The Revised D-A-CH-Reference Values for the Intake of Vitamin B$_{12}$: Prevention of Deficiency and Beyond

Alexander Ströhle, Margrit Richter, Marcela González-Gross,
Monika Neuhäuser-Berthold, Karl-Heinz Wagner, Eva Leschik-Bonnet, and Sarah Egert for
the German Nutrition Society (DGE)*

Scope: The nutrition societies of Germany, Austria, and Switzerland are the joint editors of the "D-A-CH reference values for nutrient intake", which are revised regularly.

Methods and Results: By reviewing vitamin-B$_{12}$-related biomarker studies, the reference values for vitamin B$_{12}$ were revised in 2018. For adults, the estimated intake is based on the adequate serum concentrations of holotranscobalamin and methylmalonic acid. The estimated values for children and adolescents are extrapolated from the adult reference value by considering differences in body mass, an allometric exponent, and growth factors. For infants below 4 months of age, an estimated value is set based on the vitamin B$_{12}$ intake via breast milk. The reference values for pregnant and lactating women consider the requirements for the fetus and for loss via breast milk. The estimated values for vitamin B$_{12}$ intake for infants, children, and adolescents range from 0.5 to 4.0 µg d^{-1}. For adults, the estimated values are set at 4.0 µg d^{-1}, and for pregnant and lactating women, they are set at 4.5 and 5.5 µg d^{-1}, respectively.

Conclusion: Based on the data of several vitamin B$_{12}$ status biomarkers studies, the reference value for vitamin B$_{12}$ intake for adults is raised from 3.0 to 4.0 µg d^{-1}.

1. Introduction

The D-A-CH 'reference values for nutrient intake'[1] are jointly issued by the nutrition societies of Germany, Austria, and Switzerland (the abbreviation D-A-CH arises from the initial letters of the common country identification for the countries Germany [D],

Austria [A], and Switzerland [CH]). Reference value is a collective term for recommended intake (RI) values, estimated values, and guiding values. An RI value, according to its definition, meets the requirement of nearly any person (approximately 98%) of a defined group of metabolically healthy people. Estimated values are given when human requirements cannot be determined with desirable accuracy. Guiding values are stated in terms of aids for orientation.[1]

Reference values for nutrient intake are amounts that are assumed to

- protect nearly all healthy individuals in a population from deficiency-related conditions
- ensure optimal physiological and psychological performance, and
- create a certain body reserve.[2]

Since 2012, the D-A-CH nutrition societies have published revised reference values for the intake of several nutrients[3–11] but not yet for vitamin B$_{12}$. The last update of the reference values for vitamin B$_{12}$ given by the D-A-CH nutrition societies dates to a 2000 report.[12] Since that time, a number of new scientific data have become available and have investigated the relationship between vitamin B$_{12}$ intake and biomarkers of vitamin B$_{12}$ status.[13–15] From this background, there is a need

Dr. A. Ströhle
Institute of Food Science and Human Nutrition
Leibniz University Hannover
Am Kleinen Felde 30, 30167 Hannover, Germany
Dr. M. Richter, Dr. E. Leschik-Bonnet, Prof. S. Egert
German Nutrition Society (DGE)
Godesberger Allee 18, 53175 Bonn, Germany
E-mail: richter@dge.de

Prof. S. Egert
Institute of Nutritional Medicine
University of Hohenheim
Fruwirthstr. 12, 70599 Stuttgart, Germany
Prof. M. González-Gross
ImFINE Research Group, Department of Health and Human Performance
Universidad Politécnica de Madrid
c/ Martín Fierro 7, 28040 Madrid, Spain
Prof. M. Neuhäuser-Berthold
Institute of Nutritional Science
Justus-Liebig-University
Goethestrasse 55, 35390 Giessen, Germany
Prof. K.-H. Wagner
Department of Nutritional Sciences
University of Vienna
Althanstraße 14, 1090 Vienna, Austria

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to review and update the D-A-CH reference values. In 2018, the revised reference values for vitamin B_{12} intake were published in German. This paper provides a summary of this work.

2. Nutritional Physiology of Vitamin B_{12}

Vitamin B_{12} is the collective term for a number of substances with qualitatively equivalent biological effects. These compounds consist of a corrin ring system that is similar to porphyrin with four reduced pyrrole rings and a central cobalt ion and are therefore referred to as cobalamins. Characteristically, all cobalamins have an α-axial ligand at the cobalt ion consisting of a phosphoribosyl-5,6-dimethylbenzimidazole side chain. The β-axial position can be occupied by various substituents. Depending on the group (R), a distinction is made between cyano- (R = CN), aquo- (R = H_2O), hydroxo- (R = OH), methyl- (R = CH_3), and adenosyl- (R = 5'-deoxyadenosyl) cobalamin.[16] Cobalamins are synthesized only by microorganisms, and humans and animals receive them solely through the food chain.[17] In natural foods, vitamin B_{12} is present mainly as hydroxocobalamin and adenosylcobalamin and is found in milk as methylcobalamin.[18] Due to its stability, cyanocobalamin is used in fortified foods as well as in pharmaceuticals and dietary supplements. Some pharmaceuticals and dietary supplements also contain hydroxocobalamin and methylcobalamin.[19,20]

Humans metabolize cyanocobalamin and hydroxocobalamin into the two physiologically active coenzymes methylcobalamin and 5'-deoxyadenosylcobalamin:[17,19]

- **Methylcobalamin** is a coenzyme of cytosolic methionine synthase (EC 2.1.1.13) that catalyzes the remethylation of L-homocysteine to L-methionine. This reaction also involves folate as 5-methyltetrahydrofolate (5-MTHF), which is the actual methyl donor. Methylcobalamin that is bound to the methionine synthase is demethylated in this reaction and then again remethylated through 5-MTHF. Accordingly, methylcobalamin is not only involved in the regulation of L-homocysteine and L-methionine but also required to provide free tetrahydrofolate (THF), which is an important coenzyme for the transfer of one-carbon units, for example, in purine and pyrimidine synthesis.[21]
- **5'-Deoxyadenosylcobalamin** is a coenzyme of the mitochondrial enzyme L-methylmalonyl-CoA mutase (EC 5.4.99.2). This enzyme isomerizes L-methylmalonyl-CoA to succinyl-CoA, a central intermediate in the degradation of odd-chain fatty acids and of the amino acids methionine, threonine, and isoleucine. In a first step, propionyl-CoA is converted to D-methylmalonyl-CoA in a reaction catalyzed by the biotin-dependent propionyl-CoA carboxylase (EC 3.4.1.3). Thereafter, a racemase converts D-methylmalonyl-CoA to L-methylmalonyl-CoA.[22] Furthermore, D-Methylmalonyl-CoA, which is a product of valine and thymine degradation, is also converted after epimerization to succinyl-CoA through the L-methylmalonyl-CoA mutase reaction.

The largest vitamin B_{12} pool in the human body is located in the liver (approximately 50% of the total body pool). At a mean total body pool of 3 mg,[23] this amount corresponds to a content of approximately 1.5 mg in the liver, with 61% present as adenosylcobalamin, 38% as hydroxycobalamin and only 1% as methylcobalamin.[18] Significant quantities of vitamin B_{12} are also found in skeletal muscles (approximately 9%), bone marrow (approximately 4.5%) and bowel tissue (approximately 4.5%).[18]

In the blood, cobalamins are transported bound to the β-globulins transcobalamin (TC)I (haptocorrin) or TC II. The most physiologically important carrier protein is TC II. The complex formed by vitamin B_{12} and TC II is referred to as holo-TC II (simplified synonym holo-TC).[24] The half-life of holo-TC is 60 to 90 min[18,25]; 10–30% of the circulating vitamin B_{12} is bound to TC II.[24]

3. Criteria to Assess the Vitamin B_{12} Status

There are different biomarkers for assessing vitamin B_{12} status. These biomarkers include the two status parameters vitamin B_{12} and holo-TC in serum or plasma and the functional parameters methylmalonic acid (MMA) and homocysteine in serum.[21,26–32] However, none of these parameters are sufficient on their own to adequately assess vitamin B12 status.[29] Furthermore, until now, no generally accepted cut-off values exist for any of the biomarkers. Additionally, several groups have published different cut-off values.[28,33,34]

3.1. Total Circulating Vitamin B_{12}

A serum or plasma concentration of vitamin B_{12} between 148 pmol L^{-1} and 221 pmol L^{-1} indicates marginal supply,[28] and a concentration of <148 pmol L^{-1} indicates vitamin B_{12} deficiency.[26,28,35] A serum concentration of >221 pmol l^{-1} may be considered indicative of a sufficient vitamin B_{12} status in adults.[28,36] However, a serum concentration of >221 pmol L^{-1} may not necessarily mean that the vitamin B_{12} supply is sufficient since symptoms of vitamin B_{12} deficiency and/or a functional metabolic vitamin B_{12} deficiency may also be present.[21,31] Therefore, the total serum vitamin B_{12} concentration should be determined in combination with a functional parameter such as MMA.[29,30,37]

3.2. Circulating Holo-TC

Circulating holo-TC is responsible for supplying the tissues with vitamin B_{12}.[38] Thus, the serum holo-TC concentration reflects the supply of metabolically active cobalamins.[32,39] A holo-TC concentration between 40 and 100 pmol L^{-1},[36] 40 and 200 pmol L^{-1}[39] or >37 and <190 pmol L^{-1}[40] is considered as the target concentration for a sufficient vitamin B_{12} status in adults. A holo-TC concentration of >8.4 to <20 pmol L^{-1} is indicative of a possible vitamin B_{12} deficiency and a concentration of <8.4 pmol L^{-1} is indicative of a potential vitamin B_{12} deficiency.[40] For a more reliable assessment of vitamin B_{12} supply, holo-TC should be determined in combination with a functional parameter such as MMA.[29,30]

3.3. Circulating MMA

Serum MMA concentration is considered as functional biomarker of vitamin B_{12} status. In the case of vitamin B_{12}

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deficiency, the serum concentration of MMA increases due to a reduced vitamin B_{12}-dependent isomerization of L-methylmalonic acid to succinyl-CoA through the enzyme L-methylmalonyl-CoA mutase.[29,35,41] A MMA concentration of <210 [28] or <270 nmol L^{-1}[36] is considered the target concentration for a sufficient vitamin B_{12} supply in adults. MMA concentrations of >350 to <840 nmol L^{-1} are indicative of an insufficient vitamin B_{12} status, and MMA concentrations of >840 nmol L^{-1} are indicative for a vitamin B_{12} deficiency.[40] However, an increased MMA serum concentration may also occur due to impaired renal function. During pregnancy and at advanced age, MMA also increases independent of the vitamin B_{12} intake. Therefore, the specificity of MMA as a biomarker for vitamin B_{12} is limited.[29,35] Thus, MMA should only be used in combination with a status parameter such as holo-TC.[29,30]

3.4. Circulating Homocysteine

The remethylation of homocysteine to methionine is impaired if there is an insufficient vitamin B_{12} supply; consequently, plasma homocysteine concentration increases.[42] However, the homocysteine concentration is influenced not only by vitamin B_{12} but also by folate, vitamin B_6, and riboflavin (vitamin B_2). Insufficient supply of these vitamins can also lead to increased homocysteine concentrations.[43] The latter may also occur as a consequence of renal insufficiency, dehydration, and cystathionine β-synthase deficiency as well as certain polymorphisms of the methylenetetrahydrofolate reductase.[44] Therefore, the homocysteine concentration is a sensitive but non-specific functional marker of vitamin B_{12} status.[26] Accordingly, the homocysteine concentration is suitable to assess the vitamin B_{12} status only in combination with a vitamin B_{12} status parameter (serum holo-TC or vitamin B_{12}).[30] In general, a plasma homocysteine concentration of <12 µmol L^{-1} in adults is considered to be adequate.[45]

4. Bioavailability of Vitamin B_{12}

The bioavailability of vitamin B_{12} mainly depends on the intake level and decreases with an increasing dose.[46] The approximate absorption rates for the intake of 1, 5, 10, and 20 µg are 50%, 20%, 10%, and only 5%, respectively.[47] In addition to the dosage, the food matrix determines the absorption rate of vitamin B_{12}.[17,47] The absorption rate when consuming lamb with a vitamin B_{12} content of 2.6 µg per 100 g is 56–89%, and for fish (rainbow trout with 4.9 µg per 100 g), the absorption rate is 42%. For cow's milk (0.4 µg per 100 g), a bioavailability of 65% has been determined.[17] The absorption rate for eggs with an average vitamin B_{12} content of 1.3 µg per 100 g is <9%.[17] Based on a meta-regression analysis of eight bioavailability studies, absorption rates of 37% and 29% were determined for an intake of 1.2–3.1 µg vitamin B_{12}, respectively, per meal complying with the usual dietary habits.[46] At an average vitamin B_{12} intake of 1.2 µg per meal, the bioavailability is 37%,[46] which means that for a mixed diet, which contains vitamin B_{12} also from foods with lower bioavailability,[17] an availability of 35–40% may be assumed.

Vitamin B_{12} produced by colon bacteria in the human gut cannot be absorbed due to missing receptors.[48]

5. Derivation of the Reference Values for the Intake of Vitamin B_{12}

5.1. Adults

5.1.1. Adults under 65 Years of Age

As criteria to derive the reference value of vitamin B_{12} intake in adults, the serum concentrations of holo-TC ($\geq$ 40 pmol L^{-1}) and of the functional parameter MMA in the desirable range of <270 nmol L^{-1} are considered as the most important ones. These criteria are based on the results of two studies, in which the adequate vitamin B_{12} intake was determined using the serum concentrations of total vitamin B_{12} and holo-TC as well as the functional parameters MMA and homocysteine.[13,14] In the cross-sectional study by Bor et al.[13] with subjects aged 18 to 50 years, an average serum holo-TC concentration of 65 pmol L^{-1} was reached with a daily median intake of 4.2 µg vitamin B_{12}; higher intake levels were not associated with higher holo-TC concentrations. The serum concentration of vitamin B_{12} reached an average of 325 pmol L^{-1}. For the functional parameters MMA and homocysteine, average concentrations of 210 nmol L^{-1} and 8 µmol L^{-1}, respectively, were measured,[13] which indicate adequate vitamin B_{12} supply.[28] The intake of more than 7 µg d^{-1} during this study did not result in a further increase in the plasma vitamin B_{12} concentration. With an intake of 7 µg d^{-1}, the lowest serum concentrations of MMA and homocysteine were approximately 190 nmol L^{-1} and approximately 7 µmol L^{-1}, respectively.[13]

More data on the dose-response relationship between the vitamin B_{12} intake and the serum concentrations of MMA, homocysteine and vitamin B_{12} are available from a randomized controlled intervention study including 231 subjects.[14] In this study, the lowest MMA and homocysteine concentrations were 190 nmol L^{-1} and 8.2 µmol L^{-1}, respectively, with an intake of approximately 7 µg vitamin B_{12} d^{-1} (4 µg d^{-1} from foods plus 3.4 µg d^{-1} from a supplement). This dietary intake resulted in a serum concentration of vitamin B_{12} of approximately 330 pmol L^{-1}. At a dietary intake of 4.0 µg vitamin B_{12}, the serum concentrations of vitamin B_{12}, MMA, and homocysteine were approximately 300 pmol L^{-1}, 220 nmol L^{-1}, and 8.3 µmol L^{-1}, respectively.[14]

Based on the data from the studies by Bor et al.[13] and Pentieva et al.[14] as well as on the target concentrations for total serum vitamin B_{12} (>221 pmol L^{-1}), holo-TC ($\geq$40 pmol L^{-1}), MMA (<270 nmol L^{-1}), and homocysteine (<12 µmol L^{-1}), the estimated value for the vitamin B_{12} intake is 4.0 µg d^{-1} for men and women within the age range of 19 and 65 years (**Table 1**).

5.1.2. Adults above 65 Years of Age

In a study by Bor et al.[15] on osteoporotic women and women at risk of osteoporosis at the age of 41 to 75 years, a median holo-TC serum saturation of 119 pmol L^{-1} was achieved at a daily intake of 6 µg vitamin B_{12}. At this dose, the median serum concentration of vitamin B_{12} was 380 pmol L^{-1}. Median concentrations of MMA and homocysteine were 120 nmol L^{-1} and 9.75 µmol L^{-1},

Table 1. Estimated values for adequate intake of vitamin B_{12}.

Group	Vitamin B_{12}
Infants	$\mu g\,d^{-1}$
0 to under 4 months	0.5
4 to under 12 months	1.4
Children and adolescents	
1 to under 4 years	1.5
4 to under 7 years	2.0
7 to under 10 years	2.5
10 to under 13 years	3.5
13 to under 15 years	4.0
15 to under 19 years	4.0
Adults	
19 to under 25 years	4.0
25 to under 51 years	4.0
51 to under 65 years	4.0
65 years and older	4.0
Pregnant women	4.5
Lactating women	5.5

respectively,[15] which indicate adequate vitamin B_{12} supply.[28] However, 36% of the subjects had a gastric pH of ≥ 3 or other indications of gastric dysfunction.[15] Those dysfunctions could cause an inhibition of gastric acid and pepsinogen secretion, which results in a reduced release of free vitamin B_{12} from food proteins, hence decreasing intestinal absorption of the cobalamin protein complexes from food. Furthermore, the reduced acid secretion leads to an alkalinization of the small intestine, which may result in bacterial overgrowth and thus to a further decrease of the bioavailability of vitamin B_{12}.

A cross-sectional study in German women at the age of 60 to 70 years ($n = 178$) showed that at an average intake of 5 $\mu g\,d^{-1}$, 43% of the women had a serum cobalamin concentration of less than 258 pmol L^{-1}.[49] This concentration was defined in the study as a limit for sufficient supply. To derive the reference values for vitamin B_{12} intake, a concentration range of 148 to 221 pmol L^{-1} was defined as suboptimal, while a concentration of >221 pmol L^{-1} indicated sufficient supply. However, only 10% of the subjects in the study by Wolters et al.[49] had an MMA concentration >271 nmol L^{-1}. An MMA concentration of <270 nmol L^{-1} was considered the target concentration for a sufficient supply.

Currently, the available data are not sufficient to derive reference values for adults above 65 years of age for vitamin B_{12} intake that are different from those for younger adults. Thus, the estimated value for vitamin B_{12} intake for adults above 65 years of age does not differ from that for younger adults independent of gender and is set to 4.0 $\mu g\,d^{-1}$ (Table 1).

5.2. Children and Adolescents

No data are available regarding the vitamin B_{12} requirement for children and adolescents. Therefore, the reference values for children and adolescents are based on the values compiled for adults

and take into account the differences in body weight, an allometric exponent and the growth factors to consider the requirements for growth (**Table 2**). Growth factors at the different age groups were calculated as the proportional increase in protein requirement for growth relative to the maintenance requirement according to the WHO.[1,50] When using the age groups and reference body weights the D-A-CH reference values are based upon,[1] the resulting estimated values for vitamin B_{12} intake range from 1.5 $\mu g\,d^{-1}$ (for 1 to under 4 year olds) to 4.0 $\mu g\,d^{-1}$ (for 15 to under 19 year olds) (Table 2).

5.3. Infants

The reference values for the intake of vitamin B_{12} for infants aged 0 to under 4 months were derived based on the vitamin B_{12} content of breast milk, which is considered to be the optimal diet for infants.[51,52] The cobalamin content of breast milk varies depending on the duration of breastfeeding[53] and maternal vitamin B_{12} supply.[54] It declines continuously during the lactation period.[55] In a Danish study, in which the majority of the women had taken a multivitamin supplement (1.0–4.5 μg vitamin B_{12} d^{-1}), the vitamin B_{12} content of the breast milk was 0.1 μg per 100 mL two weeks postpartum and 0.04 μg per 100 mL four months postpartum[53] with a median vitamin B_{12} content in breast milk in the first four months of 0.07 μg per 100 mL.[53] Assuming that the median vitamin B_{12} content of breast milk during the first four months is 0.07 μg per 100 mL, an exclusively breastfed infant receives 0.53 $\mu g\,d^{-1}$ vitamin B_{12} from breast milk of mothers with adequate vitamin B_{12} supply at an average breast milk intake of 750 mL d^{-1}.[56] Therefore, the estimated value for the adequate intake of vitamin B_{12} for breastfed infants aged 0 to under 4 months is set at 0.5 $\mu g\,d^{-1}$ (**Table 3**).

The consumption of breast milk declines with the introduction of solid foods. Since no data are available from Germany with regard to vitamin B_{12} intake via solid foods, the estimated value for infants aged 4 to under 12 months is based on the values compiled for adults and considers differences in the body weight, an allometric exponent and a growth factor to consider the requirements for growth. An estimated value of 1.4 $\mu g\,d^{-1}$ vitamin B_{12} for infants aged 4 to under 12 months was derived (Table 2).

5.4. Pregnancy

The requirement of pregnant women is slightly increased to meet the vitamin B_{12} requirement of the fetus. It was estimated that the fetus accumulates 0.1 to 0.2 μg vitamin B_{12} per day.[57] Considering an absorption rate of 35–40%, the estimated value for vitamin B_{12} intake for pregnant women is therefore set to 4.5 $\mu g\,d^{-1}$ (Table 1).

5.5. Lactation

In comparison to the vitamin B_{12} requirement of non-lactating women, the requirement of lactating women is increased due to vitamin B_{12} secretion from breast milk. Approximately 0.5 $\mu g\,d^{-1}$

Table 2. Estimated values for vitamin B_{12} intake for infants (4 to under 12 months), children and adolescents considering differences in body weight, allometric exponent, and growth factors.

Age [years]	Gender	Reference body weight [kg][a) [1]]	Growth factor[b) [1]]	Vitamin B_{12} intake considering reference body weight, allometric exponent and growths factor[c)] [μg d^{-1}]	Estimated value for vitamin B_{12} intake (rounded) [μg d^{-1}]
4 to under 12 months	m	8.6	0.70	1.40	**1.4**
	f	7.9	0.70	1.49	
1 to under 4	m	13.9	0.25	1.47	**1.5**
	f	13.2	0.25	1.60	
4 to under 7	m	20.2	0.06	1.66	**2.0**
	f	20.1	0.06	1.87	
7 to under 10	m	29.3	0.13	2.34	**2.5**
	f	28.7	0.13	2.60	
10 to under 13	m	41.0	0.13	3.01	**3.5**
	f	42.1	0.11	3.41	
13 to under 15	m	55.5	0.10	3.66	**4.0**
	f	54.0	0.07	3.95	
15 to under 19	m	69.2	0.07	4.20	**4.0**
	f	59.5	0.02	4.07	

a) The reference values for body weight correspond to the median body weight determined in the *German Health Interview and Examination Survey for Children and Adolescents in Germany* (KiGGS; 2003–2006).[126] In each case, the values reflect the midpoint of the respective age range; b) Growth factors at different ages were calculated as the proportional increase in protein requirement for growth relative to the maintenance requirement according to WHO[1,50]; c) Calculated from: estimated value$_{adults}$ x (reference body weight$_{infants/children/adolescents}$/reference body weight$_{adults}$)$^{0.75}$ x (1 + growth factor); Estimated value$_{adults}$: 4.0 μg d^{-1} (Table 1). Reference body weight$_{adults}$ (age group 25 to under 51 years): men 70.7 kg, women 60.0 kg.[1]; Example: Estimated value$_{Girls, 1 to under 4 years}$ = 4.0 μg d^{-1} × (13.2 kg per 60.0 kg)^{0,75} × (1 + 0.25) = 1.60.

Table 3. Calculation of the estimated value for vitamin B_{12} for breastfed infants aged 0 to under 4 months.

Age [month]	Vitamin B_{12} content of breast milk[a)] [μg per 100 mL]	Breast milk intake[b)] [mL d^{-1}]	Vitamin B_{12} intake given a breast milk intake of 750 mL d^{-1} [mg d^{-1}]	Estimated value for vitamin B_{12} intake (rounded) [mg d^{-1}]
0 to under 4 months	0.07	750	0.53	**0.5**

a) [53]; b) [56].

vitamin B_{12} is secreted with breast milk (see 5.3 Infants). Therefore, considering an absorption rate of 35–40%, lactating women require an additional 1.5 μg d^{-1}. Thus, the estimated value for vitamin B_{12} intake during lactation is 5.5 μg d^{-1} (Table 1).

6. Preventive Aspects

In the following paragraphs, currently available data on vitamin B_{12} are outlined in association with some health-related aspects. Dietary reference values are aimed at healthy individuals; thus, the requirements of patients are not addressed.

6.1. Vitamin B_{12} and Cardiovascular Diseases

Since metabolic vitamin B_{12} deficiency impairs the remethylation of homocysteine to methionine, serum homocysteine concentration increases.[42] It is well known that homocysteine is an independent risk factor for ischaemic heart disease and ischaemic stroke.[58–60] By increasing the homocysteine concentration by 5 μmol L^{-1}, the risk of coronary events increases by 18%.[60] With an increase of 3 μmol L^{-1}, the risk of stroke increases by 19%.[59]

However, it is not clear, whether this observation reflects a causal relationship.[61] Nevertheless, genetic association studies,[62–64] in vitro studies and animal studies indicate a causal link.[45,65] In contrast, the results from clinical intervention studies with vitamin B_{12}, vitamin B_6 and folate are contradictory. On the one hand, supplementation with B vitamins has the potential to reduce the relative risk of stroke by approximately 10%,[66,67] but vitamin B_{12} alone does not seem to exert a preventive effect.[67] Additionally, vitamin B_{12} supplementation was not associated with a reduced risk of coronary events and cardiovascular diseases.[66,68]

6.2. Vitamin B_{12} and Cancer

Vitamin B_{12} is essential for DNA methylation, genome integrity, and chromosomal stability, which suggests a preventive effect of vitamin B_{12} regarding the development of cancer.[69] For breast cancer, a meta-analysis of 18 observational studies showed an inverse association with the risk of disease and vitamin B_{12} intake but not for serum vitamin B_{12} concentration.[70] Furthermore, no association was observed between dietary vitamin B_{12} intake[71] or vitamin B_{12} status[72] and the risk of endometrial cancer and colorectal carcinoma.[73–77] Another meta-analysis investigated the

association between vitamin B_{12} serum concentration and the risk of renal cell cancer using data from seven cohorts. Compared with the group with the lowest intake, subjects with the highest intake had a relative renal cell cancer risk of 0.72 (95% CI = 0.52–1.00).[78]

On the other hand, it is discussed that increased serum vitamin B_{12} concentration is associated with a higher risk of cancer. For example, in a meta-analysis of observational studies, there was a positive association between vitamin B_{12} serum concentration and the risk of prostate cancer.[79] In a cohort study, an increased serum vitamin B_{12} concentration (>600 pmol L^{-1}) was associated with an elevated total risk of cancer incidence. The risk was highest within the first year of follow-up.[80] However, this association might be due to the increased intake of animal-based foods, which is associated with high vitamin B_{12} intake ("spurious correlation"). Another cause for this association may be cancer types that are already present but not yet diagnosed, as some cancer types are associated with higher serum vitamin B_{12} concentrations.[81]

6.3. Vitamin B_{12}, Miscarriages, and Neural Tube Defects

In observational studies, an insufficient supply of vitamin B_{12} during pregnancy is associated with an increased risk of miscarriage.[82,83] Furthermore, maternal vitamin B_{12} status is associated with the occurrence of neural tube defects in the child.[84,85] However, it remains unclear whether this observation reflects a causal relationship.[86]

6.4. Vitamin B_{12} and Bone-Related Diseases

Vitamin B_{12} deficiency and hyperhomocysteinaemia are linked with the stimulation of osteoclast activity and accelerated degradation of the bone matrix.[87–90] The data from observational studies on the association between vitamin B_{12} status or vitamin B_{12} intake and bone mineral density, the risk of fracture or bone turnover markers are inconsistent.[88] In a meta-analysis of four prospective studies, for each increase of total serum vitamin B_{12} concentration by 50 pmol L^{-1}, a 4% reduced fracture risk was calculated (95% CI = 0.92–1.00).[91] A meta-analysis of four randomized controlled intervention studies indicates that the effect of vitamin B_{12} on the risk of fracture depends on the basal homocysteine concentration. A protective effect of vitamin B_{12} supplementation may be expected only at homocysteine concentrations >15 μmol L^{-1} (75% risk reduction; 95% CI = 0.12–0.53). There is no evidence that vitamin B_{12} supplementation in excess of the individual requirement for subjects with adequate supply of folate and vitamin B_{12} at homocysteine concentrations <15 μmol L^{-1} results in a further fracture risk reduction.[92]

6.5. Vitamin B_{12}, Neurodegenerative Diseases, and Cognitive Impairment

There is also a discussion about the question of whether functional vitamin B_{12} deficiency, which is characterized by decreased concentrations of holo-TC and increased concentrations of MMA and/or homocysteine, is associated with an increased risk of neurodegenerative diseases and cognitive impairment.[93,94] Several epidemiological studies showed a positive correlation regarding MMA concentrations and an inverse association regarding holo-TC concentrations with the development or progression of dementia, while there is only a low or no association between total vitamin B_{12} concentrations and disease risk.[72,74,75,93,94] In older people, vitamin B_{12} deficiency is also associated with cerebral atrophy.[95,96] Vitamin B_{12} supplementation (0.5 mg d^{-1}) in combination with folate (0.8 mg d^{-1}) and vitamin B_6 (20 mg d^{-1}) in older people could slow cerebral atrophy over two years. This effect is, however, dependent on baseline homocysteine concentration.[97]

The concentrations of holo-TC and MMA have proved to be good predictors of cognitive performance, while total vitamin B_{12} concentration has not been shown to be a good predictor, as indicated by a longitudinal cohort study including 1648 senior women and men (≥65 years) over a period of 10 years. An increase of the holo-TC concentration from 50 to 100 pmol L^{-1} is associated with a 30% lower reduction of cognitive performance; the increase in MMA and homocysteine concentrations from 0.25 to 0.5 μmol L^{-1} and from 10 to 20 μmol L^{-1}, respectively, is associated with an impairment of cognitive performance by >50%.[98]

The data from intervention studies on the influence of vitamin B_{12} supplementation on cognitive performance are contradictory; the effects seem to depend on the basal homocysteine concentration and duration of the intervention. A systematic review of 25 randomized controlled studies and cohort studies concluded that there is insufficient evidence regarding an association between vitamin B_{12} intake or vitamin B_{12} status, respectively, and cognitive performance.[94] Another systematic review of ten observational studies including subjects over 50 years had shown similar results.[99]

7. Discussion and Conclusion

7.1. Vitamin B_{12} Reference Values—What has Changed?

The reference values for vitamin B_{12} intake from international expert panels vary considerably.[57,100–102] This variance of recommendations results from the fact that the scientific basis allows a certain variation (see **Table 4**). Therefore, they have been the subject of intense controversy. Since the publication of the first D-A-CH reference values for the intake of vitamin B_{12}, several vitamin B_{12} biomarker studies have questioned whether the current reference value of 3.0 μg vitamin B_{12} d^{-1} is adequate for optimal vitamin B_{12} status in healthy adults.[13–15] From this background, D-A-CH nutrition societies have reviewed and evaluated vitamin B_{12}-related biomarker studies and revised the reference values for vitamin B_{12} in 2018.

Developing new reference values for vitamin B_{12} intake requires the definition of the best target biomarkers for a supply, corresponding to adequacy of metabolic functions. For adults, the reference value for vitamin B_{12} intake is based on biomarkers of vitamin B_{12} status, notably adequate serum concentrations of holo-TC and MMA. As a result, the revised vitamin B_{12}

Table 4. Reference values for the intake of vitamin B_{12} from different nutrition societies.

	World Health Organisation (WHO), Food and Agricultural Organisation of the United Nations (FAO)[57]	European Food Safety Authority (EFSA)[102]	Institute of Medicine (IoM)[100]	Nordic Council of Ministers[101]

Infants	Infants 0 to 12 months
	• RNI vitamin B_{12}
	  0 to 6 months: 0.4 µg d^{-1}
	  7 to 12 months: 0.7 µg d^{-1}
	• Based on the concentrations of vitamin B_{12} in human milk
		Infants 0 to under 6 months[127]
		• AI vitamin B_{12}= 0.4 µg d^{-1}
		• Based on the vitamin B_{12} intake in exclusively breast-fed infants from mothers with adequate B_{12} status
		Infants 7 to under 11 months
		• AI vitamin B_{12}= 1.5 µg d^{-1}
		• Based on upwards extrapolation from the vitamin B_{12} intake in exclusively breast-fed infants and down-scaling from the AI for adults
			Infants aged 0 to 6 months
			• AI vitamin B_{12}= 0.4 µg d^{-1}
			• Based on the vitamin B_{12} intake in exclusively breast-fed infants from mothers with adequate B_{12} status
			Infants aged 7 to 12 months
			• AI vitamin B_{12}= 0.5 µg d^{-1}
			• Based on upwards extrapolation from the vitamin B_{12} intake in exclusively breast-fed infants and down-scaling from the AI for adults
				Infants 0 to 23 months
				• RI Vitamin B_{12}
				  < 6 months: -
				  6 to 11 months: 0.5 µg d^{-1}
				  12 to 23 months: 0.6 µg d^{-1}
				• No details about derivation and database

Children and adolescents	Children and adolescents 1 to 18 years
	• RNI vitamin B_{12}
	  1 to 3 years: 0.9 µg d^{-1}
	  4 to 6 years: 1.2 µg d^{-1}
	  7 to 9 years: 1.8 µg d^{-1}
	  10 to 18 years: 2.4 µg d^{-1}
	• Based on the suggestion of the same intakes for adolescents as those for adults with progressive reduction of intake for younger groups
		Children and adolescents 1 to 17 years
		• AI vitamin B_{12}
		  1 to 3 years: 1.5 µg d^{-1}
		  4 to 6 years: 1.5 µg d^{-1}
		  7 to 10 years: 2.5 µg d^{-1}
		  11 to 14 years: 3.5 µg d^{-1}
		  15 to 17 years: 4 µg d^{-1}
		• Based on downwards extrapolation from the vitamin B_{12}-values for adults
			Children and adolescents 1 to 18 years
			• RDA vitamin B_{12}
			  1 to 3 years: 0.9 µg d^{-1}
			  4 to 8 years: 1.2 µg d^{-1}
			  9 to 13 years: 1.8 µg d^{-1}
			  14 to 18 years: 2.4 µg d^{-1}
			• Based on downwards extrapolation from the vitamin B_{12}-values for adults
				Children and adolescents 2 to 17 years
				• RI vitamin B_{12}
				  2 to 5 years: 0.8 µg d^{-1}
				  6 to 9 years: 1.3 µg d^{-1}
				  10 to 13 years: 2.0 µg d^{-1}
				  14 to 17 years: 2.0 µg d^{-1}
				• Based on 0.05 µg vitamin B_{12} per kg body weight

Adults	Adults ≥ 19 years • RNI vitamin B_{12}= 2.4 µg d^{-1} • Based on the amount of vitamin B_{12} to maintain the body's store	Adults ≥ 18 years • AI vitamin B_{12}= 4.0 µg d^{-1} • Based on an adequate biomarker status of vitamin B_{12} in healthy people	Adults ≥ 19 years • RDA vitamin B_{12}= 2.4 µg d^{-1} • Based on the determination of the amount of vitamin B_{12} needed for maintenance of an adequate erythropoiesis	Adults ≥ 18 years • RI vitamin B_{12}= 2.0 µg d^{-1} • Based on the determination of the amount of vitamin B_{12} needed for maintenance of an adequate erythropoiesis
Pregnant women	• RNI vitamin B_{12}= 2.6 µg d^{-1} • Based on the vitamin B_{12} requirement for the fetus	• AI vitamin B_{12}= 4.5 µg d^{-1} • Based on the vitamin B_{12} requirement for the fetus	• RDA vitamin B_{12}= 2.6 µg d^{-1} • Based on the vitamin B_{12} requirement for the fetus	• RI vitamin B_{12}= 2.0 µg d^{-1} • Based on adequate stores to cover the additional requirement
Lactating women	• RNI vitamin B_{12}= 2.8 µg d^{-1} • Based on the vitamin B_{12} requirement for loss via breast milk	• AI vitamin B_{12}= 5.0 µg d^{-1} • Based on the vitamin B_{12} requirement for loss via breast milk	• RDA vitamin B_{12}= 2.8 µg d^{-1} • Based on the vitamin B_{12} requirement for loss via breast milk	• RI vitamin B_{12}= 2.6 µg d^{-1} • Recommendation to compensate for the loss of vitamin B_{12} in breast milk

AI: Adequate Intake; RDA: Recommended Dietary Allowance; RI: Recommended Intake; RNI: Reference Nutrient Intake.

reference value for adults was changed from a recommended intake value of 3 µg d^{-1} up to an estimated value of 4 µg d^{-1}, which is in line with the reference value of vitamin B$_{12}$ of the European Food Safety Authority (EFSA).[102]

7.2. Vitamin B$_{12}$ Reference Values—Practical Aspects

According to the analysis of the data from the National Nutrition Survey II (NVS II, 2005–2006), the median vitamin B$_{12}$ intake in Germany in women and men between the ages of 15 and 80 years is 3.8 and 5.3 µg d^{-1}, respectively.[103,104]

The intake of foods naturally rich in cobalamin as part of a mixed diet can ensure sufficient vitamin B$_{12}$ supply. To achieve sufficient vitamin B$_{12}$ intake, a regular intake of animal-based food such as milk and dairy products, fish, poultry and lean meat is recommended. It should be noted that during food processing, e.g., cooking or pasteurization, a vitamin B$_{12}$ loss up to 50% might occur.[105]

People adopting a vegan diet that excludes animal foods should be encouraged to take a dietary vitamin B$_{12}$ supplement and should have their vitamin B$_{12}$ status regularly checked by a physician.[106] Spirulina and other cyanobacteria, which are advertised as natural vitamin B$_{12}$ sources for vegans, do not contain cobalamins that are bioavailable for humans. In addition, the cobalamin analogues that they contain can even inhibit intestinal uptake of vitamin B$_{12}$ and might suppress the formation of biologically active vitamin B$_{12}$ coenzymes.[107,108] Therefore, they are unsuitable to cover the requirements of vegans.

Partly, vitamin B$_{12}$ deficiency represents a health issue for ovo-lacto vegetarians.[105,109,110] Thus, it is recommended that vegetarians should have their vitamin B$_{12}$ status checked regularly by a physician, too, and should take dietary supplements to achieve sufficient vitamin B$_{12}$ supply if necessary.[105,111–113] In particular, vegetarians with increased nutrient requirements, for example, due to pregnancy or lactation, should pay attention to sufficient vitamin B$_{12}$ intake.

In older people, the risk of vitamin B$_{12}$ deficiency is increased, mainly due to impaired absorption because of medication (e.g., proton pump inhibitors, H$_2$ blockers) or diseases (e.g., atrophic gastritis) and less due to insufficient dietary intake of vitamin B$_{12}$.[114–117] In the case of atrophic gastritis with hypochlorhydria and related malabsorption of dietary protein-bound vitamin B$_{12}$,[118,119] supplementation of vitamin B$_{12}$ is recommended to ensure adequate vitamin B$_{12}$ status.[114,116,120] In contrast to vitamin B$_{12}$ from natural foods, the absorption of crystalline vitamin B$_{12}$ from preparations is not impaired in atrophic gastritis type B-related hypochlorhydria.[116,120] However, investigations show that the use of dietary supplements or fortified foods reduces the prevalence of vitamin B$_{12}$ deficiency in older people,[96,121–123] but the risk of insufficient supply in general persists because of the dosage, which is often too low.[124,125] Thus, a regular observation of the vitamin B$_{12}$ status is also recommended for older people. For the assessment of the vitamin B$_{12}$ status, a relevant functional parameter such as MMA should be combined with a status marker such as serum vitamin B$_{12}$ or holo-TC.

Conflict of Interest

Dr. Alexander Ströhle received an honorarium from the German Nutrition Society (DGE) for developing the first draft of the dietary reference values for vitamin B$_{12}$ intake.

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