**Vitamin B12 Fortification: Methods, Technologies & Regulations**

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Editor Id **“IIPER1680036292"**

**Abstract**

Vitamin B12 is incredibly vital for human metabolism that significantly contribute to the health of neurological systems and the creation of blood cells. It is located in very low concentrations of the Pico molar level in body fluid causing anaemia and other severe conditions. Dairy products, meat, and fish are a few examples of foods high in vitamin B12. Although the B12 content of different types of milk is not high, increased milk consumption was associated with higher serum B12 levels in geriatric persons in good health, showing that milk is a useful source of B12. Deficiency of B12 is prevalent that occurs in persons of all ages that is the reason food fortification is important to combat severe conditions such as Infertility, hearing loss, Glossitis, macular degeneration, skin hyperpigmentation, bone disease and others. In this chapter, we explained the molecular structure, chemistry, physical properties, bioavailability, and deficiency of vitamin B12 followed by the strategies such as diet diversification, food fortification, challenges and vitamin B12 fortification using nanotechnology.

**Keywords:** Food, fortification, vitamin B12, deficiency, dairy products

* 1. **Introduction**

All animals and some microbes require vitamin B12, which is a well-known by-product of specific bacteria. The water-soluble vitamin B12, also known as cobalamin, is one of the necessary vitamins that significantly contribute to the health of neurological systems and the creation of blood cells (Antherjanam, 2021). Vitamin B12 is incredibly vital for human metabolism and located in very low concentrations of the Pico molar level in body fluid so, cause anaemia and also the production of huge red blood cells (K.B. Akshaya, 2020). Dairy products, meat, and fish are a few examples of foods high in vitamin B12. While the B12 content in various milk types may not be substantial, greater milk consumption correlated with elevated serum B12 levels in healthy geriatric individuals. This suggests that milk serves as a valuable source of vitamin B12 (Tomohiro et al., 2016). It can also be manufactured in a lab and is usually taken along with other B vitamins. A few examples of body parts that require vitamin B12 for proper development and operation are the brain, nerves, and blood cells. Methylocobalamin, cyanocobalamin, adenosylcobalamin, and hydroxocobalamin are the four distinct forms of cobalamin. The metabolically active forms associated with vitamin B12 are 5-deoxyadenosylcobalamin and methylcobalamin. However, after being changed into methylcobalamin or 5-deoxyadenosylcobalamin, two more forms of cyanocobalamin and hydroxycobalamin become biologically active (Zhuo Cheng, 2016).

The European Food Safety Authority recommends that adults receive 4 μg of vitamin B12 per day, and the requirements are high during lactation and pregnancy. Elderly people are also at risk for developing a deficiency in this vitamin (Obeid R, 2019). Globally, current food consumption is shifting away from fresh, unprocessed foods like fruits and vegetables but towards animal-based foods and highly processed items (Bodirsky, 2020). According to Agriculture and Rural Development at the European Commission, emerging economies like China have seen a large rise in their consumption of high-value foods like meat and dairy products. Meanwhile, trends in developed economies like those in Europe and North America reflect a shift away from the intake of red meat and toward plant-based foods like fruits and vegetables (Agriculture and Rural Development, 2019; Jones, 2020).

Since animal products are the natural source of vitamin B12 therefore, the deficiency is particularly prevalent among vegetarians. In addition to the synthesis of red blood cells and the regular operation of the nervous system, it may result in neurological, haematological, and psychiatric symptoms (Nakos, 2021). In the context of severe and ongoing vitamin B12 deficiency, neurocognitive manifestations such as dementia, cognitive impairment, depression, memory loss, delirium, and psychotic episodes are also feasible. Infertility, hearing loss, Glossitis, macular degeneration, skin hyperpigmentation, and bone disease are some additional symptoms of vitamin B12 deficiency in adult individuals (Infante, 2021). In this chapter, we begin by focusing on the physical properties, chemistry, molecular structure, bioavailability, and deficiency of vitamin B12. We then discuss strategies such as diet diversification, food fortification, challenges and vitamin B12 fortification using nanotechnology.

* + 1. ***Vitamin B12: discovery and nomenclature***

Over the course of more than a century, vitamin B12 was discovered, its role in metabolism was clarified, and the effects and remedies for its deficiency were discovered (Scott, 2012). Physicians in England had discovered the illness pernicious anaemia, a sickness that causes the body to generate few red blood cells. The illness can be fatal and makes patients feel exhausted and out of breath. A group of doctors from Harvard University determined that most patients could avoid pernicious anaemia by consuming half a pound of liver daily in 1926. As a result, scientists around the world started looking for a way to separate the liver's anemia-preventing component. Randolph West (1890–1949) of Columbia University joined with Folkers to identify participants and administer various liver extractions to them. Due to the rarity of the disease, the researchers had to work carefully and wait weeks to find any individuals with pernicious anaemia.

Mary Shorb (1907–1990) previously worked for the U.S. Department of Agriculture, had discovered a bacterium that was responsive to liver extracts. Folkers brought Shorb to Merck to expedite his research after realising that the bacteria may be used as a substitute for human test subjects. The scientists noticed that the liver extracts that had the most encouraging effects on Shorb's bacteria, which suggested that the coveted vitamin was actually a red molecule. Vitamin B12 (cobalamin) was separated in 1947 by Folkers and his team, producing tiny, vivid red crystals of the vitamin (**Figure 1**). The next year, this novel substance was examined on a patient with pernicious anaemia. Later research revealed that cobalamin is a vital component of animal growth. This insight prompted the practise of supplementing the vitamin in animal diets, which dramatically enhanced yields for cattle farmers (ACS, 2016).



**Fig. 1.** Karl Folkers discovered vitamin B12 (ACS, 2016).

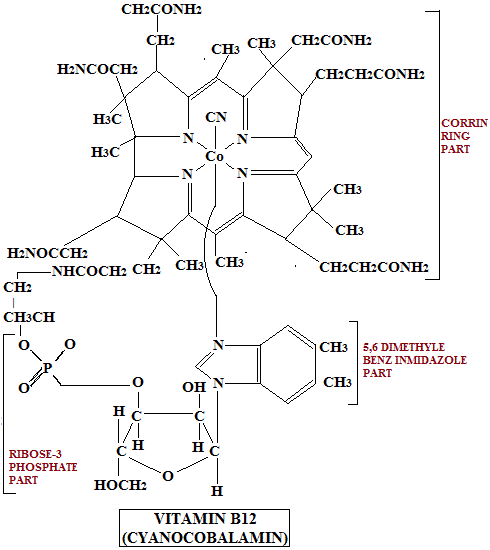
The end result of a 10-year hunt for the liver component that would regulate pernicious anaemia was the discovery of vitamin B12. Some pure red B12 crystals are magnified 240 times as shown in **Figure 2**. Vitamin B12 comes in a variety of forms, with cyanocobalamin being the main one utilised in vitamin supplements and pharmaceuticals. The cobalamins are a series of chemical compounds with a complicated structure that are closely linked and interconvertible. The larger family of corrinoids, which includes all cobalamins, consists of a planar four-membered pyrole ring (corrin-ring) with a central cobalt atom (Green, 2017). The IUPAC name for vitamin B12 (cyanocoblamine B12) is - (5, 6-dimethyl-benz-imidazolyl) cobamidcyanide. Its chemical formula is C63H88CoN14O14P, and its molecular weight is 1355.388 g mol (Mohamed, 2020; PubChem, 2022).



**Fig. 2.** Isolation of vitamin B12. Photomicrograph showing red crystals of Vitamin B12 (ACS, 2016).

* + 1. ***Biosynthesis***

The cobalt particle is arranged in a corrin ring of a porphyrin to form the octahedral cobalt (III) complexes that make up cobalamin (**Figure 3**). Four of the six coordination sites of the triply ionised cobalt atom are closely bonded by the formation of a corrin ring, while the fifth site is coupled by the formation of a dimethylbenzimidazole group (Ahmed, 2018).

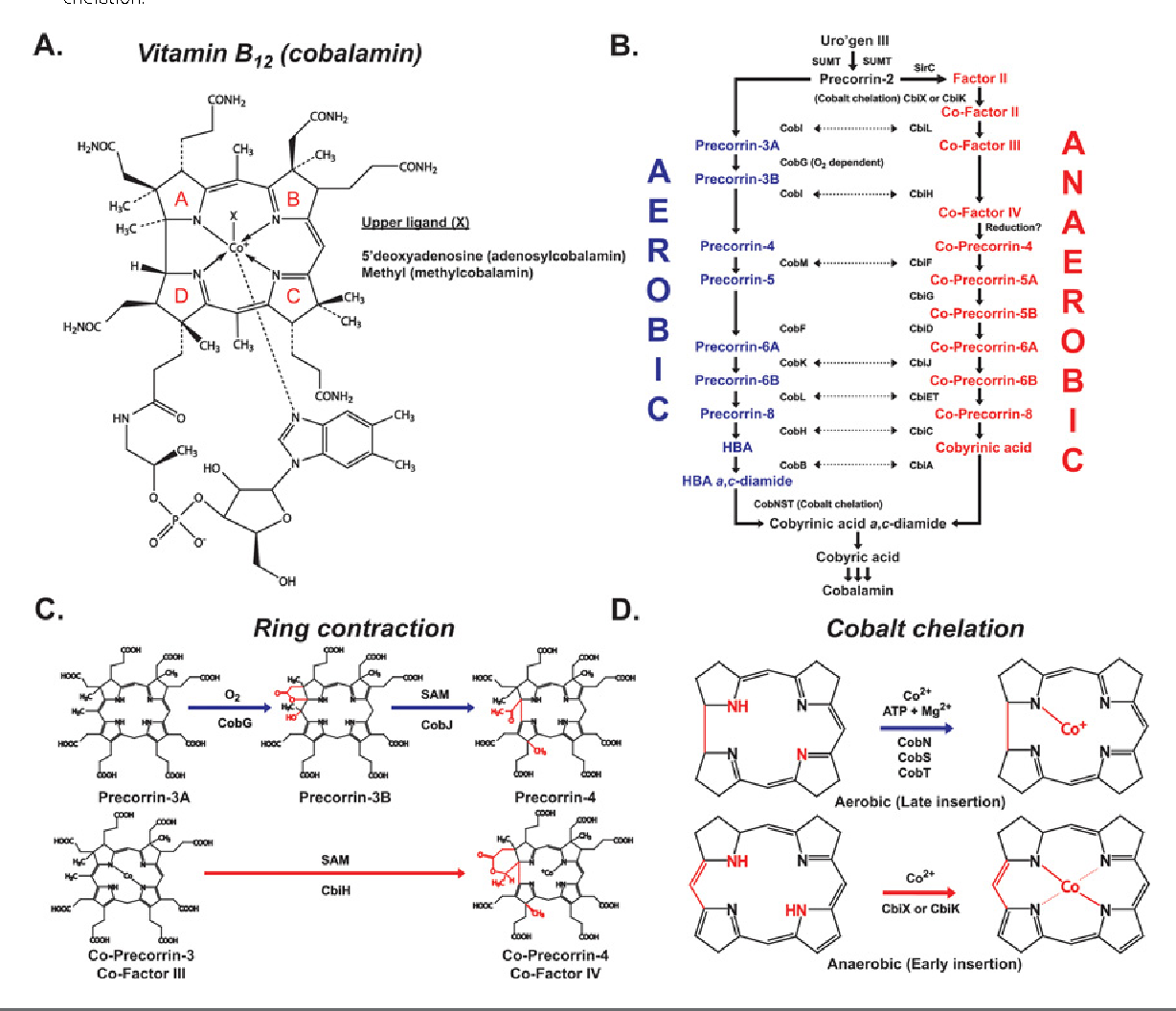


**Fig. 3.** Structure of Vitamin B12 (Warren, 2012)

Cobalamin can be produced de novo in prokaryotes through two different pathways based on the molecular oxygen and the timing of cobalt insertion. These pathways are aerobic and anaerobic pathways. Some strains can also produce cobalamin by utilising a salvage pathway to take in corrinoids. Cobalamin belongs to the family of modified tetrapyrroles, which also includes compounds like chlorophyll, haem, sirohaem, and coenzyme F430 (Moore, 2012).

***1.1.2.1. De novo pathway***

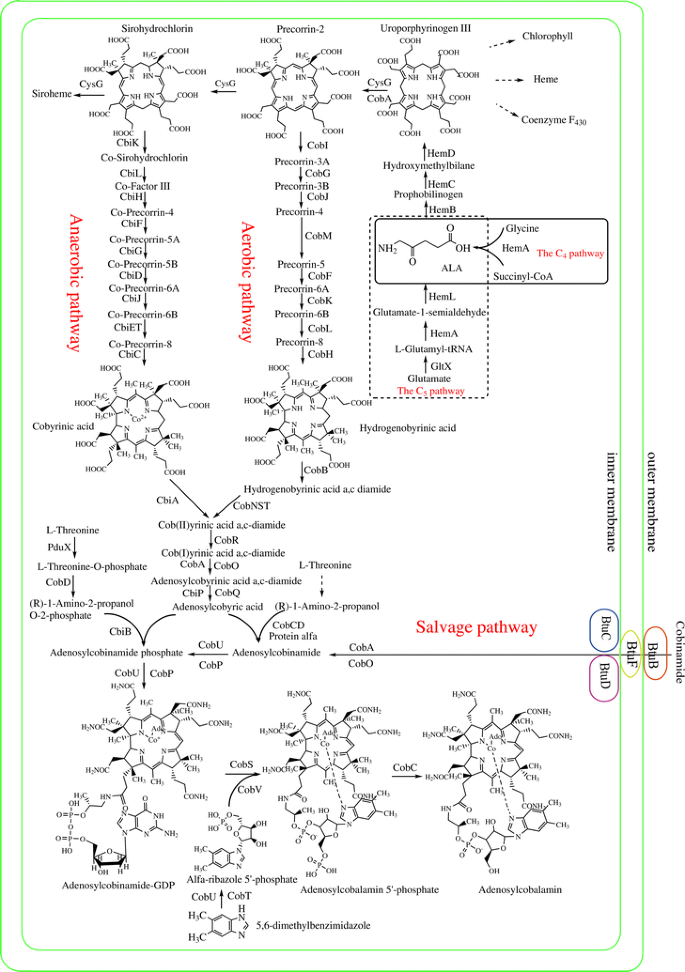
Alpha-lipoic acid (ALA) is the first committed precursor in the pathway that produces tetrapyrroles. Either the C4 pathway or the C5 pathway can produce ALA. The enzyme ALA synthase catalyses the production of ALA from glycine and succinyl-CoA in the C4 pathway. In the C5 pathway, three enzyme processes convert glutamate into ALA (Moore SJ, 2012). Porphobilinogen synthase converts two molecules of ALA into monopyrrole porphobilinogen, which is then polymerized and cyclized to form uroporphyrinogen III. The enzymes porphobilinogen deaminase and uroporphyrinogen III synthase are responsible for catalysing this process. Precorrin-2, a common precursor of cobalamin, siroheme, and coenzyme F430 are produced as a result of the methylation of uroporphyrinogen III at positions C-2 and C-7. The pathways for aerobic and anaerobic metabolism diverged at precorrin-2 and converge at coby(II)rinic acid a, c-diamide as show in **Figure 4**. During de novo cobalamin production, eight peripheral methylation processes take place in the same temporal and spatial order in the aerobic and anaerobic pathways (Avissar, 1989; Zappa, 2010; Cohen, 2014).



**Fig. 4.** De novo pathway of vitamin B12 synthesis (Moore, 2012)

***1.1.2.2. Salvage pathway***

For bacteria and archaea to get cobalamin, the salvage pathway is an efficient (in terms of energy) method (**Figure 5**). Exogenous corrinoids are taken up by gram-negative bacteria by an ATP-binding cassette (ABC) transport system, which is made up of the components BtuC (membrane permease), BtuD (ATPase), and BtuF (periplasmic-binding protein). Corrinoid is transported by BtuB, a TonB-dependent transporter, to the periplasmic corrinoid-binding protein BtuF. The latter then transports corrinoid to the inner membrane's BtuCD complex. ABC transporters are also utilised by Archaea for corrinoid absorption (Moore, 2012). Following membrane transport, ATP:co(I)rrinoid adenosyltransferases adenosylate cobinamide (ACATs). There are three ACAT families: CobA, EutT, and PduO. AdoCbi is a substrate for a bifunctional enzyme with kinase and guanylyltransferase activities found in bacteria (CobU in *S. typhimurium* or CobP in *P. denitrificans*). The cbiZ gene in archaea produces an amidohydrolase that breaks down adenosylcobyric acid into AdoCbi, which is then combined with 1-aminopropanol-O-2-phosphate by an AdoCbi-P synthase (CbiB) to produce AdoCbi-P. CobY, which contains GTP:AdoCbi-P guanylyltransferase activity, is employed to transfer guanylyl to AdoCbi-P because the archaeal enzyme lacks AdoCbi kinase activity. Similar to the de novo process, the salvage pathway involves two additional reactions that transfer lower axial ligands onto AdoCbi-GDP to create AdoCbl (Fang, 2017).



**Fig. 5.** Overall pathway for Vitamin B12 production (obeid, 2017)

**1.2. Source**

All mammals need vitamin B12, which they ultimately get from microbial synthesis products. In their rumens, ruminant animals actually harbour bacteria that produce Cbls. Animal food such as meats, fish, shellfish, etc., dairy products, and eggs provide humans with vitamin B12. The largest quantities are found in shellfish and organ meats. Improved corrinoid analytical techniques have revealed that some algae, fermented vegetable meals, and some plant products may contain B12. Meals, including cereal, sports nutrition products, and other supplemented foods have synthetic vitamin B12 added to them, particularly those sold in the United States to vegetarians (Stabler, 2020).

***1.2.1. Vitamin B12 in Animal Food***

**MEAT**

Approximately 83, 3, and 33 μg/100 g of vitamin B12 are present in cooked cow liver, lean meat, and turkey, respectively. Cooking meats caused significant vitamin B12 losses (33%), according to reports. With increases in vitamin B12 intake every meal, vitamin B12 bioavailability should fall off dramatically (USDA, 2007).

**Milk**

Milk and dairy products are important sources of vitamin B12 as the general population consumes a lot of dairy products, despite the fact that the vitamin B12 concentration of different types of milk (0.3–0.4 μg/100 g) is not high. All of the naturally occurring vitamin B12 in cow's milk is bonded to the transcobalamin, a protein that binds vitamin B12 in mammals (Fedosov, 1996). Significant vitamin B12 losses have been seen during milk processing; boiling for 2-5 minutes and 30 minutes led to 30 % to 50 % loss, microwave cooking for 5 minutes produced a 50 % loss, and pasteurisation resulted in losses of 5 % to 10 %. On the other hand, there was no visible decrease in the content of milk vitamin B12 when the pasteurised milk was chilled for 9 days under retail-simulating or household handling circumstances. In cottage cheese, hard cheese, and blue cheese, about 20 % to 60 % of the vitamin B12 that was initially contained in milk is restored.

**EGG**

The amount of vitamin B12 in an entire egg ranges from 0.9 to 1.4 μg/100 g, with the majority of this vitamin being found in the yolk. Because eggs are a common dietary item, people typically consume high amounts of vitamin B12 from them. Comparatively to other animal food sources, vitamin B12 in eggs is typically poorly absorbed.

**SHELLFISH**

Numerous shellfish are widely consumed. It is well known that mussels are good sources of vitamin B12, with concentrations often exceeding 10 μg/100 g, because they absorb significant amounts of vitamin B12-synthesizing bacteria from the water.

***1.2.2. Vitamin B12 in Plant Food***

**Vegetables**

It has been suggested that bamboo shoots are a good source of vitamin B12. But it turns out that they don't have a lot of vitamin B12 in them. Cabbage, spinach, celery, garland chrysanthermum, lily bulb, and taro produced similar outcomes. These veggies could be able to absorb the vitamin B12 present in some organic fertilisers. Mozafar showed that adding cow manure, an organic fertiliser, dramatically raises the amount of vitamin B12 in spinach leaves and barley grains (Mozafar A, 1994).

**Tea Leaves and Drinks**

Different types of tea leaves contain significant levels of vitamin B12: green tea (0.1-0.5 μg vitamin B12 per 100 g dry weight), blue tea (approximately 0.5 μg), red tea (about 0.7 μg), and black tea (0.3-1.2 μg). Only 1-2 litres of fermented tea drink are consumed on a regular basis (typical in Japan).

**Vitamin B12–Fortified Cereals**

Ready-to-eat cereals that have been fortified with vitamin B12 are known to make up a significant amount of daily vitamin B12 intake. Several research teams hypothesised that consuming a morning cereal supplemented with folic acid, vitamin B12, and vitamin B6 would raise these vitamins' blood concentrations and lower plasma, total homocysteine levels in elderly populations.

***1.2.3. Stability in foods***

Cyanocobalamin, often known as vitamin B12, is a micronutrient that must be obtained through diet. However, vitamin B12 is only present in foods made from animals, and it is highly susceptible to many factors. Given that the vitamin is present in a limited number of foods and in low concentrations, providing them with an adequate vitamin-B12 supplement would be crucial for ensuring their health. When exposed to light, an acidic or basic environment, oxidising chemicals, and continuous heating at or near neutral pH, cyanocobalamin may deteriorate quickly. In this manner, the microencapsulation procedure would be an alternative to reduce the issues brought on by its instability (Mazzocato, 2019). The success of any fortification scheme depends on the fortificants' stability in the selected food matrix. There are numerous reports of co-crystallization being used in the food sector to boost the durability of taste components, safeguard anti-oxidants and keep delicate chemicals. Co-crystals are non-ionic molecular complexes that are utilised to increase the bioactive chemicals' solubility, stability, and bioavailability (Bajaj, 2020).

***1.2.4. Vitamin B12 bioavailability***

The low bioavailability of vitamin B12 is one of the most prevalent nutritional issues in the world. The bioavailability of nutrients is significantly influenced by the lipid content of cell membranes and interactions between molecules and cell membranes. The biological action of B12 is constrained by inadequate bioavailability. Despite eating a balanced diet and getting enough of this vitamin, the human body can only use, on average, 50% of the VB12 that is taken in. Which results in B12 deficiency in roughly 15% of the population (Ramalho, 2020). The widespread consensus is that taking large amounts of vitamin B12 poses no health risks. Even at large intakes, there doesn't seem to be much of a risk of negative impacts on the general population (Institute of Medicine, 2000). The European Food Standards Agency (EFSA) created an algorithm to calculate vitamin B12 bioavailability that takes intake into account: log absorption 5 0.7694 3 log intake 2 0.9614 (EFSA NDA Panel, 2015). This equation does not account for the possibility of a day's worth of ingestion leading to potentially more effective absorption. Further testing is required for these methods of determining absorption effectiveness from intake.

**1.3. Vitamin B12 deficiency**

Symptoms of a vitamin B12 shortage might be neurological, psychological, or physical. Medication containing vitamin B12 can be used to treat it. Haematological and neurological problems might result from a B12 shortage (Ankar, 2022). Vitamin deficiency in vegans is mostly caused by insufficient food intake, and B12 malabsorption is linked to gastrointestinal disorders. Inherited conditions (such as Addison's pernicious anaemia, intrinsic factor deficiency), bariatric surgery, gastrectomies, and obesity are the main causes of B12 malabsorption. Other reasons include pancreatic insufficiency, obstructive jaundice, bacterial overgrowth, parasite infestations, tropical sprue, inflammatory bowel illnesses, and celiac disease (Guéants, 2022).

Human body performs two process in order to absorb vitamin B12. First, the hydrochloric acid in stomach dissolves the vitamin B12 in the food. Then, vitamin B12 mixes with a protein produced by stomach known as intrinsic factor that makes the digestive system to absorb vitamin B12. Pernicious anaemia is a rare illness that prevents some people to produce intrinsic factor. As a result, they suffer from a vitamin B12 deficiency since body is unable to absorb vitamin B12 effectively. In older adults, vitamin B12 insufficiency is typical and has been linked to ischemic stroke (Yahn, 2021). Humans require vitamin B12, which is also a critical component of the human gut bacteria. In newborns who are solely breastfed, vitamin B12 insufficiency is prevalent. Infants' gut microbiota, in contrast to that of adults, has been demonstrated to have a reduced potential for the de novo production of vitamin B12 and to rely on dietary sources of the vitamin (Boran, 2020).

* + 1. ***Indicators for vitamin B12***

Combining four blood markers; methylmalonic acid (MMA) total homocysteine (tHcy), holotranscobalamin (holoTC), and total B12 is a novel way to assess vitamin B12 sufficiency. The formula for this combined B12 status indicator is cB12=log10 [(holoTC.B12)/(MMA.Hcy)] - (age factor) (Fedosov, 2015). However, none of these indications alone have the best sensitivity or specificity for vitamin B12 deficiency, which is difficult to diagnose (Miller JW, 2018). It should be noted, nevertheless, that there is not perfect agreement on these definitions. Additionally, both total vitamin B12 and holoTC have a middle concentration range where the diagnosis is uncertain. Low and high blood concentrations of these substances are reliable markers of deficiency and adequacy, respectively. Although both homocysteine and methylmalonic acid have elevated levels when there is a vitamin B12 deficiency, the reason for this is renal deficiency, whereas the causes of elevated homocysteine include folate and vitamin B6 deficiencies, hypothyroidism, genetic disorders, and the use of medications that affect one-carbon metabolism and homocysteine.

**Total B12**

Measurement of serum cobalamin is used to determine vitamin B12 levels. The most popular method is the measurement of vitamin B12 in serum. However, the test also evaluates serum holohaptocorrin and serum holotranscobalamin, and as a result, it may cover up true deficiency. The test employs an automated process and competitive-binding immune chemiluminescence, and it is widely accessible and inexpensive. It is unclear exactly how much serum cobalamin is considered clinically normal. According to some research, a blood cobalamin level of 148 pmol/L (200 ng/L) would be sensitive enough to identify vitamin B12 deficiency in 97 % of individuals. What serum cobalamin level would indicate subclinical insufficiency is unclear (Devalia, 2014).

**Plasma total homocysteine (tHcy)**

A decrease in cobalamin leads to an increase in plasma total homocysteine (tHcy). A sensitive biomarker of cobalamin deficit, plasma tHcy increases early due to deficiency, sometimes prior to symptoms and advances as it gets worse. However, tHcy is not unique to cobalamin deficiency as concentrations of tHcy are elevated in individuals with renal failure, hypothyroidism, and other genetic polymorphisms, as well as in cases of folate and B6 deficiency.

**Holotranscobalamin (HoloTC)**

Compared to serum cobalamin levels, the plasma cobalamin's "active" fraction may be more accurate. The HoloTC assay outperforms the serum cobalamin assay in clinical research studies when determining deficiency based on MMA levels. For HoloTC, healthy people should have levels between 35 to 171 pmol/l.

**Plasma methylmalonic acid (MMA)**

When there is a cobalamin shortage, plasma MMA rises. Subjects with renal illness, small bowel bacterial overgrowth, and haemoconcentration may also experience artificially increased levels. Despite these restrictions, extremely high plasma MMA levels (>075lmol/l) nearly often signify cobalamin deficiency. Using mass spectrometry and gas chromatography, MMA in plasma is measured. As a result, this test is expensive, which has limited its use (Heil, 2012).

***1.3.2. Health concerns***

The most typical cause of severe vitamin B12 malabsorption is the autoimmune disease pernicious anaemia (Green, 2017). The disease affects people of all racial and ethnic backgrounds worldwide, and its frequency rises with age and female sex. Despite its rarity, pernicious anaemia can strike young people, and those of African descent may be more susceptible to its early onset (Stabler, 2020). Since the Cbl secreted in the bile cannot be bound to IF and is lost in the stool, there is impaired enterohepatic circulation of Cbl in pernicious anaemia, which causes a more rapid depletion of Cbl when treatment is stopped (Stabler, 2018). Dietary vitamin B12 deficiency is common in areas with limited resources where people cannot access animal-based foods, particularly in parts of Africa, Asia, and South America. The infant is born with a vitamin B12 deficiency even if the mother shows no symptoms. These infants have permanent disabilities as well as failures in myelination and brain development (Stabler, 2013; Huemer, 2018).

***1.3.3. Recommended dietary allowance***

A vital nutrient, vitamin B12 is crucial for many biological functions, including DNA synthesis and DNA methylation, the production of blood cells, and neuron function.  The only sources of vitamin B12 in human nutrition are animal products such meat, poultry, fish, eggs, and milk (Wolffenbuttel, 2020). Vegans are more likely to experience nutritional deficiencies, particularly vitamin B12 deficiency. Although the lowest daily intake of B12 necessary to maintain life is unknown, it is most likely less than 0.5 mcg per day. However, this would not maintain normal biochemical levels.

The Recommended Dietary Allowance (RDA) for vitamin B12 is based on the quantity required to maintain normal serum vitamin B12 levels and haematological status. The advised intake takes a 50% absorption rate into account. Adults require 2.4 μg of vitamin B12 daily. To meet their RDA, adults over 50 are advised to primarily consume foods fortified with vitamin B12 because 10 to 30% of older people may have trouble absorbing naturally occurring vitamin B12 (Brito, 2018). The advised doses for pregnant and lactating mothers were raised to 2.6 and 2.8 ug, respectively. The recommended daily allowances for children rise from 0.4 ug for newborns to 1.8 ug for teenagers (**Table 1**).

**Table 1:** Show Recommended Dietary Allowance (RDA) for children, boys and girls of different age group (k venkaiah, 2002)

|  |  |  |
| --- | --- | --- |
| **RDA for children** | 1–3 years | 0.9 μg/day of vitamin B12 |
| 4–8 years | 1.2 μg/day of vitamin B12 |
| **RDA for boys** | 9–13 years | 1.8 μg/day of vitamin B12 |
| 14–18 years | 2.4 μg/day of vitamin B12 |
| **RDA for girls** | 9–13 years | 1.8 μg/day of vitamin B12 |
| 14-18 years | 2.4 μg/day of vitamin B12 |

**1.4. Food fortification**

Food fortification is the process of adding essential vitamins or minerals during the processing of commonly consumed food to alleviate nutritional values. It is widespread in all regions of the world and especially in lower income countries. Deficiency of essential micronutrients is highly prevalent in all sections of the society. Food fortification has been demonstrated to be a cost-effective technique with higher social, economic, and most importantly better health benefits. It has direct implications in physical and cognitive skills of people who are affected by the deficiencies of essential micronutrients (Olson, 2019). An intervention study conducted stated that when compared with placebo/no intervention, MMN (multiple micro nutrient) fortification may reduce iron deficiency by 56%, iron deficiency anaemia by 72%, anaemia by 32%, vitamin B2 deficiency by 64%, vitamin B6 deficiency, vitamin A deficiency by 58%, by 91% and vitamin B12 deficiency by 58% (Das et al., 2019).

Food fortification programs have been conducted in several countries to overcome micronutrient deficiency (Dewi, 2021). Food fortification with vitamins have been on the rise in recent times owing to increasing trends of deficiencies and malnutrition as a result of it (**Figure 6**). Numerous studies have been conducted globally, including in India, to assess the status of Vitamin B12. It was observed that Vitamin B12 concentration was highest in colostrum and gradually decreased over the first 3-4 months of lactation (Dror DK, 2018). Research has also shown that decreased B12 vitamin levels and increased total choline or homocysteine in maternal blood are associated with an elevated risk of Neural Tube Defects (NTDs) (Imbard A, 2013).

Chart

Description automatically generated

**Fig. 6.** Supplementation of vitamin B12 (Imbard A, 2013)

**1.5. Fortification guidelines**

Although historically rare, vitamin B12 deficiency is now recognised as a global health problem that poses severe clinical issues, including progressive megaloblastic anaemia and potentially irreversible neurological abnormalities. Numerous studies have shown that many people, particularly the elderly populations, suffer from this asymptomatic vitamin B12 deficiency. These studies include data presented at the WHO Technical Consultation. This necessitates the fortification of vitamin B12 in foods. To ensure that fortification is effective and safe, it is essential to consider specific parameters. These include setting beneficial and safe standards, sampling, handling, micronutrient premix procurement, operational procedures, quality control, labelling with logo, storage, record-keeping, packaging, analysis, and distribution; so that fortification should be both safe and effective (Allen *et al.,* 2010; Carmel *et al.,* 2010).

***1.5.1. Foods fortified with vitamin B12***

A lack of vitamin B12 can cause severe issues and poor health. Vitamin B12 is vital for proper red blood cell development, brain function, and DNA synthesis, and its deficiency can lead to severe complications and illness. Its necessary amount can be obtained via food for those with a varied diet that includes animal products. However, those who consume a plant-based diet and older adults, those taking certain medications or suffering from gastrointestinal issues, may be more susceptible to deficiency. These people can only get vitamin B12 from fortified foods or supplements. The recommended dietary allowances (RDAs) differ depending on a person’s age and whether they are pregnant or nursing, claims the National Institutes of Health Trusted Source. Vitamin B12 is needed in doses of 2.4 mcg per day for healthy adults and 2.8 mcg per day for pregnant and lactating women. And for people over the age of 50, the RDA for B12 is 25 to 100 mcg, which should be met through supplements and fortified foods (NMCD database 2010; Mayo 2010). The RDA for vitamin B12 is shown in **Figure 7**.

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| --- |
| Vitamin B12 intake (mcg) |
| **Fig.7.** Recommended dietary allowances of vitamin B12 for different populations. |

Since vitamin B12 cannot be produced by our body, we must obtain it from outside sources. It is mostly attainable through animal products. As a result, it can be difficult for vegans and vegetarians to receive enough vitamin B12. Yoghurt, milk, other dairy products, eggs, nutritional yeast, nori, tempeh, vitamin B12 supplements, and fortified meals are a few good sources of vitamin B12 besides animal sources (Mayo 2010). The vitamin B12 source from fortified foods and its intake per saving were mentioned in **Table 2**.

|  |  |  |
| --- | --- | --- |
| **Table 2:** Dietary intake values for vitamin B12 (in mcg) from different fortified foods (NMCD database 2010) | | |
| Fortified foods | Servings | Intake (mcg) |
| Almond or oat beverage | 1 cup | 1.1 |
| Soy or rice beverage | 1 cup | 1.0 |
| Soy burger | 2 ½ oz | 1.8 |
| Cereals | 1 cup | 0.6-2.1 |

Cereals can be fortified with vitamin B12, folate, iron, and vitamin A including the bran and whole wheat oats. Regular consumption of fortified cereals can help the body to raise vitamin B12 levels. Non-dairy milk that is fortified, such as soy and almond milk, does not naturally contain vitamin B12; this vitamin is added during the fortification process. One cup of soy or almond milk contains 2.1 mcg of vitamin B12 daily (Didit et al., 2018). **Table 3** explicitly shows the amount of vitamin B12 consumed in fortified cereals and non-dairy milk based on serving size.

|  |  |  |  |
| --- | --- | --- | --- |
| **Table 3:** Dietary intake values for vitamin B12 from fortified cereals and non-dairy milk in different servings (NMCD database 2010; Mayo 2010; Didit et al., 2018). | | | |
| Fortified cereals | Vitamin B12 per 3/4 Cup | Vitamin B12 per 100g | Vitamin B12 per 200 Calories |
| 6.1μg (254% DV) | 21μg (875% DV) | 12.8μg (535% DV) |
| Fortified non-dairy milk | Vitamin B12 per 16oz Glass | Vitamin B12 per 100g | Vitamin B12 per 200 Calories |
| 6μg (249% DV) | 1.2μg (51% DV) | 7.5μg (311% DV) |

**1.6. Vitamin B12 fortification challenges**

There are various ways to fortify food. It is possible to fortify foods that are widely consumed by the general population (mass fortification). Typically, mass fortification is invariably compulsory, targeted fortification may be either obligatory or voluntary, contingent on the public health importance of the issue at hand, and market-driven fortification is consistently voluntary but constrained by regulatory boundaries. Typically, national conditions determine whether food fortification is required or optional. For instance, enforcing mandatory fortification may not be feasible in nations where small mills produce a significant portion of maize flour. If possible, in such a situation, one option would be to permit small mills to fortify their product voluntarily while adhering to predetermined rules.

Because of the wide range of national circumstances and public health objectives worldwide, many different approaches to food fortification regulation have emerged. Food fortification guidelines are established by law or through cooperative agreements in most industrialised nations. Conversely, fortified foods are manufactured without government oversight or control. Fortification can be classified as either mandatory or voluntary. These terms describe the degree of responsibility expected of food producers to adhere to governmental intentions stated in the law. Regarding food fortification, the critical distinction between mandatory and voluntary regulation is the degree of certainty over time that a specific category of foods will contain a predetermined amount of a micronutrient. Mandatory fortification increases the likelihood that the relevant population group will have a consistent supply of fortified foods to consume, which benefits public health.

**1.7. Conclusion**

The rationale behind vitamin B12 fortification is supported by several key factors. Firstly, there is a significant prevalence of deficiency globally, spanning across all age groups. Notably, the perinatal period is particularly vulnerable to the adverse consequences of deficiency. Another crucial consideration is the necessity to prevent the worsening of deficiency due to folic acid fortification. Importantly, no known risks of adverse effects on health or the quality of fortified foods have been identified, and the overall cost of implementing fortification is affordable. In conclusion, this book chapter serves as a comprehensive guide to Vitamin B12 fortification, offering a wealth of knowledge and actionable insights for policymakers, researchers, and stakeholders invested in public health and nutrition. By fostering collaborations between scientific innovation and regulatory diligence, we can pave the way towards a healthier, fortified future, where Vitamin B12 becomes more accessible, and its benefits are reaped by populations around the globe.

Acknowledgments

This research article was supported by The Gandhi Institute of Technology and Management, Visakhapatnam, Reva University, University of Delhi South Campus, and National Horticulture Research and Development Foundation (NHRDF), India.

**Conflicts of interest**

The authors declare no conflict of interest, financial or otherwise.

**Ethical approval**

Ethics approval was not required for this work.

**Reference**

1. Antherjanam, S., Saraswathyamma, B., Krishnan, R.G. *et al.* “Electrochemical sensors as a versatile tool for the quantitative analysis of Vitamin B12 ’’. *Chem. Pap.* **75,**2981–2995 (2021).
2. Agriculture and Rural Development (2019). Global Food Supply and Demand, Consumer Trends, Trade Challenges. In EU Agricultural Markets Briefs. Rome: FAO, 16.
3. American Chemical Society National Historic Chemical Landmarks. The Vitamin B Complex.http://www.acs.org/content/acs/en/education/whatischemistry/landmarks/vitamin-b-complex.html.
4. Ahmed, L.M., Saaed, S.I. and Marhoon, A.A.,. “Effect of oxidation agents on photo-decolorization of vitamin B 12 in the presence of ZnO/UV-A system”. *Indonesian Journal of Chemistry*, *18*(2), pp.272-278, 2018.
5. Ankar A, Kumar A. “Vitamin B12 Deficiency”. [Updated 2022 Jun 5]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2022 Jan.
6. Allen, Lindsay H., Joshua W. Miller, Lisette De Groot, Irwin H. Rosenberg, A. David Smith, Helga Refsum, and Daniel J. Raiten. "Biomarkers of Nutrition for Development (BOND): vitamin B-12 review." *The Journal of nutrition* 148, no. suppl\_4 (2018): 1995S-2027S.
7. Allen, L. H. (2018). “Efficacy and Safety of Vitamin B12 Fortification. Food Fortification in a Globalized World”, 255–261.
8. Avissar Y, Ormerod J, Beale S. “Distribution of δ-aminolevulinic acid biosynthetic pathways among phototrophic bacterial groups”. Arch Microbiol. 1989;151: 513–9.
9. Bodirsky, B. L., Dietrich, J. P., Martinelli, E., Stenstad, A., Pradhan, P., Gabrysch, S., et al. (2020). “The ongoing nutrition transition thwarts long-term targets for food security, public health and environmental protection”. Scientific Reports 10:19778. doi: 10.1038/s41598-020-75213-3
10. Boran, P., Baris, H.E., Kepenekli, E., Erzik, C., Soysal, A. and Dinh, D.M., 2020. “The impact of vitamin B12 deficiency on infant gut microbiota. European journal of paediatrics”, 179(3), pp.385-393.
11. Brito A, Habeych E, Silva-Zolezzi I, Galaffu N, Allen LH. “Methods to assess vitamin B12 bioavailability and technologies to enhance its absorption”. Nutr Rev. 2018;76: 778e792.
12. Bajaj, S. R., & Singhal, R. S. (2020). “Enhancement of stability of vitamin B12 by co-crystallization: A convenient and palatable form of fortification. Journal of Food Engineering”, 110231. doi:10.1016/j.jfoodeng.2020
13. Cohen GN. “Biosynthesis of cobalamins including vitamin B12 “. In Microbial biochemistry. Dordrecht: Springer; 2014. p. 555–565
14. Chittaranjan, Y. (2020). “Vitamin B12: An Intergenerational Story”. Nestlé Nutrition Institute Workshop Series, 91–102. doi:10.1159/000503358
15. Carmel, R., 2013. “Diagnosis and management of clinical and subclinical cobalamin deficiencies: why controversies persist in the age of sensitive metabolic testing”. Biochimie 95 (5), 10471055.
16. Devalia V, Hamilton M, Molloy A. “Guidelines for the diagnosis and treatment of cobalamin and folate disorders”. Br J Haematol 2014;166: 496-513.
17. Duggan, C., Srinivasan, K., Thomas, T., Samuel, T., Rajendran, R., Muthayya, S., et al., 2014. “Vitamin B-12 supplementation during pregnancy and early lactation increases maternal, breast milk, and infant measures of vitamin B-12 status”. J. Nutr. 144 (5), 758764.
18. EFSA NDA Panel (EFSA Panel on Dietetic Products Nutrition and Allergies, 2015. Scientific opinion on dietary reference values for cobalamin (vitamin B12). EFSA J. 13, 41504213.
19. FAO (2017). “Nutrition-Sensitive Agriculture and Food Systems in Practice. Options for Intervention”. In Nutrition-Sensitive Agriculture and food Systems in practice. Options for Intervention. Rome: FAO.
20. Fang, Huan, Kang, Jie, Zhang, Dawei (2017) “Microbial production of vitamin B12: a review and future perspectives, Microbial Cell Factories, 1475-2859
21. Fedosov SN, Petersen TE, Nexø E. “Transcobalamin form cow milk: isolation and physico-chemical properties”. Biochim Biophys Acta 292: 113–119, 1996.
22. Fedosov SN, Brito A, Miller JW, Green R, Allen LH. “Combined indicator of vitamin B12 status: modification for missing biomarkers and folate status and recommendations for revised cut-points”. Clin Chem Lab Med. 2015 Jul;53(8):1215-25.
23. Green R, Allen LH, Bjørke-Monsen AL, et al. “Vitamin B (12) deficiency”. Nat Rev Dis Primers. 2017;3, 17040.
24. Green, R., Allen, L.H., Bjorke-Monsen, A.L., Brito, A., Gueant, J.L., Miller, J.W., et al., 2017. “Vitamin B12 deficiency”. Nat. Rev. Dis. Primers 3, 17040.
25. Guéant JL, Guéant-Rodriguez RM, Alpers DH. “Vitamin B12 absorption and malabsorption”. Vitam Horm. 2022;119:241-274.
26. Green, R., Miller, J.W., Zempleni, J., Rucker, R.B., McCormick, D.B. and Suttie, J.W., 2007. Vitamin B12.
27. Heil, S.G., de Jonge, R., de Rotte, M.C., van Wijnen, M., Heiner-Fokkema, R.M., Kobold, A.C., Pekelharing, J.M., Adriaansen, H.J., Sanders, E., Trienekens, P.H., Rammeloo, T. & Lindemans, J. (2012) “Screening for metabolic vitamin B12 deficiency by holotranscobalamin in patients suspected of vitamin B12 deficiency: a multicentre study”. Annals of Clinical Biochemistry, 49, 184–189.
28. Huemer M, Baumgartner MR. The clinical presentation of cobalamin-related disorders: from acquired deficiencies to inborn errors of absorption and intracellular pathways. J Inherit Metab Dis. 2018;Dec 27.
29. Institute of Medicine, food and nutrition board. [Dietary Reference Intakes for Thiamin, Riboflavin, Niacin, Vitamin B(6), Folate, Vitamin B(12), Pantothenic Acid, Biotin, and Choline.](https://www.ncbi.nlm.nih.gov/books/NBK114310/)Washington ,DC:National Academies press;1998.
30. Infante M, Leoni M, Caprio M, Fabbri A. Long-term metformin therapy and vitamin B12 deficiency: An association to bear in mind. World J Diabetes. 2021 Jul 15;12(7):916-931.
31. Institute of Medicine, 2000. Dietary Reference Intakes: Thiamin, Riboflavin, Niacin, Vitamin B6, Folate, Vitamin B12, Pantothenic Acid, Biotin, and Choline. National Academies Press, Washington, D.C.
32. Jones, L. (2020). Veganism: Why are Vegan Diets on the Rise?–BBC News. In BBC News. London: BBC News, 1–2.
33. K.B.AkshayaVargheseAnithaM.NidhinY.N.SudhakarGeorgeLouis. Electrochemical sensing of vitamin B12 deficiency marker methylmalonic acid using PdAu-PPy tailored carbon fiber paper electrode. Volume 217,(2020),121028,ISSN 00399140.
34. Moore TC, Newmister SA, Rayment I, Escalante-Semerena JC. Structural insights into the mechanism of four-coordinate Cob(II)alamin formation in the active site of the Salmonella enterica ATP:Co(I)rrinoid adenosyltransferase enzyme: critical role of residues Phe91 and Trp93. Biochemistry. 2012;51:9647–57.
35. Mazzocato, M. C., Thomazini, M., & Favaro-Trindade, C. S. (2019). *Improving stability of vitamin B12 (Cyanocobalamin) using microencapsulation by spray chilling technique. Food Research International, 108663.*
36. Miller JW. Proton Pump Inhibitors, H2-Receptor Antagonists, Metformin, and Vitamin B-12 Deficiency: Clinical Implications. Adv Nutr. 2018 Jul 1;9(4):511S-518S. doi: 10.1093/advances/nmy023.
37. Mozafar A. Enrichment of some B-vitamins in plants with application of organic fertilizers. Plant Soil 167:305–311, 1994
38. Moore, S. J., & Warren, M. J. (2012). The anaerobic biosynthesis of vitamin B12. Biochemical Society Transactions, 40(3), 581–586.
39. Mohamed, G.G., Fekry, A.M., Abou Attia, F.M., Ibrahim, N.S. and Azab, S.M., 2020. Simultaneous determination of some antidepressant drugs and vitamin B12 in pharmaceutical products and urine sample using HPLC method. *Journal of Chromatography B*, *1150*, p.122178.
40. Nakos, M. (2016). Quantitative Determination of Vitamin B 12 in Plants [Gottfried Wilhelm Leibniz Universität Hannover].
41. National Center for Biotechnology Information. "PubChem Compound Summary for CID 91820207, Adenosylcobalamin" *PubChem*, https://pubchem.ncbi.nlm.nih.gov/compound/Adenosylcobalamin. Accessed 21 August, 2022.
42. Obeid R, Heil SG, Verhoeven MM, Van den Heuvel EG, De Groot LC, Eussen SJ. Vitamin B12 intake from animal foods, biomarkers, and health aspects. Frontiers in Nutrition. 2019 Jun 28;6:93.
43. Ramalho, M. J., Andrade, S., Coelho, M. A. N., Loureiro, J. A., & Pereira, M. C. (2020). *Molecular interactions between vitamin B12 and membrane models: a biophysical study for new insights into the bioavailability of Vitamin. Colloids and Surfaces B: Biointerfaces, 111187.*
44. Scott, J. M., & Molloy, A. M. (2012). The Discovery of Vitamin B12. Annals of Nutrition and Metabolism, 61(3), 239–245.
45. Stabler, S. P. (2020). Vitamin B12. Present Knowledge in Nutrition, 257–271. doi:10.1016/b978-0-323-66162-1.00015-9 10.1016/b978-0-323-66162-1.00015-9
46. Siddiqua, T.J., Allen, L.H., Raqib, R., Ahmed, T., 2014. Vitamin B12 deficiency in pregnancy and lactation: Is there a need for pre and post-natal supplementation? J. Nutr. Disorders Ther. 4, 142.
47. Stabler, S. P. (2020). Vitamin B12. Present Knowledge in Nutrition, 257–271.
48. Stabler SP. Megaloblastic anemias. In: Goldman L, Schafer AI, eds. Goldman-Cecil Medicine. 26th ed. Philadelphia, PA: Elsevier; 2018.
49. Stabler SP. Clinical practice: vitamin B12 deficiency. N Engl J Med. 2013;368:149e160
50. Tomohiro Bito, Mariko Bito, Yusuke Asai, Shigeo Takenaka, Yukinori Yabuta, Kazunori Tago, Masato Ohnishi, Toru Mizoguchi, and Fumio Watanabe *Journal of Agricultural and Food Chemistry* **2016** *64* (45), 8516-8524
51. USDA National Nutrient Database for Standard Reference, Release 18. Reports by single nutrients. Vitamin B-12 (lg) content of selected foods per common measure, sorted by nutrient content. USDA Nutrient Data Laboratory.
52. Wolffenbuttel, Bruce HR, M. Rebecca Heiner-Fokkema, Ralph Green, and Rijk OB Gans. "Relationship between serum B12 concentrations and mortality: experience in NHANES." *BMC medicine* 18, no. 1 (2020): 1-14.
53. Yahn GB, Abato JE, Jadavji NM. Role of vitamin B12 deficiency in ischemic stroke risk and outcome. Neural Regen Res. 2021 Mar;16(3):470-474.
54. Zhuo Cheng, Haruki Yamamoto, Carl E. Bauer,Cobalamin's (Vitamin B12) Surprising Function as a Photoreceptor, Trends in Biochemical Sciences,Volume 41, Issue 8,2016,Pages 647-650,ISSN 0968-0004Zappa S, Li K, Bauer CE. The tetrapyrrole biosynthetic pathway and its regulation in Rhodobacter capsulatus. Adv Exp Med Biol. 2010;675:229–50.
55. Zayas CL, Escalante-Semerena JC. Reassessment of the late steps of coenzyme B12 synthesis in Salmonella enterica: evidence that dephosphorylation of adenosylcobalamin-5′-phosphate by the CobC phosphatase is the last step of the pathway. J Bacteriol. 2007;189:2210–8.
56. Dror DK, Allen LH. Vitamin B-12 in Human Milk: A Systematic Review. Adv Nutr. 2018 May 1;9(suppl\_1):358S-366S. doi: 10.1093/advances/nmx019.
57. Imbard A, Benoist JF, Blom HJ. Neural tube defects, folic acid and methylation. Int J Environ Res Public Health. 2013 Sep 17;10(9):4352-89.
58. Rathee S, Nayak V, Singh KR, Ojha A. Nanofortification of vitamin B-complex in food matrix: Need, regulations, and prospects. Food Chem (Oxf). 2022 Mar 14;4:100100.
59. Olson R, Gavin-Smith B, Ferraboschi C, Kraemer K. Food Fortification: The Advantages, Disadvantages and Lessons from *Sight and Life* Programs. Nutrients. 2021 Mar 29;13(4):1118.
60. Das JK, Salam RA, Mahmood SB, Moin A, Kumar R, Mukhtar K, Lassi ZS, Bhutta ZA. Food fortification with multiple micronutrients: impact on health outcomes in general population. Cochrane Database Syst Rev. 2019 Dec 18;12(12):CD011400. doi: 10.1002/14651858.CD011400.pub2.
61. Das JK, Salam RA, Kumar R, Bhutta ZA. Micronutrient fortification of food and its impact on woman and child health: a systematic review. Syst Rev. 2013 Aug 23;2:67.
62. Detzel P, Klassen-Wigger P. Market-Driven Food Fortification to Address Dietary Needs. World Rev Nutr Diet. 2020;121:81-88.
63. Dewi, N.U.; Mahmudiono, T. Effectiveness of Food Fortification in Improving Nutritional Status of Mothers and Children in Indonesia. Int. J. Environ. Res. Public Health **2021**, 18, 2133.
64. Peña‐Rosas JP, Mithra P, Unnikrishnan B, Kumar N, De‐Regil LM, Nair NS, Garcia‐Casal MN, Solon JA. Fortification of rice with vitamins and minerals for addressing micronutrient malnutrition. Cochrane Database of Systematic Reviews 2019, Issue 10. Art. No.: CD009902.