Vitamin B12

Disclaimer

This chapter describes the background for setting dietary reference values for "vitamin B12" in the 6th edition of the Nordic Nutrition Recommendations (NNR2022). Anne-Lise Bjørke Monsen and Vegard Lysne has been assigned as authors. The present version of the chapter has been peer reviewed by David Smith and Ebba Nexø, and considered by the NNR2022 Committee. The chapter is now open for public consultation. The hearing responses will be publicly available and carefully considered by the NNR2022 Committee. All input considered by NNR2022 Committee as scientifically valid and relevant will be forwarded to the authors for consideration. Please note that sustainability aspects and other issues such as obesity, physical activity, and burden of diseases will be integrated at a later stage, if relevant. The NNR Committee is responsible for setting the dietary reference values. The suggestion for setting of dietary reference values will be open for public consultation at a later stage, before the NNR2022 Committee reach the final conclusion, and are not included in the document now available for public consultation.

1. Abstract:

Vitamin B12 is essential for normal metabolic function and even moderate deficiency of this vitamin has negative health effects. Vitamin B12 is found in animal food and as vegetarian diets are increasingly popular in Western countries, one might expect a higher prevalence of vitamin B12 deficiency in the Nordic population.

Setting recommendations for vitamin B12 intake has proven to be difficult, as uptake of vitamin B12 varies substantially, the clinical symptoms are often diffuse and there is no clear agreement on the decision limits for vitamin B12 deficiency.

The vitamin B12 intake needed just to compensate for daily losses is estimated to be in the range from 3.8 to 20.7 µg per day. Vitamin B12 deficiency is reported to be particularly common among pregnant women and infants, despite the fact that less than 1 % of Norwegian pregnant women have a cobalamin intake below the recommended level of 2.0 µg/day. In addition, the assumption that breast milk contains sufficient vitamin B12 for optimal health and neurodevelopment during the first 6 months of life does not comply with high prevalence of insufficient vitamin B12 status in this age group.

Recommended intake of vitamin B12 for various age-groups must depend on what is considered an adequate status, according to levels of serum vitamin B12, holotranscobalamin and the metabolic markers total homocysteine and methylmalonic acid, and not only on the absence of clinical signs of vitamin B12 deficiency.

A de novo NNR2022 systematic review will be conducted in 2022.

2. Introduction:

The aim of this chapter is to describe the totality of evidence for the role of vitamin B12 for health related outcomes as a basis for setting and updating DRVs. Vitamin B12 is the common term for a group of cobalt-containing compounds (corrinoids) that are biologically active in humans. Cobalamin is used synonymously with vitamin B_{12}.

Vitamin B12 have two metabolic functions. Methylcobalamin serves as cofactor for methionine synthase, involved in the transfer of a methyl group from folate to homocysteine, regenerating methionine. Adenosylcobalamin is a cofactor for methylmalonyl coenzyme A mutase, involved in the metabolism of odd-chain fatty acids, amino acids, and cholesterol. Insufficient activity of these enzymes, due to vitamin B12 deficiency, results in the accumulation of homocysteine and/or methylmalonic acid, which therefore are considered functional markers of vitamin B12 status(1).

Vitamin B12 is produced by soil bacteria and is found in animal food and in soil-contaminated food(2). Vegetarian and vegan diets are increasingly popular in Western countries, particularly among young women(3). Although these diets are considered nutritionally adequate and healthy when appropriately planned, substantial amount of nutritional knowledge is needed to achieve an optimal vegetarian diet with regard to vitamin B12 intake and status. However, this happens not to be the case, even among well-educated people and a higher prevalence of vitamin B12 deficiency in the Nordic population might be expected(4).

Vitamin B12 is essential for normal metabolic function and even moderate deficiency of this vitamin has negative health effects. Even moderate vitamin B12 deficiency is associated with impaired neurodevelopment in young infants(5-7). Vitamin B12 deficiency is reported to be common among Norwegian pregnant women(8) and infants(9). However, only 0.8% of Norwegian mothers in pregnancy week 18 had a vitamin B12 intake below the recommended level of 2.0 µg/day (10), indicating that the current recommendations may be too low.

The recommendation of vitamin B12 intake is difficult and there are a number of uncertainties to consider. The absorption rate of vitamin B12 varies from 30 to 90%, reported estimated body stores vary from 2 to 3 mg, and daily losses range from 1.4 to 5.1 µg. Vitamin B12 intakes needed to compensate for these losses have been reported to range from 3.8 to 20.7 µg(11). Additionally, there is no clear agreement on what levels of serum vitamin B12, plasma holotranscobalamin (holo-TC), and the metabolic markers plasma total homocysteine (tHcy) and serum methylmalonic acid (MMA) constitute an optimal vitamin B12 status. It is however important to remember that, as long as vitamin B12 is taken orally, either in food or in

supplements containing a moderate vitamin B12 content, the body will actively regulate the uptake in ileum, and there is no evidence that intakes up to 100 µg/d from foods and supplements represent a health risk(12).

3. Methods:

This review follows the protocol developed within the NNR2022 project "The Nordic Nutrition Recommendations 2022 – Instructions to authors of chapter" which can be found on the official NNR2022 website. The sources of evidence used in the chapter follow the eligibility criteria described in the paper "The Nordic Nutrition Recommendations 2022 – Principles and methodologies"(2).

The main literature search for this chapter was performed in MEDLINE July 1st, 2022, with a search string: (b12, vitamin[MeSH Terms] OR cobalamin[MeSH Terms]) AND review[Publication Type] AND ("2011"[Date - Publication] : "3000"[Date - Publication]) AND Humans[Filter]. The number of hits was 453. Based on the title and abstract, a total of 32 articles were picked up, of which 18 were considered as relevant (11, 13-29). Three additional articles were identified through a supplementary search (30-32). We also identified relevant literature for this chapter via "snowballing"/citation chasing that was relevant for the background information. No published articles were considered by the committee as a qualified systematic review (33), and a de novo NNR2022 systematic review will be conducted (Bärebring et al, 2022).

4. Physiology:

Vitamin B12 refers to a group of cobalt-containing compounds (corrinoids) that are biologically active in humans. Corrinoids also includes vitamin B12 analogues which are not biologically active, such as cobamides and cobinamides. The vitamin B12 molecule consists of a cobalt-containing corrin ring, a 5,6-dimethylbenzimidazole base, a sugar, and an aminopropanol group. A functional ligand is covalently bound to the cobalt at the upper axial position, which can be one of either a methyl-, adenosyl-, cyano-, or hydroxyl group.

Absorption and intestinal transport

Dietary vitamin B12 is absorbed via an active receptor-mediated physiological process and via a less efficient passive diffusion pathway. In the acidic environment of the stomach, dietary protein-bound vitamin B12 is cleaved from the protein by gastric enzymes and bound to salivary derived haptocorrin (HC). Stimulated by food intake, intrinsic factor (IF) is produced by the parietal cells in the stomach, and both IF

and the haptocorrin-vitamin B12 complex are transported to the small intestine. In the less acidic environment of the upper small intestine, vitamin B12 is released from HC, and the free vitamin B12 binds to IF. While HC binds all corronoids, IF for all practical purposes only recognizes and binds biologically active cobalamins.

The IF-vitamin B12 complex is transported to the distal ileum, where it binds to the cubam receptor and enters the enterocytes through endocytosis. The availability of cubam receptors is the rate-limiting factor for receptor-mediated absorption of cobalamin. It has been reported the fractional absorption decreases with increasing dose (Allen2018). Biovailability studies have demonstrated that while about 50% of a 1µg oral dose is absorbed, only 5% of a 25µg dose is retained. Further, during the subsequent 4-6 hours, active uptake is restricted due to regeneration of cubam receptors (Allen2018). In total, a maximum of ~2µg vitamin B12 can be absorbed from a meal through the active route. Additionally, approximately 1.2% of vitamin B12 is absorbed passively (34). Normally, this accounts for a small proportion of total vitamin B12 absorption but becomes relevant if vitamin B12 is consumed in high doses (>50µg). Bioavailability of vitamin B12 from various foods, as assessed by whole-body retention or faecal excretion, ranges from about 20% up to 90% at single doses of 0.25 µg to 5 µg (35). It is estimated that approximately 50% of dietary vitamin B12 is absorbed by healthy adults with normal gastric function (36, 37).

Transport in blood and enterohepatic circulation

Upon entering the enterocyte, IF is degraded and free vitamin B12 is released into the cytosol and transported into the circulation through multidrug resistance protein 1 (MRP1). In the circulation, vitamin B12 is transported bound to either HC or to transcobalamin (TC). About 70-90% of circulating vitamin B12 is bound to HC, which can be taken up by the liver through specific receptors. About 20-30% of circulating vitamin B12 is bound to TC (holoTC), which is responsible for delivering vitamin B12 to all tissues through endocytosis facilitated by the transcobalamin receptor (TCR) (Allen 2018). HC-bound vitamin B12 has a half-life of several days compared to the half-life of about an hour for holoTC (38). HC-bound vitamin B12 and vitamin B12 analogues are excreted from the liver through bile and cleaved from HC in the intestines. While the analogues are excreted in faeces vitamin B12 may be reabsorbed, referred to as the enterohepatic circulation.

Intracellular metabolism

Once bound to the transcobalamin receptor (TCR) on the target cells membrane, the holoTC-TCR complex is taken up into the cell by endocytosis. Within the lysosome, vitamin B12 is released, the transcobalamin is degraded, and the TCR is recycled back to the cell surface. A series of vitamin B12 processing proteins facilitates the transport out of the lysosome (CblF/CblJ), removal of the upper axial ligand (CblC/CblX), partitioning to the cytosolic or mitochondrial compartment (CblD), and then formation of the two active cobalamin cofactors methylcobalamin (CblE/CblG) and adenosylcobalamin (CblA/CblB/CblH/mut)(1)..

Molecular functions

Vitamin B12 is a cofactor for only two enzymes in the human metabolism (2). Methylcobalamin is a cofactor for methionine synthase – the enzyme that catalyses the conversion of homocysteine to methionine. This reaction requires the transfer of a methylgroup from 5'-methyl tetrahydrofolate. Intracellular deficiency of folate and/or vitamin B12 therefore result in increased plasma concentrations of tHcy. Adenosylcobalamin is a cofactor for methylmalonyl-CoA mutase in the isomerization of methylmalonyl-CoA to succinyl-CoA. Vitamin B12 insufficiency therefore results in increased serum MMA concentrations (2).

Specific groups

Infants, children and adolescents

The vitamin B12 status in the newborn is a result of maternal status during pregnancy, gestational age, and birth weight. Infants born premature, with low birth weight or with a vitamin B12 deficient mother are prone to develop vitamin B12 deficiency during the first weeks of life, something that may impair neurodevelopment (5, 39). During the first weeks of life, there is a considerable decrease in serum cobalamin, accompanied by a marked increase in plasma tHcy and MMA (40-42). The lowest vitamin B12 levels and the highest plasma tHcy and MMA levels in childhood are seen in infants 6 weeks to 6 months of age. In older children (>6 months), serum vitamin B12 increases and peaks at 3–7 years and then decreases, median plasma tHcy remains low (<6 µmol/L) and increases from the age of 7 years(42, 43).

Pregnancy and lactation

All markers of vitamin B12 status are reduced in pregnant compared to non-pregnant women(8). Serum vitamin B12 decreases during pregnancy. This is accompanied by a gradual increase in the metabolic markers, indicative of a functional intracellular vitamin B12 depletion, despite the fact that both plasma tHcy and MMA are substantially reduced compared to non-pregnant women(8).

Serum vitamin B12 is increased by more than 40% at six weeks postpartum and remains so as long as the mother are lactating. An additional moderate increase in the metabolic markers plasma tHcy and MMA are observed, suggestive of maternal intracellular vitamin B12 depletion, despite high serum concentrations(8). Vitamin B12 concentrations in breast milk decrease during the lactational period with the highest levels seen in the first four weeks(44).

Inborn errors of metabolism

There are several inborn errors of vitamin B12 metabolism, which can be roughly divided into genetic mutations affecting absorption and transport, and mutations affecting intracellular vitamin B12 processing. The former category includes Intrinsic factor deficiency and Immerslund-Gräsbeck syndrome, which inhibits absorption either through the lack of intrinsic factor or a defect in the cubalamin receptor facilitating intestinal uptake, ultimately resulting in vitamin B12 deficiency. Haptocorrin deficiency is

considered benign and results in low serum vitamin B12 and normal concentrations of the metabolic markers. The inborn errors affecting intracellular processing are categorized based on whether they result in accumulation of Hcy and or MMA. CblF, CblJ, CblC, CblX, or CblD are characterized by increased levels of both tHcy and MMA, CblE and CblG yield isolated hyperhomocysteinemia, whereas CblA and CblB yield isolated methylmalonic aciduria. Most inborn errors of vitamin B12 metabolism are rare, and the most common mutation is CblC which has been reported in >500 patients (45).

5. Assessment of nutrient status.

Vitamin B12 status is judged based on the estimated dietary vitamin B12 intake and relevant symptoms that may suggest vitamin B12 insufficiency. However, clinical symptoms are often diffuse and vitamin B12 deficiency may be difficult to diagnose. Further, biomarkers of vitamin B12 status include serum vitamin B12 and holoTC (bioavailable fraction in the circulation), and the functional biomarkers total tHcy and MMA. Because B12 is essential for folate metabolism, it is also important to consider folate status. The importance of biomarkers is reflected by increasingly common practice of defining vitamin B12 insufficiency as a “biochemical” or “subclinical” vitamin B12 insufficiency/deficiency, which is frequently used synonymously with vitamin B12 insufficiency/deficiency.

Many laboratories still use the 2.5 percentile, ranging from 145 - 200 pmol/L for serum cobalamin, to define B12 deficiency. A reference interval is typically defined as the interval between the two reference limits (2.5th and 97.5th percentiles) derived from the distribution of results from an apparently healthy reference population(46). As reference intervals merely describe the B12 status in a specific population and will differ according to the diet in the tested population, one must have clinical decision limits in order to interpret vitamin B12 status. Clinical decision limits defines a value above or below a threshold associated with a significantly higher risk of adverse clinical outcomes or diagnostic for the presence of a specific disease (46). However, all four vitamin B12 biomarkers have limitations as standalone markers, and there is currently no clear agreement on the decision limits for any of these parameters concerning vitamin B12 deficiency (1, 2). MMA is considered the most specific biomarker of vitamin B12 status and has been utilized when suggesting cutoffs for the other biomarkers. However, as only less than 17% of the MMA concentrations in blood are determined by cobalamin, renal function, age and gender, neither MMA is the perfect vitamin B12 marker(47) and there is no consensus regarding which MMA concentration to use in this context. In both children and adults, the metabolic markers tHcy and MMA start to increase when serum vitamin B12 falls below 500 pmol/L, indicating suboptimal intracellular vitamin B12 stores, with a steeper increase in both markers when serum vitamin B12 falls below 250 pmol/L indicating biochemical deficiency(47, 48). In the following we will comment on the individual biomarkers and the biological variations that should be taken into account when employing them.

Serum or plasma cobalamin

Serum vitamin B12 is the primary marker of vitamin B12 status in both children, adults, and pregnant women (2). The assays used to analyze human samples are widely available, cheap, and specific for biologically active cobalamins. A limitation of this biomarker is that it measures the total circulating cobalamin, of which the majority (70-90%) is bound to haptocorrin, which may be affected by high estrogen levels, cancer and genetically low haptocorrin levels. In these circumstances serumvitamin B12 concentrations may not accurately reflect intracellular vitamin B12 status (1, 2).

The serum concentrations are not substantially affected by recent dietary intake, meaning prandial status is not a major concern when collecting samples. In the longer term (months), the concentrations increase with increasing habitual dietary intake and has been reported to plateau at intakes of 7-10 µg (13). A meta-analysis of 37 RCT's and 19 observational studies aimed to quantify the dose-response relationship and reported that each doubling of vitamin B12 intake increased circulating concentrations by 11% (95% CI 9.4%-12.5%). However, substantial heterogeneity between the studies, not explained by study design, age, or vitamin B12 dose, warrant caution when interpreting the reported associations (14).

Diagnostic cut-offs vary from below 148 up to 300 pmol/L depending on the outcome(2). The lower decision levels (<148 pmol/L) are generally based on the presence of clinical deficiency symptoms, while studies show that hypomethylation, chromosome breaks, uracil incorporation and micronucleus formation are minimised when serum vitamin B12 are > 300 pmol/L(49).

All vitamin B12 markers are decreased during pregnancy, so specific decision limits must be used. A maternal vitamin B12 concentration >275 pmol/L (measured by immunoassay) in week 18 of pregnancy has been recommended to secure an optimal infant vitamin B12 status the first six months of life (8, 50).

HoloTC

Circulating holoTC represents the active fraction (10-30%) of serum vitamin B12 and may therefore be considered a better measure of vitamin B12 status. However, there is a lack of knowledge regarding factors influencing holoTC homeostasis, and the diagnostic value may thus be questioned (1). Compared to circulating cobalamin, the assay for HoloTC is less widely available, more expensive and decision limits are missing for children and pregnant women. Unlike the other biomarkers, HoloTC increases in the postprandial period, and is therefore a better indicator of recent intake (13). This is taken advantage of when evaluating vitamin B12 absorption with the CobaSorb test, by measures the holoTC response after an oral cyanovitamin B12 dose (51). Circulating concentrations also increase with increasing habitual vitamin B12 intakes and stabilizes at intakes > 4 µg (13, 52). Diagnostic cut-offs based on the relation between holoTC and serum MMA concentration, suggest that holoTC concentrations <35-40 pmol/L reflect

deficiency in adults(13). While the other biomarkers change during pregnancy, holoTC has been reported to be unaffected(13).

Serum methylmalonic acid

Plasma MMA also accumulates with insufficient intracellular vitamin B12 status, due to reduced activity of methylmalonyl-CoA mutase. As this reaction is not directly influenced by other micronutrients, MMA has for a long time been considered the most specific and informative of the vitamin B12 biomarkers. However, MMA concentrations are influenced by several other factors. Higher concentrations are seen with impaired renal function, and with increasing age, especially after 70 years (47). The latter is not entirely explained by age-related decline in kidney function. Further, two genetic variants have been reported to explain 12% of the variance in circulating MMA (53). In a meta-analysis, a 7% decrease (95% CI 4%-10%) in MMA was reported for each doubling in vitamin B12 intake (14).
In adults with normal kidney functions, cutoffs in the range >0.376 - >0.271 µmol/L has been suggested as indicating elevated MMA, suggesting vitamin B12 insufficiency. In order to rule out impaired renal function as the cause of elevated MMA, renal function should be evaluated (13) (Allen 2018).

MMA, has been reported to increase throughout pregnancy indicating vitamin B12 insufficiency (8, 54). However, compared to non-pregnant women lower concentrations have been reported in pregnant women (8). In infants and toddlers the MMA concentrations are higher across the entire vitamin B12 spectrum compared to older children and adults (42) and age-specific decision limits must be used for all children.

Plasma total homocysteine

Plasma tHcy accumulates with insufficient intracellular vitamin B12 status, due to reduced function of methionine synthase, referred to as hyperhomocysteinemia. Plasma tHcy concentrations is not a specific vitamin B12 status marker, as it is also increases with insufficient intakes of folate, and to a lesser degree with low intake of vitamin B6.
Plasma tHcy concentrations increase with age and decreased renal function and are influenced by sex and pregnancy, so it is necessary to use age specific decision limits (2). The most common decision limit to define hyperhomocysteinemia is >15 µmol/L in adults.
Plasma tHcy is the preferable metabolic marker for vitamin B12 status in infants and toddlers, whereas in older children and adults, tHcy is primarily a folate marker and MMA is the preferable vitamin B12 marker(2). In infants and toddlers up to 3 years, a plasma tHcy concentration > 6.5 µmol/L is indicating vitamin B12 deficiency (43, 55, 56).

6. Dietary intake in Nordic and Baltic countries.

With a few exceptions, only animal foods contain cobalamin. Meat, liver, dairy products, fish, and shellfish, are particularly good sources and are the main vitamin B12 sources in the average diet.
The average vitamin B12 intake in the Nordic countries, according to national dietary surveys ranged from 4.0-6.4 and 5.5-8.9 µg per day in adult women and men, respectively (see chapter on dietary intake in Nordic countries).

Vegans/Vegetarians

Vegetarian diets, especially vegan diets, tend to contain low or no amounts of vitamin B12 and these diets are associated with an increased risk of vitamin B12 deficiency (22, 57-60). Plant foods might contain trace amounts due to bacterial or soil contamination or as a result of fermentation. Green and purple lavers (Nori) may contain some vitamin B12 and can be used by humans, whereas blue-green algae or cyanobacteria (Spirulina) contain biologically inactive B12 analogues (2, 61). Some plant-based milk substitutes might be fortified with vitamin B12 and might be an important source of vitamin B12 in vegans.

Pregnant women

In pregnancy week 18 women from the Norwegian Mother, Father and Child Cohort Study (MoBa) had a median vitamin B12 intake of 8.5 µg/day from diet and supplements (10).

Vitamin B12 in breast milk and formula and intake in infants

In breast milk from Danish mothers, most of them taking daily multivitamin supplements containing 1.0–4.5 µg cobalamin, the median vitamin B12 concentration at two weeks was 0.76 (0.21–1.88) nmol/L, at four months 0.29 (0.14–0.69) nmol/L and at nine months 0.44 (0.16–1.94) nmol/L (44). In breast milk from Norwegian mothers, users of supplements versus nonusers, median (IQR) vitamin B12 concentration was at 6 weeks 0.34 (0.23, 0.46) versus 0.29 (0.16, 0.36) nmol/L, at 4 months 0.31 (0.21, 0.40) versus 0.27 (0.18, 0.48) nmol/L and at 6 months 0.35 (0.22, 0.54) versus 0.24, (0.19, 0.47) nmol/L.
Mean vitamin B12 content in 11 different formula milk was 2.2 µg/L in a Danish study published in 2016(62). Table 1 shows the estimated vitamin B12 intake in infants from breast milk from these Danish and Norwegian mothers and from formula milk. Based on these data, formula fed infants receive from 2.1 (at 2 weeks) up to 5.2 (at 4 months) times more vitamin B12 per day compared to breast fed infants.

Table 1. Median (IQR) vitamin B12 intake from breast milk versus formula milk in infants

	2 weeks	**6 weeks**	**4 months**	**6 months**	**9 months**
Mean weight, kg	3.7	4.8	6.7	7.6	8.6
Mean milk intake/day	150 ml/kg	130 ml/kg	120 ml/kg	120 ml/kg	100 ml/kg

Median infant vitamin B12 intake from:						
Danish mothers, +Suppl.(44)		0.57 µg/day		0.31 µg/day		0.51 µg/day
Norwegian mothers(8)	+Suppl.		0.29 µg/day	0.34 µg/day	0.43 µg/day	
	-Suppl.		0.24 µg/day	0.29 µg/day	0.30 µg/day	
Formula(62)		1.22 µg/day	1.37 µg/day	1.77 µg/day	2.00 µg/day	1.89 µg/day

Despite a higher milk intake with increasing age, daily vitamin B12 intake is reduced from 2 weeks to 6 months in exclusively breast fed infants (8, 44, 63, 64).

In a Norwegian study, the estimated vitamin B12 intake at age 12 months was median 1.7 (IQR 1.1, 2.2) µg per day from solid food and breastmilk versus 2.3 (1.8, 3.0) µg per day from solid food and formula (64).

Infants born premature or with low birth weight (LBW)

Infant born premature or with a low birthweight have smaller vitamin B12 stores and an increased risk of deficiency during the first months or year of life(65). However, as many premature and LBW infants have problems breastfeeding, this group is often mainly formula fed(7).

Elderly

With increasing age, the production of both hydrochloric acid and intrinsic factor declines, putting elderly at increased risk of developing vitamin B12 deficiency or insufficiency (2), and it has been estimated that 10-30% of elderly have some form of vitamin B12 malabsorption due to atrophic gastritis which is often undiagnosed. Dietary surveys in the Nordic countries suggest that vitamin B12 intake in the elderly is slightly higher compared to younger adults, with estimated intakes ranging from 5.2-6.6 and 5.2-10.8 µg/day in women and men, respectively.

7. Health outcomes relevant for Nordic and Baltic countries.

Deficiency

An adequate supply of vitamin B12 is essential for normal development, neurological function and blood formation(2). B12 deficiency associated with classic haematological manifestations is relatively uncommon (66). Neurological manifestations, such as numbness and paresthesias in the extremities, loss of position and vibratory sensation, difficulty walking, depression and irritability, diminished cognitive function and psychosis may be observed even in the absence of hematologic disease, which may be difficult to diagnose(66, 67). Subclinical deficiency detected by metabolic dysfunction biomarkers such as high homocysteine and MMA biomarkers, which are predictors of developmental and degenerative diseases, is

much more common in the general population(49). The observed biochemical insufficiency at B12 levels below 550 pmol/L raises the question whether such vitamin B12 insufficiency has clinical consequences(48). A review has shown a high prevalence of vitamin B12 insufficiency in the world and has identified several examples of adverse clinical outcomes, mainly in relation to functions of the nervous system (68). Others have suggested that vitamin B12 deficiency/insufficiency is related to increased pro-oxidant and reduced anti-oxidant status(31). There is currently no clear agreement on what symptoms constitute clinical vitamin B12 deficiency and what decision limits for vitamin B12 status one should use(2), but one guiding principle is that if the symptoms are alleviated by treatment with cobalamin, then it is likely that there was a degree of vitamin B12 insufficiency.

Vitamin B12 deficiency during pregnancy and infancy

Vitamin B12 deficiency in women of fertile age is associated with infertility, early pregnancy loss(69), increased prevalence of preeclampsia, preterm birth (70)(ref) possibly low birth weight (26, 28)and a moderate risk of neural tube defects(71). A review published including 23 studies from 1961 to 2017 reported a positive effects of vitamin B12 on semen quality, including increasing sperm count, enhanced sperm motility and reduced sperm DNA damage(72).

Maternal vitamin B12 deficiency during pregnancy is associated with vitamin B12 deficiency in the infant(8). Symptoms of vitamin B12 deficiency in children differ with age, presenting a continuum from subtle developmental delay to life-threatening clinical conditions. The first 4 months of life is the most rapid period of brain development, with a brain growth ranging starting from 1% per day and gradually decreasing to 0.4% at 3 months(73). An optimal micronutrient status is particularly important during this period. In infants symptoms of vitamin B12 deficiency include irritability, failure to thrive, apathy, anorexia, gastrointestinal reflux, constipation and developmental regression(5, 6), all of which respond remarkably rapidly to supplementation(6). Symptoms of vitamin B12 deficiency are difficult to detect in all age groups, but particularly in infants, and there tends to be a diagnostic delay of several months in this age group(5, 39). Several reports show that even moderate deficiency in children may be harmful, and long-term consequences of neurological deterioration may persist after vitamin B12 deficiency has been treated(39).

Toxicity

As long as vitamin B12 is taken orally, either in food or in supplements containing a moderate vitamin B12 content, the body will actively regulate the uptake in ileum, and excess vitamin B12 will be excreted in urine. There are no known adverse effects of excess vitamin B12(12). Potential negative effects of megadoses, i.e. on the microbiome, are largely unknown.

Obesity

The association between circulating vitamin B12 concentrations and body mass index (BMI) was investigated in a systematic review of 19 observational studies including 7055 participants (32). Overall it

was reported that those with BMI categorized as overweight or obese had 21-56 pmol/L lower observations compared to those with normal weight. In meta-regression analyses, no inverse or J-shaped association was established, but the authors concluded that the results from the direct pairwise comparisons supported further investigation on the association between vitamin B12 status and overweight/obesity. Lower vitamin B12 levels with higher BMI are also reported in pregnant women (74).

CVD and diabetes type 2

In a Cochrane review of 15 RCTs, Marti-Carvajal et al reported on the effect of homocysteine-lowering intervention with B-vitamin supplements (Vitamin B6, folate, and vitamin B12 alone or in combination) on cardiovascular disease outcomes and mortality (19). Compared to placebo, they reported reduced risk of stroke (RR [95% CI] 0.90 [0.82-0.99]), but no effect on myocardial infarction risk (1.02 [0.95-1.10]) or all-cause mortality (1.01 [0.96-1.06]). Rafnsson et al performed a systematic review of 7 cohort studies, and found only very limited evidence that low serum vitamin B12 was associated with the risk of mortality and morbidity from either cardiovascular diseases or diabetes type 2 in adults (25).

Cancer

Observational studies find an association between higher circulating ~~cobalamin~~ vitamin B12 concentrations and increased risk of prostate (75), and total cancers (76), and a high serum ~~cobalamin~~ vitamin B12 has been proposed as a cancer biomarker (77). The elevated vitamin B12 levels appear to be due to increased haptocorrin concentrations and not to raised holoTC (75, 78). Furthermore, clinical trials do not support an association between higher vitamin B12 intakes and cancer risk (79-81). A meta-analysis of 18 randomized clinical trials including approximately 75,000 individuals, found that supplements containing B vitamins, including 20 to 2,000 µg/dayvitamin B12, had little or no effect on cancer incidence, cancer deaths, or all-cause mortality (80). A dose-response meta-analysis including data from 10 600 patients suggested an inverse association between both total and dietary vitamin B12 intakes and colorectal cancer risk(82). This was supported in a meta-analysis of 13 case-control studies reporting a inverse association between circulating vitamin B12 and colorectal cancer risk (not statistically significant), and a positive association with circulating tHcy(29). However, a meta-analysis of 10 observational studies with 3164 cases observed a higher risk of esophageal cancer when comparing the highest vs lowest intake (OR [95% CI] 1.30 [1.05-1.62]), and a linear dose-response per 1 µg/d increment (1.02 [1.00-1.03]) (24).

Osteoporosis and bone health

The association of vitamin B12 status with bone mineral density and osteoporotic fractures was investigated in a systematic review by Macedo et al, including 6 longitudinal studies, 9 cross-sectional studies and two RCTs(18). The authors concluded that the potential effect of vitamin B12 deficiency on bone health, and the underlying mechanisms, warrants further research, especially in vulnerable groups such as postmenopeusal and elderly women.

Mental health

Vitamin B12 deficiency in adults may result in neurological and psychiatric symptoms and/or macrocytic and megaloblastic anemia (2, 66, 83). Lindenbaum et al reported vitamin B12 deficient patients with psychiatric symptoms, but without anemia or macrocytosis (66). The reason why some patients mainly present with megaloblastic anemia and others with neurologic symptoms remains unknown.

Data on vitamin B12 status and associated cognitive function and depression in elderly people are conflicting. A Cochrane Review in 2003 found that the evidence for any effect of vitamin B12 on improving the cognitive function of people with dementia and low serum vitamin B12 levels was insufficient (84). The same conclusion was reached in a systematic review and meta-analysis of 19 RCTs (15), concluding that supplementation of cobalamin, vitamin B6 or folic acid alone, or in combination, did not appear to improve cognitive functions in inficiduals with or without cognitive impairment at baseline. The same authors performed an updated systematic review of 31 RCTs, and in a meta-analysis of 12 RCTs using the Mini Mental State Examination, they observed no meaningful effect in individuals with, and no effect in individuals without, cognitive impairment at baseline(30).Accordingly, there is limited evidence from observational studies to suggest an association of vitamin B12 status with cognitive decline or dementia in elderly people (20, 85). In a systematic review of observational studies, the authors concluded that there is insufficient evidence to establish an overall association, but it was highlighted that all fourstudies that used more specific biomarkers of vitamin B12 status (MMA and holoTC) showed consistent associations between poor vitamin B12 status and increased risk of cognitive decline or dementia diagnosis (21). Another systematic review identified poor vitamin B12 status as being associated with increased risk of vascular dementia, primarily based on cross-sectional studies (23). Treatment with a combination of folic acid and vitamin B12 has also shown cognitive benefits in people with mild cognitive impairment (86, 87).

Vitamin B12 insufficiency has been found to increase the risk of depression in the elderly (88), and a recent review concluded that early vitamin B12 supplementation can delay the onset of depression and improve the effect of anti-depressants(89). On the other hand, a systematic review of 11 randomized controlled trials concluded that short-term treatment with folic acid and/or vitamin B12 did not improve depressive symptoms. However, the results suggested that longer-term treatment may decrease the risk of relapse or onset of symptoms in those at increased risk (90). A systematic review pooling data from 10 observational studies reported consistently lower circulating vitamin B12 concentrations in patients with Parkinson's Disease (overall standardized mean difference [95% CI] -0.38 [-0.51, -0.25])(27). However, no association was observed for dietary vitamin B12 intake and Parkinson's disease risk.

Eye disease

In a systematic review and meta-analysis of 11 case-control studies, including 1072 cases, Huang et al reported that tHcy was higher (mean difference [95% CI] 2.67 [1.6-3.74] µmol/L), and serum vitamin B12 was lower (64.2 [19.3-109] pmol/L) in cases compared to controls(16). Together, the results demonstrated

that AMD is associated with elevated tHcy levels and decreased vitamin B12 levels, Another systematic review and meta-analysis by Li et al included 9 studies reporting on the serum vitamin B12 levels and different types of glaucoma (17). In random-effect meta-analyses, they reported no statistically significant differences in serum vitamin B12 between cases and controls.

8. Requirement and recommended intakes.

The recommended intake of vitamin B12 published in NNR 2009 and 2012 was based on a study published in 1958, where 20 patients with pernicious anaemia were treated with an intramuscular dose of 0.5–2.0 µg vitamin B12 per day and the outcome parameter was normalizing of haematological status(91). An average physiological requirement of vitamin B12 was set at 0.7 µg/day, based on this and other studies (36, 92). With correction for absorption efficiency (50 %), the Average Requirement (AR) was set at 1.4 µg/day for adults. By assuming a CV of 15 % and adding two SD to allow for individual variation, the recommended intake (RI) for adults was set at 2 µg/day.

The same recommendations applied to elderly and pregnant women, while an additional 0.6 µg/day was recommended to lactating women. The recommendations for children were based on an assumed requirement of 0.05µg/kg body weight/day, and the RI's were set at 0.8 (2-5y), 1.3 (6-9y), and 2.0 (10-13y) µg/day, respectively. The NNR2012 RI was unchanged from the previous recommendations in NNR2004.

The NNR2012 DRV's are comparable to most other vitamin B12 intake recommendations by authoritative scientific bodies worldwide (13) , with one notable exception. In 2015 the EFSA Panel concluded that it is impossible to determine an AR and RI, and therefore set an Adequate Intake (AI) for vitamin B12 at 4 µg/day for men and women > 14y, based on available data on different vitamin B12 biomarkers (93). An additional 0.5µg/d was added for pregnancy (AI 4.5µg/day), and 1µg/day was added during lactation (AI 5.0 µg/day) based on fetal accumulation of vitamin B12 and excretion through breast milk. For children, the AI's were extrapolated from adult values and was set at 1.5µg/day from 7 months of age.

The de novo NNR2022 systematic review conducted concluded that for all included populations (pregnancy, lactation, infants, elderly, and vegetarians and vegans) there was insufficient evidence to assess whether habitual intakes in line with the previously recommended intakes (NNR 2012) was sufficient to maintain adequate status (Bärebring et al, not yet published). No eligible studies were identified for young adults or children other than breastfed infants.

Adults

Daily vitamin B12 losses in apparently healthy adults and elderly probably range from 1.4 to 5.1 µg. A systematic review estimated that the vitamin B12 intakes needed to compensate for these losses ranged from 3.8 to 20.7 µg (11). However, these estimates should be interpreted with

caution, as the rate-limited absorption from a single dose was not considered. To better estimate the dose needed, both the bioavailability and the division of the dose throughout the day must be considered. Based on biomarker concentrations, it has been reported that in healthy young adults and postmenopausal women, circulating concentrations of cobalamin, homocysteine, and MMA appear to plateau at intakes of 4-10 µg/day(52, 94, 95). This suggests that previous recommended intakes may be insufficient to maintain optimal biomarker status, although currently, no consensus exist regarding what should be considered optimal in terms of metabolic profile. Based on available data on different biomarkers of vitamin B12 status, a vitamin B12 intake in the range of 5-7 µg/day seem to be adequate for adults.

Elderly

In NNR2012, the RI for elderly was the same as for younger adults. This is in line with the IOM, who emphasize that because dietary vitamin B12 can be assumed to be poorly absorbed due to atropic gastritis, increasing the recommendations was unlikely to be sufficient.

Vegetarian and vegan diets

As vitamin B12 only occur in foods of animal origin, strict vegan diets will eventually result in vitamin B12 deficiency unless supplemented. However, lower intake and status is also observed with less restrictive diets limiting animal food (96). It should be emphasized that symptoms of deficiency may not occur until years after adopting such diets. As repleting the body stores through supplements would take a long time, initial treatment with injections is warranted once symptoms of deficiency are present.

Pregnancy and lactation

A maternal vitamin B12 concentration >275 pmol/L in week 18 of pregnancy has been recommended to ensure an adequate infant vitamin B12 status the first 6 months of life(8, 50). Additionally serum vitamin B12 concentration above 300 pmol/L in early pregnancy have been associated with lower incidence of neural tube defects (97).

In pregnancy week 18 women from the Norwegian MoBa study reported a median vitamin B12 intake of 8.5 µg/day from diet and supplements (10). Median (IQR) serum vitamin B12 concentration in 2911 women from the MoBa study was 309 (249 – 378) pmol/L(98). Based on this, a vitamin B12 intake of 8.5 µg/d can be expected to give more than 50% of the women a sufficient vitamin B12 status in pregnancy week 18.

Infants

Infants born at term with an appropriate weight for gestational age

The calculated adequate intake (AI) for infants aged 0–6 months ranges from 0.3 – 0.5 µg/day and is based on the assumption that breast milk contains sufficient vitamin B12 for optimal health during this period of life. The data for estimated AI include an average intake of breast milk (~800 ml/d) and an average vitamin B12 concentration in breast milk (~0.45 µg/L).

Only 28% of infants at 6 weeks and 34% at 4 months achieve the recommended intake of 0.4 µg per day from breast milk (8). Additionally, whether this amount of vitamin B12 is enough will also depend on the infant vitamin B12 stores, which will be low in infants born to women with vitamin B12 deficiency due to gastrointestinal disease or a diet with a low content of animal food. Formula fed infants receive from 1.2 to 2 µg vitamin B12 per day from 2 weeks to 6 months (Table 1) and are reported to have lower plasma tHcy concentrations, indicating a better vitamin B12 status compared to exclusively breast fed infants(39).

For infants aged 7–12 months, the recommended AI ranges from 0.7-1.5 µg/day. EFSA has the highest AI (1.5 µg/day) based on an extrapolation from the AI for adults using allometric scaling and applying a growth factor(93). At 12 months, infants with median vitamin B12 intake of 2.3 (25^{th}-75^{th} percentile 1.8-3.0) µg per day had a lower geometric mean plasma tHcy: 4.5 (95%CI 4.1-4.9) µmol/L, compared to infants with a lower median vitamin B12 intake (1.7 (1.1-2.2) µg per day) plasma tHcy 5.5 (5.1-5.8) µmol/L. Given that a plasma tHcy <6.5 µmol/L indicate an adequate vitamin B12 status in infants, a vitamin B12 intake of approximately 2.3 µg per day should be sufficient for most infants aged 12 months.

Infants born premature or with low birth weight (<2500 grams) (LBW)

Infants born premature or with a low birthweight have an increased risk of deficiency during the first year of life(65). A study showed that breast-fed and formula-fed LBW infants had similar plasma tHcy until 20 postnatal days, after this plasma tHcy subsequently increased in breast fed infants, but not in formula-fed infants (99). The vitamin B12 intake would be 0.17 to 0.23 µg vitamin B12 per day (based on a vitamin B12 concentration in breast milk of ~0.45 µg/L and 0.4 -0.5 L milk intake/day) in the breast fed infant. A formula fed infant would get 0.8 to 1 µg vitamin B12 per day (based on 2.2 µg/L vitamin B12 and 0.4 -0.5 L milk intake /day) during the period from 3 to 6 weeks. As plasma tHcy did not increase in the formula-fed infants, 0.8 to 1.0 µg vitamin B12 per day seems to be the necessary amount of vitamin B12 intake for LBW infants aged 3 to 6 weeks.

Norwegian infants with a birth weight between 2 to 3 kg, those who were exclusively breast-fed for >1 month had lower vitamin B12 and higher tHcy levels at 4 and 6 months compared to infants who were fed formula. Additionally, the breast fed infants had lower gross motor scores at 6 months compared to the formula-fed infants(7), indicating that even moderate vitamin B12 insufficiency may impair neurodevelopment. Given a vitamin B12 concentration in formula of 2.2 µg/L, and a milk intake of 0.120 L/kg/day; the estimated vitamin B12 intake associated with a better metabolic status and neurodevelopment would range from 1.1 µg/day at 4 months to 1.5 µg/day at 6 months.

Children

In healthy, non-breast-fed Norwegian toddlers at age 24 months a plateau in serum vitamin B12 and holoTC was reached at a vitamin B12 intake of ~ 3 µg/day(100). Neither MMA nor tHcy concentrations decreased with increasing vitamin B12 intakes, which may indicate that a vitamin B12 intake of ~ 3 µg/day at this age secures an adequate vitamin B12 status.

In a survey published in 2015, Ungkost 3 (101), vitamin B12 intake in Norwegian children aged 9 years was mean 4.9 (SD 2.2) µg/day and in children aged 13 years mean 5.3 (SD 3.7) µg/day (Ungkost 3, 2015). In a study published in 2003(102), Norwegian children aged 1-10 years had a median serum vitamin B12 of 551 (25^{th}-75^{th} percentile 456, 683) pmol/L, in age group 10.5 -15 years median serum vitamin B12 was 436 (295, 529) pmol/L and in age group 15.5-19 years median vitamin B12was 369 (294, 452) pmol/L. As a serum vitamin B12 > 300 pmol/L indicate an adequate vitamin B12 status, approximately 25% of the older children (>10.5 years) had a risk of vitamin B12 deficiency, whereas this was not an issue for the younger children.

In Canadian children aged 6 years and adolescents, a daily consumption of vitamin B12 supplements was associated with higher serum vitamin B12 concentrations, with no additional increase in serum vitamin B12 at doses above 10 µg/day (103).

Based on this, a vitamin B12 intake from 3-4.9 µg/day may be adequate for younger children, whereas children > 10 years need a vitamin B12 intake in the range of 5.3-10 µg/day.

9. Integration

Will be included later.

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