VITAMIN B_{12} FORTIFICATION: METHODS, TECHNOLOGIES & REGULATIONS

Abstract

Vitamin B_{12} 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 B_{12}. Although the B_{12} content of different types of milk is not high, increased milk consumption was associated with higher serum B_{12} levels in geriatric persons in good health, showing that milk is a useful source of B_{12}. Deficiency of B_{12} 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 B_{12} followed by the strategies such as diet diversification, food fortification, challenges and vitamin B_{12} fortification using nanotechnology.

Keywords: Food, Fortification, Vitamin B_{12}, deficiency, Dairy Products

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I. INTRODUCTION

All animals and some microbes require vitamin B_{12}, which is a well-known by-product of specific bacteria. The water-soluble vitamin B_{12}, 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 et al., 2021). Vitamin B_{12} 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 (Akshaya et al., 2020). Dairy products, meat, and fish are a few examples of foods high in vitamin B_{12}. While the B_{12} content in various milk types may not be substantial, greater milk consumption correlated with elevated serum B_{12} levels in healthy geriatric individuals. This suggests that milk serves as a valuable source of vitamin B_{12} (Bito 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 B_{12} 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 B_{12} are 5-deoxyadenosylcobalamin and methylcobalamin. However, after being changed into methylcobalamin or 5-deoxyadenosylcobalamin, two more forms of cyanocobalamin and hydroxycobalamin become biologically active (Cheng et al., 2016).

The European Food Safety Authority recommends that adults receive 4 µg of vitamin B_{12} per day, and the requirements are high during lactation and pregnancy (Siddiqua & Allen, 2014). Elderly people are also at risk for developing a deficiency in this vitamin (Obeid et al., 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 et al., 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 B_{12} 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, 2016). In the context of severe and ongoing vitamin B_{12} 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 B_{12} deficiency in adult individuals (Carmel, 2013; Infante et al., 2021). In this chapter, we begin by focusing on the physical properties, chemistry, molecular structure, bioavailability, and deficiency of vitamin B_{12}. We then discuss strategies such as diet diversification, food fortification, challenges and vitamin B_{12} fortification using nanotechnology.

1. **Vitamin B_{12}- Discovery and Nomenclature:** Over the course of more than a century, vitamin B_{12} was discovered, its role in metabolism was clarified, and the effects and remedies for its deficiency were discovered (Scott & Molloy, 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 B_{12} (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 National Historic Chemical Landmarks, 2016).

Figure 1: Karl Folkers discovered Vitamin B_{12} (Adapted from ACS National Historic Chemical Landmarks, 2016).

The end result of a 10-year hunt for the liver component that would regulate pernicious anaemia was the discovery of vitamin B_{12}. Some pure red B_{12} crystals are magnified 240 times as shown in **Figure 2**. Vitamin B_{12} 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 *et al.,* 2017). The IUPAC name for vitamin B_{12} (cyanocoblamine B_{12}) is - (5, 6-dimethyl-benz-imidazolyl) cobamidcyanide. Its chemical formula is C63H88CoN14O14P, and its molecular weight is 1355.388 g/mol (Mohamed *et al.,* 2020; National Center for Biotechnology Information, 2023).

Figure 2: Isolation of vitamin B_{12}. Photomicrograph showing red crystals of Vitamin B_{12} (Adapted from ACS National Historic Chemical Landmarks, 2016).

2. **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 *et al.,* 2018).

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 F_{430} (Zayas & Escalante-Semerena, 2007; Moore *et al.,* 2012).

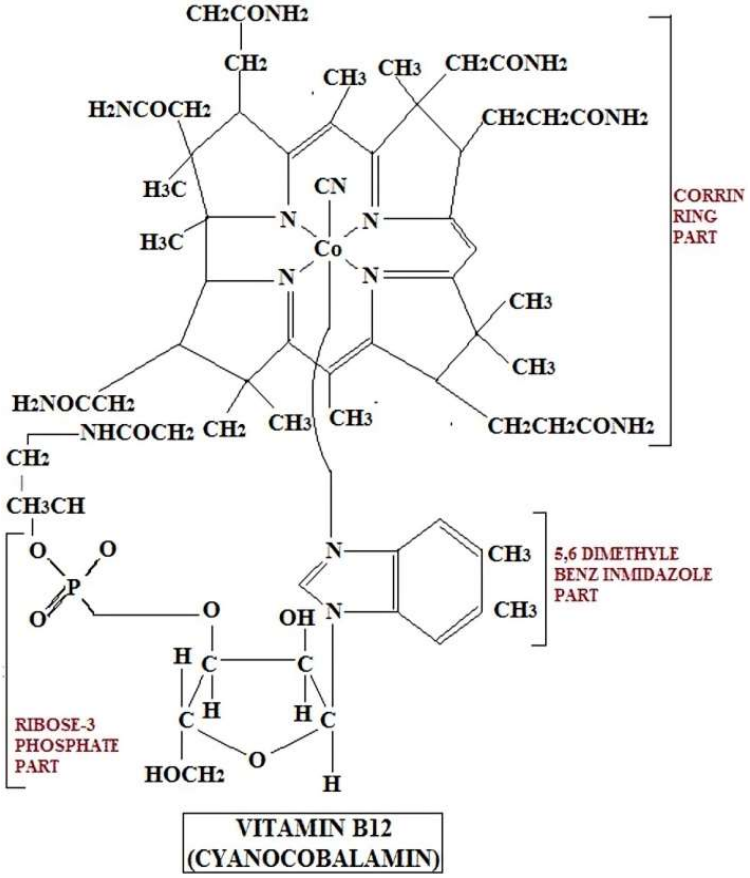


Figure 3: Structure of Vitamin B_{12} (Adapted from Moore & Warren, 2012).

- **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 & Warren, 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 F_{430} 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 *et al.,* 1989; Zappa *et al.,* 2010; Cohen, 2014).

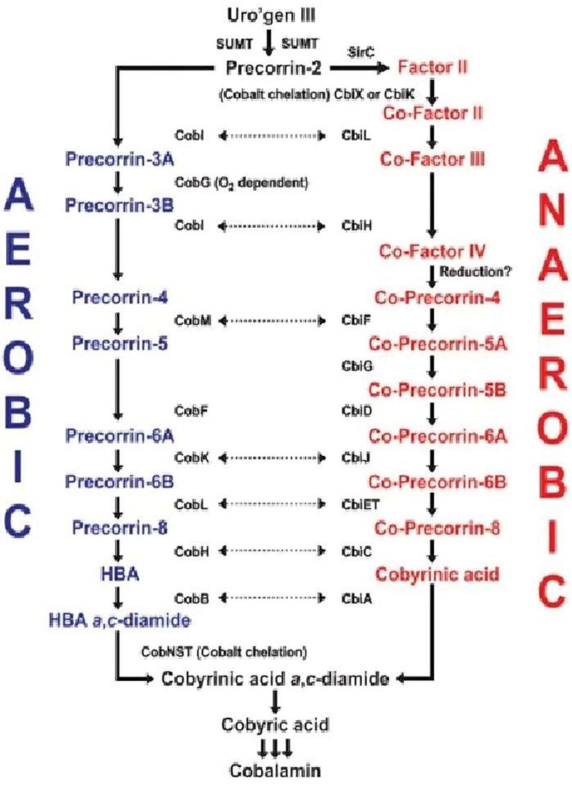


Figure 4: De novo pathway of Vitamin B_{12} synthesis (Adapted from Moore & Warren, 2012).

- **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 & Warren, 2012; Moore *et al.,* 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 *et al.,* 2017).

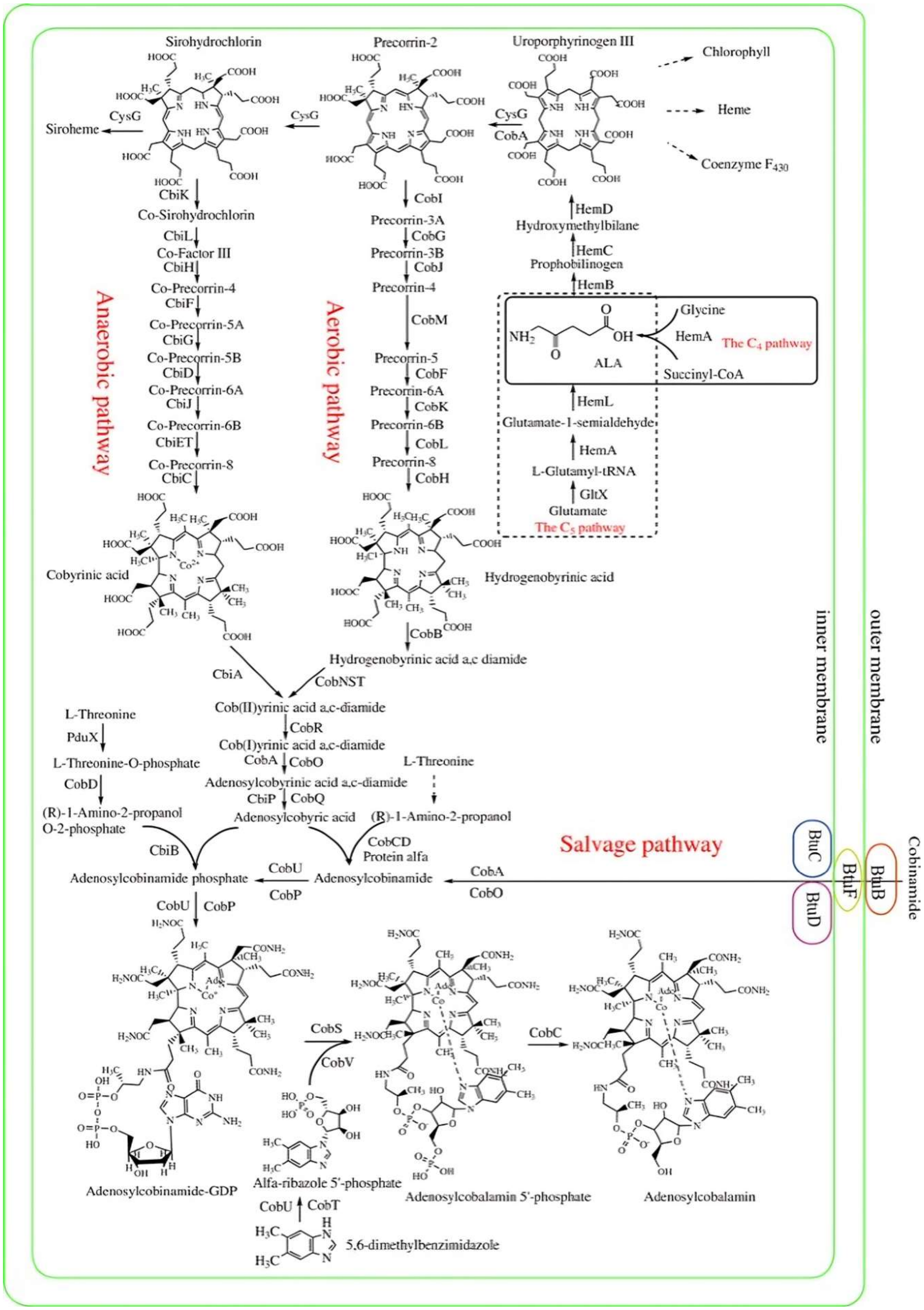


Figure 5: Overall pathway for Vitamin B_{12} production (Adapted from Obeid *et al.,* 2019).

II. SOURCE

All mammals need vitamin B_{12}, 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 B_{12}. 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 B_{12}. Meals, including cereal, sports nutrition products, and other supplemented foods have synthetic vitamin B_{12} added to them, particularly those sold in the United States to vegetarians (Stabler, 2020).

1. Vitamin B_{12} in Animal Food

- **Meat:** Approximately 83, 3, and 33 μg/100 g of vitamin B_{12} are present in cooked cow liver, lean meat, and turkey, respectively. Cooking meats caused significant vitamin B_{12} losses (33%), according to reports. With increases in vitamin B_{12} intake every meal, vitamin B_{12} bioavailability should fall off dramatically (USDA, 2008).

- **Milk:** Milk and dairy products are important sources of vitamin B_{12} as the general population consumes a lot of dairy products, despite the fact that the vitamin B_{12} concentration of different types of milk (0.3–0.4 μg/100 g) is not high. All of the naturally occurring vitamin B_{12} in cow's milk is bonded to the transcobalamin, a protein that binds vitamin B_{12} in mammals (Fedosov *et al.,* 1996). Significant vitamin B_{12} 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 B_{12} 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 B_{12} that was initially contained in milk is restored.

- **Egg:** The amount of vitamin B_{12} 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 B_{12} from them. Comparatively to other animal food sources, vitamin B_{12} in eggs is typically poorly absorbed.

- **Shellfish:** Numerous shellfish are widely consumed. It is well known that mussels are good sources of vitamin B_{12}, with concentrations often exceeding 10 μg/100 g, because they absorb significant amounts of vitamin B_{12}-synthesizing bacteria from the water.

2. Vitamin B_{12} in Plant Food

Vegetables: It has been suggested that bamboo shoots are a good source of vitamin B_{12}. But it turns out that they don't have a lot of vitamin B_{12} in them. Cabbage, spinach, celery, garland chrysanthermum, lily bulb, and taro produced similar outcomes. These veggies could be able to absorb the vitamin B_{12} present in some organic fertilisers. Mozafar showed that adding cow manure, an organic fertiliser, dramatically raises the amount of vitamin B_{12} in spinach leaves and barley grains (Mozafar, 1994).

- **Tea Leaves and Drinks:** Different types of tea leaves contain significant levels of vitamin B_{12}: green tea (0.1-0.5 µg vitamin B_{12} 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 B_{12}–Fortified Cereals:** Ready-to-eat cereals that have been fortified with vitamin B_{12} are known to make up a significant amount of daily vitamin B_{12} intake. Several research teams hypothesised that consuming a morning cereal supplemented with folic acid, vitamin B_{12}, and vitamin B_6 would raise these vitamins' blood concentrations and lower plasma, total homocysteine levels in elderly populations.

3. **Stability in Foods:** Cyanocobalamin, often known as vitamin B_{12}, is a micronutrient that must be obtained through diet. However, vitamin B_{12} 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-B_{12} 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 *et al.,* 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 & Singhal, 2020).

4. **Vitamin B_{12} Bioavailability:** The low bioavailability of vitamin B_{12} 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 B_{12} 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 VB_{12} that is taken in. Which results in B_{12} deficiency in roughly 15% of the population (Ramalho *et al.,* 2020). The widespread consensus is that taking large amounts of vitamin B_{12} 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 B_{12} bioavailability that takes intake into account: log absorption 5 0.7694 3 log intake 2 0.9614 (EFSA NDA Panel, 2013). 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.

III. VITAMIN B_{12} DEFICIENCY

Symptoms of a vitamin B_{12} shortage might be neurological, psychological, or physical. Medication containing vitamin B_{12} can be used to treat it. Haematological and neurological problems might result from a B_{12} shortage (Stabler, 2013; Ankar & Kumar, 2022). Vitamin deficiency in vegans is mostly caused by insufficient food intake, and B_{12} 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 B_{12} malabsorption. Other reasons include pancreatic insufficiency, obstructive jaundice, bacterial overgrowth, parasite infestations, tropical sprue, inflammatory bowel illnesses, and celiac disease (Guéant, 2022).

Human body performs two process in order to absorb vitamin B_{12}. First, the hydrochloric acid in stomach dissolves the vitamin B_{12} in the food. Then, vitamin B_{12} mixes with a protein produced by stomach known as intrinsic factor that makes the digestive system to absorb vitamin B_{12}. Pernicious anaemia is a rare illness that prevents some people to produce intrinsic factor. As a result, they suffer from a vitamin B_{12} deficiency since body is unable to absorb vitamin B_{12} effectively. In older adults, vitamin B_{12} insufficiency is typical and has been linked to ischemic stroke (Yahn *et al.,* 2021). Humans require vitamin B_{12}, which is also a critical component of the human gut bacteria. In newborns who are solely breastfed, vitamin B_{12} 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 B_{12} and to rely on dietary sources of the vitamin (Boran *et al.,* 2020).

1. **Indicators for Vitamin B_{12}:** Combining four blood markers; methylmalonic acid (MMA) total homocysteine (tHcy), holotranscobalamin (holoTC), and total B_{12} is a novel way to assess vitamin B_{12} sufficiency. The formula for this combined B_{12} status indicator is $cB_{12}=\log10\ [(holoTC \cdot B_{12})/(MMA \cdot Hcy)]$ - (age factor) (Fedosov *et al.,* 2015). However, none of these indications alone have the best sensitivity or specificity for vitamin B_{12} deficiency, which is difficult to diagnose (Miller, 2018). It should be noted, nevertheless, that there is not perfect agreement on these definitions. Additionally, both total vitamin B_{12} 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 B_{12} deficiency, the reason for this is renal deficiency, whereas the causes of elevated homocysteine include folate and vitamin B_6 deficiencies, hypothyroidism, genetic disorders, and the use of medications that affect one-carbon metabolism and homocysteine.

   - **Total B_{12}:** Measurement of serum cobalamin is used to determine vitamin B_{12} levels. The most popular method is the measurement of vitamin B_{12} 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 B_{12} deficiency in 97 % of individuals. What serum cobalamin level would indicate subclinical insufficiency is unclear (Devalia *et al.,* 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 B_6 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 *et al.,* 2012).

2. **Health Concerns:** The most typical cause of severe vitamin B_{12} malabsorption is the autoimmune disease pernicious anaemia (Green *et al.,* 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 (Antony, 2018). Dietary vitamin B_{12} 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 B_{12} 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 & Baumgartner, 2018; Chittaranjan, 2020).

3. **Recommended Dietary Allowance:** A vital nutrient, vitamin B_{12} 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 B_{12} in human nutrition are animal products such meat, poultry, fish, eggs, and milk (Wolffenbuttel *et al.,* 2020). Vegans are more likely to experience nutritional deficiencies, particularly vitamin B_{12} deficiency. Although the lowest daily intake of B_{12} 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 B_{12} is based on the quantity required to maintain normal serum vitamin B_{12} levels and haematological status. The advised intake takes a 50% absorption rate into account. Adults require 2.4 µg of vitamin B_{12} daily. To meet their RDA, adults over 50 are advised to primarily consume foods fortified with vitamin B_{12} because 10 to 30% of older people may have trouble absorbing naturally occurring vitamin B_{12} (Brito *et al.,* 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**).

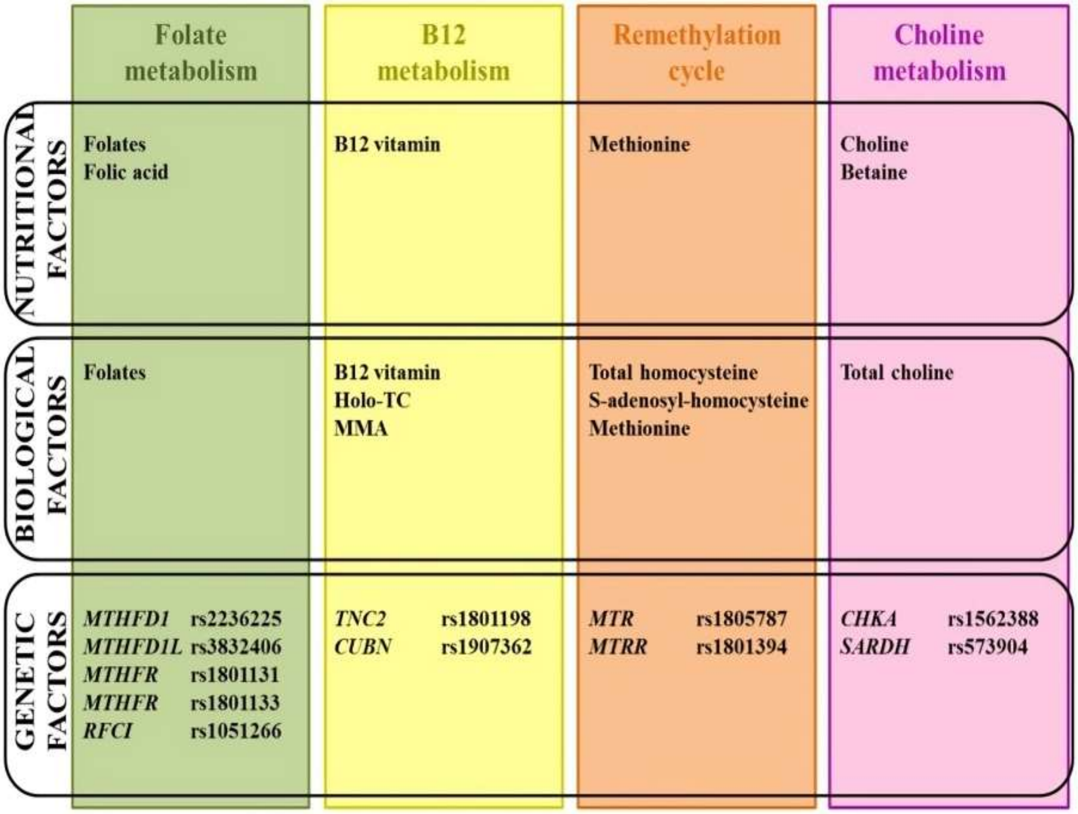
Table 1: Show Recommended Dietary Allowance (RDA) for children, boys and girls of different age group (Allen, 2018)

RDA for Children	1–3 years	0.9 µg/day of vitamin B_{12}
	4–8 years	1.2 µg/day of vitamin B_{12}
RDA for Boys	9–13 years	1.8 µg/day of vitamin B_{12}
	14–18 years	2.4 µg/day of vitamin B_{12}
RDA for Girls	9–13 years	1.8 µg/day of vitamin B_{12}
	14-18 years	2.4 µg/day of vitamin B_{12}

IV. 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 *et al.,* 2021). 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 B_{12} deficiency by 58% (Das *et al.,* 2019).

Food fortification programs have been conducted in several countries to overcome micronutrient deficiency (Dewi & Mahmudiono, 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 B_{12}. It was observed that Vitamin B_{12} concentration was highest in colostrum and gradually decreased over the first 3-4 months of lactation (Dror & Allen, 2018). Research has also shown that decreased B_{12} vitamin levels and increased total choline or homocysteine in maternal blood are associated with an elevated risk of Neural Tube Defects (NTDs) (Imbard *et al.,* 2013).



	Folate metabolism	B12 metabolism	Remethylation cycle	Choline metabolism
NUTRITIONAL FACTORS	Folates Folic acid	B12 vitamin	Methionine	Choline Betaine
BIOLOGICAL FACTORS	Folates	B12 vitamin Holo-TC MMA	Total homocysteine S-adenosyl-homocysteine Methionine	Total choline
GENETIC FACTORS	*MTHFD1* rs2236225 *MTHFD1L* rs3832406 *MTHFR* rs1801131 *MTHFR* rs1801133 *RFCI* rs1051266	*TNC2* rs1801198 *CUBN* rs1907362	*MTR* rs1805787 *MTRR* rs1801394	*CHKA* rs1562388 *SARDH* rs573904

Figure 6: Supplementation of Vitamin B_{12} (Adapted from Imbard *et al.,* 2013).

V. FORTIFICATION GUIDELINES

Although historically rare, vitamin B_{12} 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 B_{12} deficiency. These studies include data presented at the WHO Technical Consultation. This necessitates the fortification of vitamin B_{12} 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 (Das *et al.,* 2013; Allen*,* 2018; Detzel & Klassen-Wigger, 2020).

1. **Foods Fortified with Vitamin B_{12}:** A lack of vitamin B_{12} can cause severe issues and poor health. Vitamin B_{12} 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 B_{12} 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 B_{12} 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 B_{12} is 25 to 100 mcg, which should be met through supplements and fortified foods (NMCD database 2010; Mayo 2010; Allen, 2018). The RDA for vitamin B_{12} is shown in **Figure 7**.

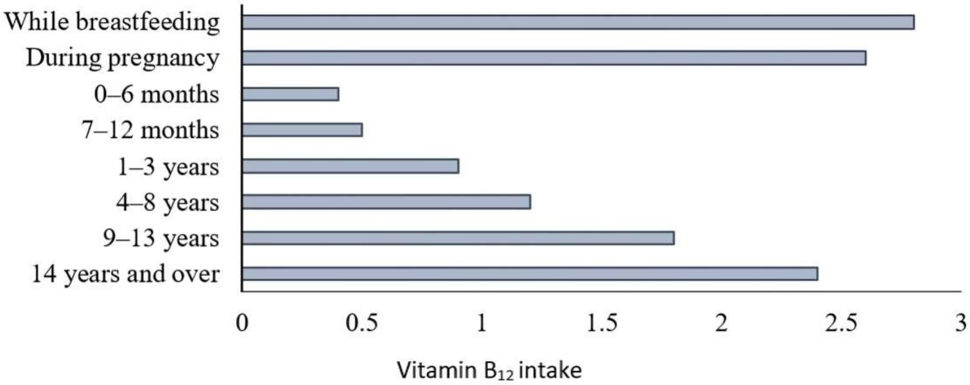


Figure 7: Recommended Dietary Allowances of Vitamin B12 for different Populations.

Since vitamin B_{12} 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 B_{12}. Yoghurt, milk, other dairy products, eggs, nutritional yeast, nori, tempeh, vitamin B_{12} supplements, and fortified meals are a few good sources of vitamin B_{12} besides animal sources (Mayo, 2010). The vitamin B_{12} 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 B_{12}, folate, iron, and vitamin A including the bran and whole wheat oats. Regular consumption of fortified cereals can help the body to raise vitamin B_{12} levels. Non-dairy milk that is fortified, such as soy and almond milk, does not naturally contain vitamin B_{12}; this vitamin is added during the fortification process. One cup of soy or almond milk contains 2.1 mcg of vitamin B_{12} daily (Didit *et al.,* 2018). **Table 3** explicitly shows the amount of vitamin B_{12} 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 B_{12} per 3/4 Cup	Vitamin B_{12} per 100g	Vitamin B_{12} per 200 Calories
	6.1μg (254% DV)	21μg (875% DV)	$_{12}$.8μg (535% DV)
Fortified non-dairy milk	Vitamin B_{12} per 16oz Glass	Vitamin B_{12} per 100g	Vitamin B_{12} per 200 Calories
	6μg (249% DV)	1.2μg (51% DV)	7.5μg (311% DV)

VI. VITAMIN B_{12} 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.

VII. CONCLUSION

The rationale behind vitamin B_{12} 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 B_{12} 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 B_{12} becomes more accessible, and its benefits are reaped by populations around the globe.

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