of serum vitamin B₁₂ in the normal range [36]. Antiepileptic drugs have been associated with low concentrations of vitamin B₁₂, but this is controversial with some studies showing no change and others increased levels of vitamin B₁₂ [37].

8. Vitamin B₁₂ and Neural Tube Defects (NTD)

NTD include spina bifida, anencephaly, and encephalocele. These are caused by the failure of the neural tube to close during gestation. The aetiology of NTD is not fully understood but risk factors include folate deficiency, genetic and environmental factors [26,27]. A significant reduction in NTD has been reported since folate fortification of the US food supply [38]. As folate has reduced but not eliminated rates of NTD, research is ongoing to determine further strategies to minimise risk. Low vitamin B₁₂ status has been postulated as a potential risk factor for NTD [39] since vitamin B₁₂ acts as a cofactor for methionine synthase in the folate cycle. When vitamin B₁₂ supply is low, the folate needed for DNA synthesis remains trapped in the methylation cycle and cell replication is impaired. Studies assessing the impact of vitamin B₁₂ status on NTD are shown in Table 2. The studies consistently report a 2-4 fold increased risk of NTD with low vitamin B₁₂ status. The studies were undertaken in a range of population groups including those that are exposed to folate fortified foods, as well as non-fortified populations.

Table 2. Case control and cohort studies of vitamin B₁₂ and Neural Tube Defects.

Design and reference	Study Details	Main Outcome
Case control [39]	81 NTD cases and 247 controls	In cases only, plasma vitamin B ₁₂ and plasma folate affected maternal Red Cell Folate (multiple $r = 0.68$, $p < 0.001$).
Case control [40]	84 NTD pregnancies and 110 controls	Women with lower vitamin B ₁₂ have increased risk of NTD.
Cohort [41]	Vitamin B ₁₂ at 15 weeks' gestation	Vitamin B ₁₂ <185 pmol/L associated with the highest risk of NTD.
Case control [42]	46 NTD pregnancies and 44 controls	Lower serum vitamin B ₁₂ ($p = 0.005$) in cases compared to controls
Case control [43]	35 NTD neonates and parents vs 24 normal neonates.	Low vitamin B ₁₂ in both the parents of child with NTD.
Case control [44]	89 NTD and 422 controls	Increased NTD risk with lower holo-TC.
Case control [45]	36 NTD vs normal pregnancy.	Low vitamin B ₁₂ associated with 2-3 x increased risk for NTD
Case control [46]	46 NTD and 73 control mothers	For NTD holo-TC % (holo-TC/total TCII) Q1 vs Q4 OR = 5.0 (95% CI: 1.3, 19.3).

Table 2. Cont.

Case control ¹ [47]	57 NTD cases and 186 controls	Q1 vs Q5 of vitamin B ₁₂ OR = 3.0 (95% CI:1.4, 6.3)
Case control ¹ [48]	45 mothers and NTD children vs 83 controls	Case mothers with vitamin B ₁₂ ≤ 185 pmol/L OR = 3.5-fold (95% CI:1.3, 8.9) for NTD risk.
Case control [49]	56 NTD babies and mothers vs 97 control children and mothers.	Low vitamin B ₁₂ levels increase risk of NTD.
Case control ¹ [50]	60 NTD cases and 94 controls	NTD for mothers for vitamin B ₁₂ levels ≤ 5 th % vs ≥95 th
Case control [51]	32 NTD pregnancies and 132 control pregnancies.	MMA higher in cases vs controls.

¹Study performed in folate fortified population, NTD = Neural tube defects, OR (95%CI) = Odds Ratio and 95% confidence interval, Q4 = 4th quartile, Q5 = 5th quintile, RBC = red blood cell, tHcy = total homocysteine concentration, holo-TC = holotranscobalamin II, total TCII = total transcobalamin II, MMA = methylmalonic acid

9. Vitamin B₁₂ and Cardiovascular Disease (CVD)

Nutritional risk factors for CVD include hypercholesterolaemia, hypertension and obesity. Elevated tHcy concentrations are also considered a risk factor, however, it is unclear if tHcy is a modifiable risk factor or an independent marker of the disease process. Much of the research into CVD and tHcy is related to the effects of folate supplementation with or without the addition of vitamins B₁₂ and B₆. Investigations of the relationship between CVD and vitamin B₁₂ per se are limited.

Meta-analyses of prospective studies (Table 3) have consistently shown associations between tHcy and increased risk of CVD. Supplementation with vitamin B₁₂ of doses ranging from 0.02–1 mg/d produces approximately 7% reduction in tHcy, while folate produces 10–30% reduction in risk. Vitamin B₆ has been shown to have little effect [52].

Table 3. Meta-analyses of studies assessing vitamin B₁₂ and CVD.

Trial Type	Study Details	Main Outcome
Meta-analysis [53]	9 case-control studies. Assessed associations between tHcy and CVD risk.	5μM tHcy increment associated with increased risk of CAD, OR = 1.6 (95% CI:1.4 to 1.7) for males and 1.8 (95% CI:1.3 to 1.9) for females.
Meta-analysis [54]	30 prospective or retrospective studies assessed tHcy and CVD risk.	25% lower tHcy associated with lower risk of IHD & stroke.
Meta-analysis 7 RCTs [55]	B vitamin supplementation and tHcy lowering, assessed effect of vitamin B ₁₂ (range 0.02–1.0 mg/day)	Vitamin B ₁₂ (median dose 0.4 mg/d) - further decrease (-7%) in tHcy

Table 3. Cont.

Meta-analysis 12 RCTs [56]	Preexisting CVD or renal disease- included 3 studies of vitamin B ₁₂ supplementation, with doses 0.4–1.0 mg B ₁₂ /day.	Reduction in stroke risk in vitamin B ₁₂ (1 mg/d) intervention OR = 0.76 (95% CI:0.59, 0.96)
Meta-analysis 8 RCTs [57]	4 studies assessed vitamin B ₁₂ supplementation (0.018–1 mg) and stroke risk	Reduction in stroke greater in longer trials with more tHcy lowering and no stroke history. No specific effect of vitamin B ₁₂ .
Meta-analysis 24 RCTs [58]	Assessed CIMT: 3 with vitamin B ₁₂ : 0.4–0.5 mg/d; endothelial function: 5 with B ₁₂ : 6 µg–1 mg/day	↓ CIMT, ↑ FMD found in short-term not long term trials

µM = micromolar, tHcy = total homocysteine, CAD = coronary artery disease, OR = odds ratio, CI=confidence intervals, CVD = coronary vascular disease, IHD=ischemic heart disease, CIMT = carotid intima media thickness, FMD = flow mediated dilation

The recent B vitamin supplementation trials investigating the effect of tHcy reduction and CVD did not show the expected reductions in risk of CVD [59-63]. All of these randomised controlled trials (RCTs) included vitamin B₁₂ supplementation (ranging from 6 µg-1 mg) in tandem with folate, and it is not possible to determine the individual impact of vitamin B₁₂. A number of reviews have discussed the limitations of these trials [64-66] and identified inadequate treatment with vitamin B₁₂ as one of the limitations.

Subgroup analysis of the VISP Trial found that patients with higher baseline vitamin B₁₂ concentrations, taking high dose vitamins, had the best outcomes and those with lower baseline vitamin B₁₂ taking low-dose vitamins had the poorest outcomes for stroke, death, and coronary events, suggesting higher vitamin B₁₂ doses may be needed in some patients [67]. Vitamin B₁₂ has been shown to be a major determinant of tHcy concentrations in subjects with adequate folate status [68] and the existence of vitamin B₁₂ deficiency could be one reason for the lack of effect of intervention with folate [69].

10. Cognitive Decline

The assessment of vitamin B₁₂ status forms part of the screening process for dementia, however, the effects of sub-clinical levels of vitamin B₁₂ on cognitive status are unclear. Studies investigating cognitive decline and vitamin B₁₂ status using serum vitamin B₁₂ concentrations alone, have been inconclusive [70]. Raised MMA concentrations are associated with cognitive decline and Alzheimer's Disease [71]. It has been suggested that holo-TC and MMA and the ratio holo-TC:vitamin B₁₂ [72,73] are better correlated with cognition and the rate of cognitive decline in elderly subjects. In older people with low vitamin B₁₂ status, a high serum folate concentration was associated with increased odds of cognitive impairment, but in subjects with normal vitamin B₁₂ status, high serum folate was found to be protective against cognitive impairment [74].

To date, there are few intervention studies that examine the relationship between vitamin B₁₂ and cognitive function. A Cochrane review, based on 2 studies, identified no effect of supplementation with vitamin B₁₂ alone on cognitive score in older adults [75]. A meta-analysis and review identified a correlation between tHcy and Alzheimer's Disease, and suggested the effect was due to lower levels of vitamins B₁₂, B₆ and folate [76]. These studies suggest a role for vitamin B₁₂ in the prevention of cognitive decline. However, more long-term studies using biomarkers of vitamin B₁₂ status and intervention studies from mid-life are needed to determine the effects of B vitamins on cognition.

11. Osteoporosis

Dietary factors associated with the development of osteoporosis include inadequate protein, calcium and vitamin D. More recently, there has been interest in the effect of other nutrients, including vitamin B₁₂ on bone health.

Elevated tHcy has been associated with an increased risk of bone fractures, however it is not clear whether this is related to tHcy per se, to the level of vitamins B₁₂, B₆ or folate which are required for its metabolism, or to other causes of elevated tHcy such as environmental factors or underlying disease. A recent systematic review found that there is evidence for the association between tHcy and increased fracture risk, but less conclusive evidence for tHcy and low bone mineral density (BMD) or for the association between vitamin B₁₂ and either fracture risk or low BMD [77]. Intervention trials of the association between B vitamin supplementation have also shown mixed results.

Positive effects of the supplementation of B vitamins on BMD have been found in a subgroup of osteoporotic patients with high tHcy and stroke patients at risk for osteoporosis [78,79], but none in a group of healthy older people or from the secondary analysis of the HOPE Trial for CVD reduction [80,81]. Cohort studies with more than 1,000 subjects or smaller intervention trials are summarized in Table 4. Some of the inconsistencies in study results may be due to differences in the study populations [82,83], differences in the cut points used to define vitamin B₁₂ status, and the reliance on serum vitamin B₁₂ concentrations rather than more specific biomarkers.

Table 4. Studies of vitamin B₁₂ and risk of osteoporosis or fracture.

Design and reference	Study Details	Main Outcome	Reduced risk
Cohort [84]	Elderly, fracture risk	Low vitamin B ₁₂ and/or HHcy: RR = 3.8 (95% CI:1.2, 1.6) males and 2.8 (95% CI:1.3, 5.7) females	Yes
Cohort [85]	Elderly, fracture risk	tHcy > 14, hip fracture HR = 1.49; (95% CI: 0.91, 2.46)	No
Cohort [86]	Hip fracture risk	fracture for high vs low tHcy (≥15 vs <9 μM), HR=2.42 (95% CI:1.43, 4.09) in women	Yes
Cohort [87]	Elderly, fracture risk	For 1 SD in tHcy fracture RR =1.4 (95% CI:1.2, 1.6)	Yes

Table 4. Cont.

Cohort [88]	Elderly BMD, tHcy, MTHFR polymorphisms	OR for low BMD w HHcy $\geq 15 \mu\text{M}$ vs. low tHcy OR=1.96 (95% CI:1.40, 2.75) for females.	Yes
Cohort [89]	Elderly BMD and plasma vitamins	Vitamin B ₁₂ <148 pM had lower BMD at hip (males) and spine (females) $p < 0.05$.	Yes
Cohort [90]	Elderly subjects (n=1550)	Serum vitamin B ₁₂ <15 th percentile: OR of osteoporosis/osteopenia = 2.0 (95% CI:1.0, 3.9).	Yes
RCT [79]	559 subjects:5 mg folate, 1.5 mg vitamin B ₁₂ or placebo	RR for hip fracture = 0.20 (95% CI: 0.08, 0.50)	Yes
RCT [78]	47 Osteoporotic subjects 2.5 mg folate, 0.5 mg vitamin B ₁₂ and 25 mg B ₆ or placebo.	No changes in BMD or bone metabolism markers.	No
RCT [80]	Healthy older n = 276; folate 1 mg, vitamin B ₁₂ 0.5 mg, B ₆ 10 mg or placebo.	No differences in bone markers in vitamin vs placebo groups.	No
CT [81]	5522 subjects with vascular disease, 2.5 mg folic acid, 50 mg B ₆ , 1 mg vitamin B ₁₂ or placebo	HR =1.06 (95% CI:0.81, 1.40) for fracture risk in supplemented vs non supplemented	No

HHcy = hyperhomocysteinaemia, tHcy = total homocysteine, CI = confidence intervals, SD = standard deviation, RR = relative risk, OR = odds ratio, HR = hazard ratio.

12. Other Aspects of Vitamin B₁₂ and Ageing

Vitamin B₁₂ has been associated with the development of age related macular degeneration (AMD) and risk of frailty, both leading causes of disability in the elderly.

AMD is the leading cause of vision loss in the elderly. Risk factors include increasing age, family history, hypertension, smoking, obesity, sunlight exposure and hypercholesterolemia [91]. Some [91,92] but not all [93] cross sectional studies have found lower vitamin B₁₂ concentrations in AMD cases. However, a recent RCT with 5205 female health professionals at risk of vascular disease found a 34% reduction in the relative risk of AMD after supplementation with vitamins B₁₂, B₆ and folate (daily doses of 1 mg, 50 mg, 2.5 mg respectively) [94].

Frailty is characterized by muscle wasting, diminished strength, often with weight loss with or without reduced nutritional intake. Frailty is associated with an increased vulnerability to stresses, causing longer and more complicated recovery from illness or surgery [95].

Increased risk of frailty and disability has been associated with poor B vitamin status. Subjects with vitamins B₁₂ and B₆ in the lowest quintiles and subjects with elevated MMA and tHcy concentrations, have been found to have increased risk of decline in physical function and the development of frailty [96,97]. Two cross sectional studies found the length of hospital stay was associated with poor vitamin B₁₂ status as assessed by MMA and serum vitamin B₁₂ concentrations [98,99]. To date there

are limited studies, however, if improvements in nutrition can delay frailty progression, it could significantly enhance the independence of the increasing numbers of older people.

13. Conclusion

Vitamin B₁₂ is a particularly important vitamin for women of childbearing age and for older people, however, adequate vitamin B₁₂ status over the whole of the lifecycle is needed for optimal health. There has been renewed interest in vitamin B₁₂ since the reporting of associations between homocysteine and chronic disease, particularly vascular disease. The effects of sub-clinical deficiency are not fully known and many aspects of vitamin B₁₂ absorption, bioavailability and metabolism are yet to be determined. The identification of sensitive biomarkers of vitamin B₁₂ status will help elucidate the relationships between vitamin B₁₂ and chronic disease, and help to identify those at risk of clinical and sub-clinical deficiency.

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