Is There a Carcinogenic Risk Attached to Vitamin B$_{12}$ Deficient Diets and What Should We Do About It? Reviewing the Facts

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The number of individuals partaking in veganism has increased sharply in the last decade. Therefore, it is critical to look at the implications of vegan diets for public health. Although there are multiple health benefits of a vegan diet, studies have also linked the diet with deficiencies in various micronutrients. This study focuses on vitamin B$_{12}$, because of its critical role in DNA synthesis and methylation. In light of these connections, a critical review of recent scientific literature is conducted to understand the effects of a B$_{12}$ deficient diet on the genome and epigenome, and whether it can give rise to cancer. It is observed that a B$_{12}$ deficiency leads to increased uracil misincorporation, leading to impaired DNA synthesis and genomic instability. The deficiency also leads to global hypomethylation of DNA, a hallmark of early carcinogenesis. The findings of this study highlight the need for increased awareness among vegans to ensure adequate B$_{12}$ intake through supplementation or consumption of fortified products as a preventative measure. Additionally, the biofortification of staple crops and an improved version of fermented products with increased B$_{12}$ content can be developed when inadequate intake seems otherwise inevitable.

1. Introduction

Although the concept of a plant-based diet is not novel, recently an impressive rise in individuals adopting a vegan diet has been observed globally. Veganism is defined as the practice of not consuming animal-derived products, such as meat, fish, poultry, eggs, dairy products, and honey.[1] Followers of the vegan diet are commonly referred to as "vegans" and this terminology will be used in this review. Taking the United States as an example, a recent study showed that 6% of the total population identified as followers of the vegan diet,[2] of which 11% are young adults (age 25–34).[3] Similarly, there was an increase of 45,0000 vegans in the United Kingdom in the last 5 years.[4] In Portugal, there were 12,0000 individuals who claimed to follow a plant-based diet in 2018, compared to a mere 30,000 in 2007.[2] These statistics could be attributed to studies linking a vegan diet with improved health, sustainability, and reducing the global carbon footprint.[3,5–8] Despite its benefits, plant-based diets tend to result in a deficiency of certain micronutrients, namely iron, zinc, calcium, vitamin D, omega-3 (*n*-3) fatty acids, iodine, and vitamin B$_{12}$ (B$_{12}$).[3]

This review will focus on B$_{12}$, also known as cobalamin, an essential micronutrient that is present in meat, fish, egg, and dairy products.[9,10] B$_{12}$ is produced by microorganisms that are present in the bowel of ruminants (e.g., cattle, sheep, etc.) and omnivorous animals (e.g., chicken, pigs). In aquatic environments such microorganisms are found in phytoplankton, which are consumed by fishes and crustaceans. Once absorbed in the small intestine, the vitamin is then stored in the liver and muscle of these animals or secreted into milk.[11] This water-soluble vitamin acts as a co-factor for methionine synthase (MS; the enzyme that catalyzes the conversion of homocysteine to methionine) and L-methylmalonyl-CoA mutase (the enzyme that catalyzes the conversion of methylmalonic acid (MMA) to succinyl-CoA). Thus, B$_{12}$ plays a crucial role in red blood cell production, neurologic development and function, DNA synthesis, and DNA methylation.[12] A B$_{12}$ deficiency is correlated with both aberrant DNA synthesis and methylation.[13] Errors in these physiological processes lead to a variety of clinical manifestations, resulting in megaloblastic anemia, neurological impairment, cognitive decline, and neurodevelopmental disorders.[14,15] Therefore, it is imperative to have sufficient amounts of B$_{12}$ in one's diet.

Multiple studies have shown that due to the dietary intake restrictions of a vegan diet, vegans have the highest risk of developing a B$_{12}$ deficiency, in comparison to those consuming other vegetarian diets or omnivorous diets. In these studies, a B$_{12}$ serum deficiency is defined as having less than 200 pmol L^{-1} of B$_{12}$.[16,17] In the United States, the prevalence of a B$_{12}$ deficiency in young adults (age 20–39) was less than 3%; however, amongst vegans, the prevalence was a stunning 43%.[16,18]

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Furthermore, individuals who adopted a vegan diet at a younger age were found to have a higher risk of depleted B_{12} levels later in life.[16] Having a deficiency at a young age could take a few years to trigger the onset of clinical manifestations.[19] Previous research focused heavily on the effects of maternal B_{12} status on neonatal development.[20–22] However, concurrent with the rising percentage of vegans among the younger demographic, research focus has shifted to how a B_{12} deficiency affects adult health.[15]

Recent studies have indicated a possible link between a B_{12} deficiency and the onset of cancer.[23,24] In 2019, cancer was considered one of the leading causes of death in high-income countries, with almost twice as many deaths as from cardiovascular diseases, the leading cause of death worldwide.[25] Despite the increasing amount of research, there is a substantial lack of systematic overviews regarding cancer as a potential clinical manifestation of a B_{12} deficiency. Therefore, this review aims to evaluate the effects of a B_{12} deficiency on both genome stability and the epigenome, providing a comprehensive overview of possible contributions to cancer development. We limited our sources to peer-reviewed scientific papers, with the exception of online news articles from reputable websites for vegans (e.g., The Vegan Society).

The number of vegans, especially of the younger demographic, is predicted to further increase in the coming years due to the heightened awareness of the impact of non-vegetarian, meat-based diets on greenhouse gasses emissions, leading to climate change.[3] Hence, it is critical to build an in-depth understanding of the metabolic implications of this global dietary trend on individual health. We should also recognize the need for appropriate prevention strategies to ensure adequate B_{12} intake, mitigating the potentially adverse effects on public health.

2. Vitamin B_{12}

Though the vegan diet has benefits, it also has been linked to a deficiency of multiple essential micronutrients, B_{12} (see **Figure 1**) amongst them.[3] As described above, B_{12} is a water-soluble vitamin found predominantly in animal derived dietary products. [14] B_{12} was first isolated in 1948 and later synthesized in 1973.[26] It is part of the corrinoid family, as its chemical structure includes a corrin ring. This ring is made up of four reduced pyrrole rings, which are linked together in a macro-cyclic ring, coordinately linked to a central cobalt atom. The structure of the corrin ring appears similar to the structure of heme in hemoglobin, apart from one less methylene bridge and the cobalt atom at its center. Also linked to the cobalt atom is a 5,6,-dimethylbenzimidazole nucleotide part of the molecule.[27] Four main active forms of B_{12} exist, cyanocobalamin, hydroxocobalamin, methylcobalamin ($CH_3 \bullet B_{12}$), and adenosylcobalamin; these differ in the "R" groups attached to the cobalt atom depicted in Figure 1. The three latter forms are typically found in animal products, while cyanocobalamin is the commercially available form used in supplements to prevent and treat a B_{12} deficiency.[28]

2.1. B_{12} Absorption

In the human body, B_{12} is extracted and absorbed from food in a series of steps that involve a complex network of proteins. Upon
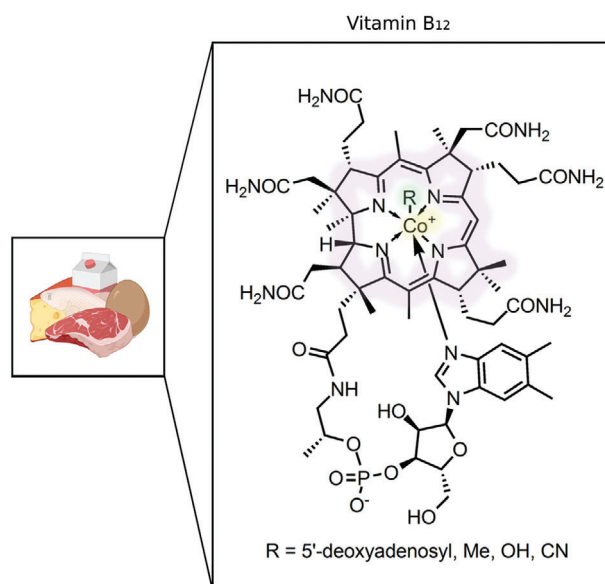

Figure 1. Chemical structure of B_{12}. The structure of the vitamin is centered around a corrin ring (highlighted in purple) coordinately linked to a center cobalt atom (highlighted in yellow). There are four forms of B_{12}, each differing in the functional group (highlighted in green) attached to the center cobalt atom. The R group could either be an adenosine (adenosylcobalamin), a methyl (methylcobalamin), a hydroxyl (hydroxocobalamin), or a cyanide group (cyanocobalamin), as indicated. Based on Jägerstad and Arkbåge, 2003.[27]

dietary intake, gastric acid and pepsin in the stomach release B_{12} from the proteins it was previously bound to. The vitamin then proceeds to attach to haptocorrin (HC), a glycoprotein primarily secreted by the salivary gland, until it reaches the duodenum. In the duodenum, HC is digested by pancreatic proteases, in the process releasing B_{12}. Once released, B_{12} binds to intrinsic factor (IF), a glycoprotein secreted by gastric parietal cells. Binding to IF is essential for the absorption of B_{12} to occur, and an absence of IF leads to only 1–2% absorption of B_{12}. Next, the vitamin is absorbed in the terminal ileum by IF receptors. [26,28,29] Once absorbed, B_{12} complexes with transcobalamin (TC) in the enterocyte, before release into the circulation (**Figure 2**). This complex allows B_{12} to be delivered to the tissues; 50% of B_{12} is taken up by the liver.[30] The body stores about 3–4 mg of B_{12} in the liver. This is a substantial amount and, if all dietary B_{12} uptake is discontinued, is sufficient for approximately 3 to 6 years before symptoms begin manifesting clinically. Thus, a B_{12} deficiency is relatively slow to develop.[31] However, upon bariatric surgery, symptoms are observed earlier (most likely by a combination of interrupting the enterohepatic cycle and presurgical reductions in food intake).

2.2. The Role of B_{12}

B_{12} is a fundamental nutrient, especially for two significant processes in the body, namely DNA synthesis and DNA methylation. Both processes form part of the one-carbon metabolism pathway, a set of linked cyclical cytosolic reactions that result in the formation of methionine from homocysteine and donation
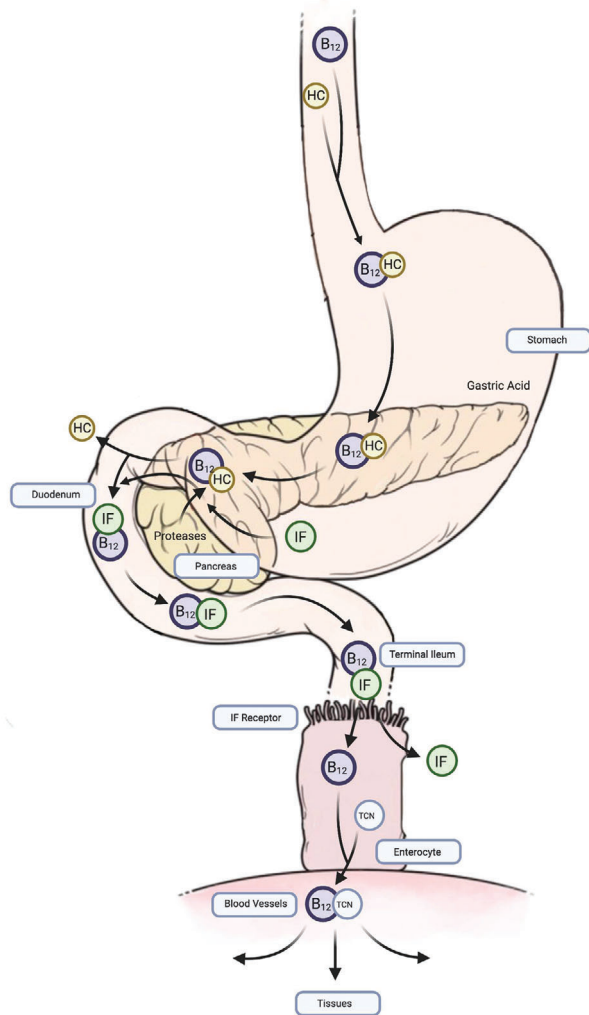
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Figure 2. Absorption of B_{12}. B_{12} gets extracted from food in the stomach by gastric juices and pepsin. It then binds to HC. Once the B_{12}-HC complex reaches the duodenum, HC is degraded by pancreatic proteases, and B_{12} binds to IF. In the terminal ileum, the B_{12}-IF complex is absorbed by the IF receptors, causing B_{12} to be released from IF. In the enterocyte, B_{12} then complexes with TC and is released into the circulation. This complex allows B_{12} to be absorbed by the tissues, especially the liver. Based on Lieberman and Marks, 2017.[30]

of methyl groups. Methionine is crucial for the regeneration of S-adenosylmethionine (SAM), the universal methyl donor; combining methionine with adenosine triphosphate (ATP) results in the "activated" SAM molecule. SAM is responsible for reactions that require the transfer of methyl groups to oxygen or nitrogen atoms of the acceptor molecule.[32] When SAM donates a methyl group, it consequently forms S-adenosylhomocysteine (SAH), which can then be further hydrolyzed to form homocysteine and adenosine.[29] SAM is critical for the maintenance of methylation patterns in DNA.[33a,b]

B_{12} works in unison with vitamin B_9 (folate), vitamin B_6, and vitamin B_2 in the one-carbon metabolism pathway.[13,34] All four B vitamins act as cofactors for enzymes which participate in the pathway. The coenzyme form of folate, tetrahydrofo-

late (THF), acts as the primary acceptor for one-carbon groups. A methyl group from serine is transferred to THF in order to form 5,10-methylenetetrahydrofolate (5,10-MTHF). This process is catalyzed by serine hydroxymethyltransferase with vitamin B_6 as a cofactor. 5,10-MTHF can then be further utilized in the de novo formation of thymidine, the synthesis of purines, as well as in the re-synthesis of methionine.[13] B_{12}, in the form of $CH_3\bullet B_{12}$, is a cofactor for the enzyme MS, the enzyme responsible for catalyzing the central reaction of the one-carbon pathway. The steps are as follows: (1) a methyl group from 5-methyltetrahydrofolate (5-MTHF), the predominant form of dietary folate, is transferred to B_{12} to form $CH_3\bullet B_{12}$; (2) this methyl group is then transferred to homocysteine by MS, resulting in the formation of methionine; (3) the remaining tetrahydrofolate (THF) can then be converted to 5,10-MTHF, which can be further reduced by methylenetetrahydrofolate reductase (MTHFR), a vitamin B_2 containing enzyme, to become 5-MTHF (**Figure 3**).[13,15,26,28]

3. B_{12} Deficiency

Due to the essential role the vitamin plays, insufficient levels of B_{12} can be highly detrimental to one's health.[19] The European Food Safety Authority (EFSA) Panel on Dietetic Products, Nutrition and Allergies concluded that the average adult requires a daily intake of 4 µg, and a pregnant woman requires up to 6 µg of B_{12}.[35] Despite this, individual intake typically consists of 2.4 µg day^{-1} of B_{12}, from which approximately 50–60% of the vitamin is absorbed.[32,36] Typically, a healthy individual has a serum level of approximately 200–900 pg mL^{-1} (147-662 pmol L^{-1}) of B_{12}. A patient is considered deficient when they have less than 200 pg mL^{-1} of serum B_{12}, and critically deficient when they have 150 pg mL^{-1} (111 pmol L^{-1}) or less.[37] However, it should be noted that a B_{12} deficiency may be difficult to diagnose and the lower limit of B_{12} serum level may vary.[38] Also, liver damage, e.g., stemming from alcoholism, as well as systemic low-grade inflammation, may temporarily allow B_{12} serum levels to rise due to loss from liver stores, masking real deficiency.[39]

There are multiple ways to try to assess B_{12} status. Largely, it is determined through an analysis of total serum B_{12}. However, this method often lacks specificity in the early stages of the deficiency. Due to this, other tests scoring B_{12} analytes have been developed in order to assess B_{12} status. When B_{12} binds to TC it is called holo-TC.[40] Some studies observed that the serum holo-TC concentrations sufficiently reflect B_{12} status with an increased degree of specificity compared to measuring serum B_{12} levels. Of note, in plasma, B_{12} is not only found bound to TC, but to HC as well. In addition, multiple studies that examine the effects of a B_{12} deficiency also measure homocysteine and MMA concentrations (which will increase because the enzymes converting these compounds need B_{12} as a co-factor), to achieve higher accuracy of results, as well.[39,41–43] In order to strenuously evaluate and assess the possibilities of a B_{12} deficiency, researchers may thus choose to conduct multiple analyte testing and test for two or more measurements of B_{12} status simultaneously. An improved indicator of B_{12} status analysis seems to have been proposed by Fedosov et al. (2015), encompassing combined measurements of serum B_{12}, plasma holo-TC, homocysteine, and MMA.[44] Altogether, an accurate diagnosis of this deficiency is necessary to further reveal

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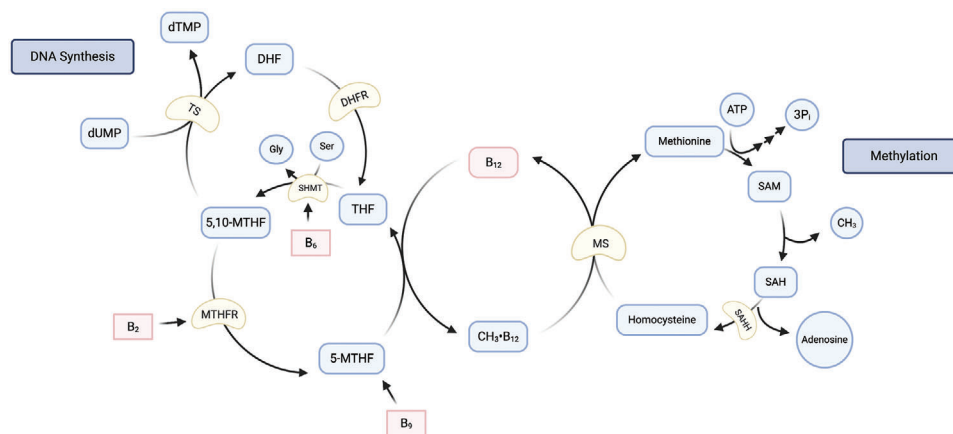Food Research**
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Figure 3. The one-carbon metabolism pathway. Serine donates a methyl group to THF to create 5,10-MTHF, leaving glycine. This (reversible) reaction is catalyzed by serine hydroxymethyltransferase (SHMT), using vitamin B_6 as a cofactor. 5,10-MTHF is converted to 5-MTHF by methylenetetrahydrofolate reductase (MTHFR), with vitamin B_2 as a cofactor and NADPH as electron donor (not depicted). 5-MTHF, which can also be acquired from dietary folate (B_9), donates a methyl group to B_{12} to create $CH_3 \bullet B_{12}$, a cofactor for the enzyme methionine synthase (MS), which catalyzes the conversion of homocysteine to methionine, by in turn transferring this CH_3 group. Methionine is combined with ATP to become SAM, which maintains methylation reactions. When SAM donates a methyl group, it becomes SAH, which can be further hydrolyzed to form homocysteine and adenosine (by S-adenosylhomocysteine hydrolase; SAHH). THF, left after $CH_3 \bullet B_{12}$ creation, can be converted back into 5,10-MTHF. 5,10-MTHF is a carbon donor for the enzyme thymidylate synthase (TS), which catalyzes the conversion of deoxyuridine monophosphate (dUMP) to deoxythymidine monophosphate (dTMP); hence, playing a role in de novo DNA synthesis. Upon this reaction, 5,10-MTFH is converted into dihydrofolate (DHF), which can be reduced by dihydrofolate reductase (DHFR), using NADPH, regenerating THF. B-family vitamins coming from food indicated in red. Based on Friso et al., 2017 and Green et al., 2017.[13,15]

the presence or absence of correlations between potential clinical manifestations and respective target groups.

4. Vitamin B_{12} and Carcinogenesis

Carcinomas are cancers derived from epithelial tissue, which make up approximately 80% of all human cancer cases.[45] Past studies have postulated that there might be a causal relationship between a localized insufficiency of B_{12} and carcinomas.[46] In support of this hypothesis, Wu et al. (1999) performed a nested case-control study and saw that in both menopausal and postmenopausal breast cancer patients, there were significantly lower concentrations of serum B_{12}, and patients who possessed the lowest B_{12} had an increased risk of breast cancer.[47] This inverse association of serum levels of B_{12} and the risk of cancer is also evident in patients with cervical cancer. Pathak et al. (2014) found that cervical cancer patients had significantly lower serum B_{12} concentrations when compared to control patients.[48] Furthermore, there was a significantly higher risk of human papillomavirus (HPV) infection when B_{12} levels were insufficient. HPV infection has been implicated in the etiology of 70% of cervical cancers. [49]

This inverse relationship between B_{12} serum concentration and cancer risk is further seen in cancers of the gastrointestinal tract,[50,51] the liver,[33a,b] the colon, and the rectum.[52,53] An overview of studies into relationships between B_{12} serum concentrations and cancer risk is given in **Table 1**.

Certain studies also suggest onco-protective properties of B_{12}. Zhang et al. (2003) recorded that higher levels of B_{12} (>572.7 pg mL^{-1}) were significantly associated with a lower risk of breast cancer amongst premenopausal women.[39] Similar results were obtained in a cervical cancer study. Piyathilake et al. (2009) reported that sufficient concentrations of B_{12} ($\geq$200.6 pg mL^{-1})

correlated with a 50% reduction in cervical intraepithelial neoplasia (CIN) grade 2 diagnosis.[54] Studies have implicated CIN in the pathogenesis of cervical cancer. In essence, low-grade lesions (i.e., CIN1) are primarily associated with benign tumors and higher-grade lesions (i.e., CIN2, CIN3) are associated with highly malignant cervical cancer cases.[55] Interestingly, women who had low plasma folate concentrations but sufficient concentrations of B_{12} remained 50% less likely to be diagnosed as CIN2 positive.[54] This inverse relationship is also apparent with regards to colorectal cancer risk and was further highlighted in a dose-response meta-analysis done by Sun et al. (2015). These authors observed a significant relationship between the dietary intake of B_{12} and colorectal cancer risk. They found a slight reduction in colorectal cancer risk with every 4.5 µg increase in dietary B_{12} (µg d^{-1}) intake.[53] Research by Banjari and Hjartaker (2018) further confirmed this observation.[56] Last, we should point out that MMA, which increases upon B_{12} deficiency, seems generally upregulated in serum of the elderly and, as was very recently found, might mediate tumor progression via its induction of SOX4 expression.[57]

5. Possible Mechanisms

Two possible mechanisms could especially explain the role of B_{12} in the etiology of some cancers. As previously mentioned, B_{12} plays a role in the central pathway of the one-carbon metabolism cycle, which results in the formation of crucial components for the maintenance of DNA synthesis, repair, and methylation. An insufficient amount of B_{12} could lead to a disruption in this pathway, hence resulting in potentially adverse effects.[32] When B_{12} levels are inadequate, MS activity is downregulated, hence impairing the remethylation of homocysteine to methionine. As a result, there is a decrease in methionine and subsequently SAM,

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Table 1. Studies highlighting a relationship between B_{12} and cancer risk.

Cancer type	Author (Year)	Outcome	Serum B_{12} concentration [pg mL^{-1}]
Breast	Wu et al. (1999) [47]	• Significantly higher risk of breast cancer	<280
Cervical	Pathak et al. (2014) [48]	• Significant inverse correlation between homocysteine concentration and B_{12} • Lower serum B_{12} in patients with CIN or cervical cancer • Higher risk of HPV infection	(a) 376.54 a) (b) 341.36
Gastric	Murphy et al. (2016) [50]	• Significantly increased risk of non-cardia gastric adenocarcinoma and gastric carcinoid tumors	<200 b)
	Miranti et al. (2017) [51]	• 5.8-fold times significantly increased risk of developing non-cardia gastric adenocarcinoma • B_{12} can serve as a biomarker for atrophic gastritis that precedes non-cardia gastric adenocarcinoma	<291–300
Hepatic	Brunaud et al. (2003) c) [33a,b]	• Reduced MS activity and DNA methylation • Increased risk of hepatocellular carcinoma	<200
Colorectal	Dahlin et al. (2008) [52]	• Inverse association between plasma B_{12} levels and risk of rectal cancer	≤221
	Sun et al. (2015) [53]	• Insignificant reduction in colorectal cancer risk when B_{12} intake was below 12.8 µg d^{-1} • A slight reduction of colorectal cancer risk with every 4.5 µg d^{-1} increment vitamin intake	- d)

a) Median serum B_{12} concentrations of (a) patients who were diagnosed with CIN while (b) correlates with patients diagnosed with cervical cancer; b) All subjects were patients with pernicious anemia; c) Animal study with F344 rats; d) Average serum B_{12} concentration not indicated
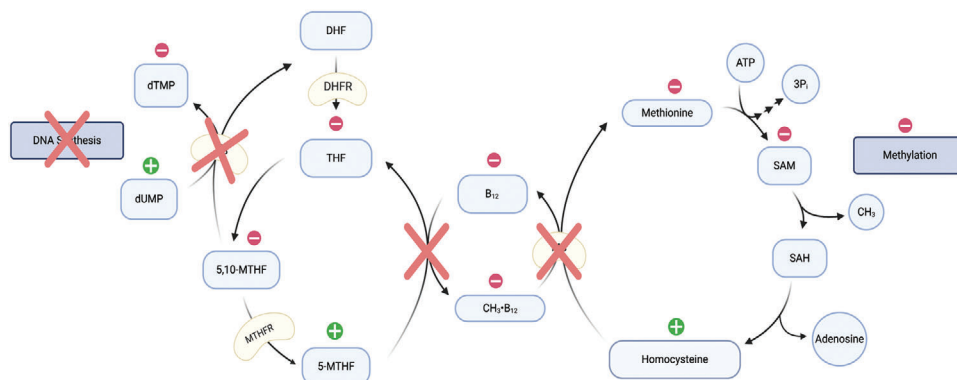

Figure 4. The methyl-trap hypothesis. B_{12} deficiency impairs MS activity. MS is downregulated and homocysteine cannot be converted to methionine, hence resulting in an increase of homocysteine. This leads to a decrease in SAM production and global hypomethylation. Reduced SAM levels also manifest as a deficiency in THF. This results in a decrease of 5,10-MTHF, the carbon donor for TS and an accumulation of dUMP, which may lead to increased misincorporation of uracil into DNA strands and halted DNA synthesis. Compare with Figure 3. Green "+" indicates rises in steady state concentrations; Red "–" in general indicates lower steady state concentrations; Based on Choi, 1999 and Lieberman and Marks, 2013.[58,30]

as well as a concomitant increase in homocysteine. Such a down-regulation of SAM could have detrimental effects for both DNA synthesis and repair, as well as for DNA methylation, possibly explaining some of the findings summarized in Tables 1 and 2.

5.1. B_{12} and DNA Synthesis

A deficiency in B_{12} disrupts deoxythymidine monophosphate (dTMP) biosynthesis, necessary for DNA synthesis, due to the downregulation of essential precursors for dTMP synthesis. Reduced SAM levels, as a result of deficient B_{12}, manifest as a deficiency in THF as the methyl group from 5-MTHF becomes "trapped"; this model is called the methyl-trap hypothesis.[30,58]

Consequently, this results in a decrease of 5,10-MTHF, the precursor to 5-MTHF and the carbon donor for thymidylate synthase (TS); see **Figure 4**. TS methylates deoxyuridine monophosphate (dUMP) to become dTMP, which is further phosphorylated to become deoxythymidine triphosphate (dTTP), an essential precursor for DNA synthesis and repair.[59,60] Thus, when the enzyme cannot function properly, it leads to a downregulation of deoxythymidine production and an imbalance in the dUTP/dTTP ratio, which could culminate in an increase of uracil misincorporation in DNA (again, see Figure 4).[61] When uracil is misincorporated, it results in the recruitment of the enzyme uracil-DNA glycosylase that can recognize and excise the incorrect base by creating single-strand or double-strand breaks (DSBs) in the DNA.[62] Repair pathways for DSBs are difficult and error-prone;

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thus, leading to further complications such as genomic instability, increased mutagenesis, chromosomal breakages, and eventually apoptosis.[63] All of these enhance an individual's susceptibility to developing cancer.[50] Hence, deficient B_{12} levels could lead to a domino effect, culminating in an increased risk of developing cancer.

The presence of chromosomal damages was first seen in the form of "Howell-Jolly bodies" in erythrocytes of patients with megaloblastic anemia, a clinical manifestation of a B_{12} deficiency.[19] Other studies (both in vivo and in vitro) have also highlighted the association of low serum B_{12} with an increase of chromosome damage and micronucleus formation.[41,64] An animal study conducted by Choi et al. (2004) illustrated a causal relationship between a B_{12} deficiency, anomalies in base substitution, and colorectal carcinogenesis. The study showed that in the colonic mucosa of rats, after 10 weeks of consuming a diet low in B_{12}, there was a 105% increase of uracil incorporation in colonic DNA. In order to establish an independent association, dietary folate and total folate was measured throughout the experiment. No depletion of folate in the colon was evident; thus, supporting the assumption that the base-insertion anomalies occurred independently of folate levels.[65]

5.2. Vitamin B_{12} and DNA Methylation

In addition to effects in DNA synthesis, insufficiency of B_{12} also leads to aberrant DNA methylation. DNA cytosine methylation is an epigenetic mechanism which involves the transfer of a methyl group to the 5-carbon position of CpG dinucleotides by DNA methyltransferases (DNMTs), the activity of which is stimulated by SAM. CpG dinucleotides refer to parts of the genome in which a cytosine nucleotide is immediately followed by a guanine nucleotide in the 5' to 3' direction.[66] Chemical exposure and nutritional status have both been implicated in the alteration of methylation patterns.[67] Broadly speaking, when DNA is hypermethylated, chromatin is compacted and consequently, locally encoded genes are no longer expressed. Conversely, when DNA is hypomethylated, chromatin unravels, and genes can become expressed.[13] This is particularly dangerous as it could result in the activation of transposable elements,[23] which are highly mutagenic and have been linked with multiple instances of cancer.[68] Hence, dysregulation of this process can result in changes in gene expression independent of the primary DNA sequence.[13] When B_{12} is lacking, MS is unable to catalyze the conversion of homocysteine to methionine, leading to an up-regulation of homocysteine, and subsequently reduced synthesis of SAM (Figure 4). This limits available methyl groups and results in global hypomethylation of DNA, which may result in changes in gene expression. [13,33a,b] Global hypomethylation has been proven to be a risk factor for a variety of cancer types and is commonly seen during early carcinogenesis. [69–72]

The causative association of B_{12}, global hypomethylation, and carcinogenesis has been studied extensively with regards to cervical cancer. As stated previously, HPV infection has been heavily implicated in the etiology of cervical cancer. However, it is important to note that only about 10% of all HPV infection cases lead to the development of CIN and only 8% of these cases eventually lead to cervical cancer. The transformative ability of HPV

relies heavily on the expression of two viral oncoproteins, E6 and E7. In vitro studies have shown that cervical cancer cells undergo apoptosis when E6 and E7 are not expressed.[73] According to Huang et al. (2018), B_{12} and folate work synergistically to maintain the methylation of the HPV E6 promoter and enhancer; thus, blocking the integration of HPV into the host genome.[10] When B_{12} is deficient, a decrease in methylation of HPV-16 E6 promoter and enhancer sites occurs, as reported by Piyathilake et al. (2014).[74] Of note, HPV-16 is one of the most frequent causative strains for cervical and anal cancer.[75] It was observed that patients with less than 406.58 pg mL^{-1} of B_{12} displayed a substantial decrease in HPV-16 E6 methylation and were 37% more likely to develop CIN3. Inversely, a high concentration of serum B_{12} led to an increase of methylation of the E6 promoter and a 60% reduction of being diagnosed with CIN2. Again, a weak correlation between serum B_{12} and serum folate in this experiment also seems to establish that the two nutrients work independently in modulating the methylation of HPV and increasing the risk of CIN.[73] Similarly, Ragsudha et al. (2011) found that having deficient B_{12} and folate levels increased the risk of developing CIN1 (about 15-fold) and cervical cancer (approximately 9-fold).[76]

Comparable trends of aberrant methylation have been identified in cases of hepatic, [33a,b] lung, [77,78] colorectal, [23,65] and head and neck cancer (**Table 2**).[79] Here, it should be pointed out that in this last example the correlation between B_{12} levels, methylation and cancer risk is not straight forward. In this instance, high B_{12} intake correlated with lower amounts of local (inactivating) tumor suppressor gene methylation, and thus lower cancer risk.

6. Mitigating and Preventative Treatments

The evidence mentioned, illustrates a possible link between cancer and B_{12} deficiencies. Thus, we want to highlight possible preventative and mitigating treatments for the deficiency. As an individual's capability of absorbing B_{12} deteriorates with age, it might be vital to begin preventative measures early.[80] Here, it must be said that to date, there are no well controlled studies showing that B_{12} supplements, as such, lower the risk of cancer in general and studies of different cancer types give conflicting results. [81,82] However, extensive comparative studies regarding oncogenesis specifically in vegans are not available either.

To successfully apply early intervention, a diagnostic system for a B_{12} deficiency is important. Presently, the available on-demand tests to diagnose the deficiency are serum B_{12}, homocysteine, and MMA blood tests (see above). However, these diagnostic measures are typically only recommended for elderly patients or patients who demonstrate symptoms consistent with a deficiency.[12] In light of the shifted trend of diet, incorporation of such a diagnostic test in the routine blood analysis for teenagers and young adults, in case they follow a restrictive diet, would in principle allow for effective mitigation and potentially prevent clinical impacts of B_{12} deficiencies at later (st)ages.

As for preventative measures, there are currently two techniques available to avert a B_{12} deficiency: supplementation and fortification. With regards to B_{12} supplementation, there are four routes of administration utilized: oral, nasal, sublingual, and intramuscular.[28] The United States predominantly utilizes B_{12} in the form of cyanocobalamin, while hydroxocobalamin is predominantly used in Europe. [83] Originally, the intramuscular

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Table 2. Studies highlighting correlations between B_{12} deficiency and aberrant DNA methylation.

Cancer type	Author (Year)	Outcome
Cervical	Piyathilake et al. (2014)[74]	• Patients with less than 406.58 pg mL^{-1} of B_{12} had less HPV-16 E 6 promoter methylation • 37% chance more likely to develop CIN3 • A high concentration of serum B_{12} led to increased methylation of E6 promoter and 60% reduction in risk of being diagnosed with CIN2 • Weak correlation between serum B_{12} and folate
	Ragasudha et al. (2012)[76]	• <160 pm mL^{-1} B_{12} serum concentration led to 14.9 times increased risk of developing CIN1 • <160 pm mL^{-1} B_{12} serum concentration led to 8.72 times increased risk of developing cervical cancer • Dependent on low folate levels
Hepatic	Brunaud et al. (2003) [33 a-b]	• Low B_{12} activity led to reduced methionine synthase activity • Low methionine synthase activity and low B_{12} led to DNA hypomethylation • Livers of rats fed a B_{12} deficient diet showed methylation patterns similar to animals exposed to chemical carcinogens
Lung	Piyathilake et al. (2000)[77]	• Localized deficiency of folate and B_{12} made cells more susceptible to carcinogens present in tobacco smoke • Smokers have lower serum B_{12} levels than non-smokers • B_{12} levels are lower in squamous cancer cells (3.98 ± 1.3 pg μg^{-1} DNA) than in adjacent non-infected tissue (8.83 ± 1.3 pg μg^{-1}) • Lower DNA methylation in squamous cell cancer cells • Decreased activity of MTHR
	Johanning et al. (2002)[78]	• Lung squamous cell cancer tissues had localized deficiencies of both folate and B_{12} • These cells also had global DNA hypomethylation
Colorectal	Choi et al. (2004)[65]	• Impaired DNA methylation was present in colonic tissue of colorectal cancer cells in rats • Supplementation of B_{12} alleviated the deficiency after 10 weeks
	Hasan et al. (2019)[23]	• B_{12} led to increased homocysteine levels • Increased homocysteine levels led to an increase of cellular proliferation in Caco-2 cell lines[a]
Head and Neck	Colacino et al. (2012)[79]	• Individuals with high B_{12} intake (32 μg d^{-1}) showed the least amount of tumor suppressor gene methylation

[a] A cell line of human epithelial colorectal adenocarcinoma cells.

delivery was the preferred method of supplementation for a B_{12} deficiency, and remains such for clinical B_{12} deficiencies caused by malabsorption. However, due to the invasive nature of an intramuscular injection, and its dependency on the patient having access to a medical facility, as well as higher costs, this method can lead to low compliance.[83,84]

Currently, oral B_{12} supplements are the preferred route of B_{12} supplementation for B_{12} deficiencies that result from nutritional deficiencies or low consumption. Some studies have found that B_{12} supplementation via oral administration (whether cyanocobalamin or hydroxocobalamin) had similar efficacies as intramuscular injections.[85,86] However, as these studies were limited in number and quality, doubts regarding the replacement of intramuscular injections linger. A widely cited panel by The Institute of Medicine (US) Standing Committee on the Scientific Evaluation of Dietary Reference Intakes (1998) suggests that a small dosage of 5 μg per day of oral cyanocobalamin at a time would be sufficient in ameliorating the deficiency.[87] Others have suggested that rather a daily oral dosage of 10 μg of B_{12} is sufficient.[4] Despite the difference in numbers, supplementation is generally advised for individuals who consume a diet low in B_{12} and may have a positive impact on public health [88] and possibly reduce the risk of developing cancer (but see above).[61]

Although not commonly used, B_{12} supplements can be administered nasally and sublingually as well. Results from these two administration methods have been positive in ameliorating a B_{12} deficiency. [89–91] Despite initial success, more research needs to be done to further test the efficacy and effectiveness of these methods in comparison with that of oral B_{12} supplements and B_{12} injections. Furthermore, vegans may choose to consume non-animal B_{12} supplements, such as those derived from algae. However, supplements made from algae (e.g., spirulina) have been under debate, and several studies have found that many supplements produced from cyanobacteria can be labeled as "pseudovitamins", without bioavailability for humans. [34,92] Whether such supplements might further complicate clinical presentations by a residual influence on serum detection and/or unpredictable effects on B_{12} dependent pathways remains to be seen.

Another method of ensuring adequate intake of B_{12} for individuals partaking in a vegan diet would be consuming B_{12} fortified food products. Typically, fortification of food is preferred over oral supplementation due to its cost-effectiveness.[93] Currently, no country has implemented a fortification plan for B_{12},[94] but products such as nutritional yeast, B_{12} fortified plant-based milk, and a variety of meat analogues have been marketed to successfully alleviate a possible B_{12} deficiency.[95] In light of the lack of any structural implementation so far, concomitant with the rising percentage of individuals partaking in a vegan diet, concerns have been raised regarding the need for a fortification plan.[96] One specific problem associated with general fortification of food must be mentioned: for those with specific B_{12} resorption issues (see above) normal serum B_{12} levels might mask cellular deficiencies. This does not invalidate the need for fortification, but physicians should be much more acutely aware of this. When,

even though serum B_{12} levels react positively to diet changes, typical neurocognitive symptoms indicating a deficiency persist, B_{12} might still be the problem. Future development of a robust B_{12} dependent enzymatic assay with cell extracts might put a stop to such uncertainties.

Different research groups have proposed different vehicles for B_{12} fortification to see which option would be the most feasible. Winkels et al. (2008) suggested that the implementation of flour co-fortified with 9.6 µg B_{12} and 138 µg folic acid would be beneficial in raising serum B_{12} concentrations and providing necessary dietary requirements. They found that after 12 weeks, the consumption of fortified bread successfully improved serum B_{12} levels by 49%, decreased homocysteine concentrations by 13%, and MMA concentrations by 10%.[97] Comparably, Siebert et al. (2017) looked into fortifying toothpaste with B_{12} for vegans in order to ensure adequate intake of the vitamin and found similar results. In addition to high compliance within groups of participants, the toothpaste was successful in raising both B_{12} serum concentrations, while simultaneously lowering homocysteine and MMA concentrations.[91] Further efforts have also been made in fortifying tempeh; a traditional, widely consumed, Indonesian food product made from fermented soybeans.[98]

7. Limiting Factors and Suggestions for Future Research

It is worth noting that although a B_{12} deficient diet seems to have carcinogenic effects, there exists contradictory research regarding its onco-protective nature. Hence, the overconsumption of B_{12} is not recommended.[99–101] Some studies have suggested that once a threshold value is reached, a diet high in B_{12} could potentially lead to carcinogenesis as well. Patients who had greater than 1000 pmol of serum B_{12} (∼50% above the currently used upper "healthy" limit) had a higher risk of developing solid cancer.[102,103] A potential explanation for these findings could be that higher B_{12} levels led to the hypermethylation, and subsequently, the silencing of certain key genes (i.e., tumor suppressor genes; but see above). Local hypermethylation of promoter regions in tumor-suppressor genes, genes involved in cell adhesion or apoptosis, and genes which encode for upstream regulators of essential processes, has been implicated to play a critical role in carcinogenesis.[104] Hence, in the future, a follow-up study should be conducted to see whether high B_{12} consumption past a certain threshold could prove to be cytotoxic and/or carcinogenic. Therefore, a meta-analysis of the available global data or a large-scale study to reach a consensus of the threshold level would be useful. Furthermore, relevant underlying molecular processes should be investigated. Here, a first, important but difficult question is of course whether such high levels of B_{12} are indeed carcinogenic/toxic by themselves, or can, e.g., be understood as markers for excessive food intake (with or without toxic contaminants).

In light of the potential implementation of diagnostic approaches to detect B_{12} deficiencies, currently the techniques generally used are heavily based on serum B_{12} levels. However, this is problematic as it was found to be unreliable in diagnosing certain clinical manifestations of a B_{12} deficiency.[12] A further improvement of this diagnostic system would be beneficial in accurately mapping deficient patients and developing a potential mitigation strategy.

Another limitation is related to the greatly interconnected role B_{12} and folate play in the one-carbon metabolism. Due to this, it is difficult to establish whether B_{12} works independently in potentially promoting or impeding carcinogenesis, or synergistically with folate. This is of particular interest with regards to a vegan diet, which is high in folate intake, while being deficient in B_{12}.[105] Additionally, studies have reported that high folate concentration could mask the symptoms of a B_{12} deficiency; thus, making it harder to diagnose quickly.[106] Future studies should look into the effects of a diet high in folate and deficient in B_{12} and whether high folate intake could compensate for the lack of B_{12} and/or rather mask an existing deficiency.

Moreover, with regards to opportunities for future research, concurrent with the rise of further improved genome editing technology, a potential area of future research would be the application of these innovations to biofortify staple foods of the vegan diet with B_{12}. This might prove to be a challenge as plants are not capable of synthesizing B_{12} on their own; thus, there would need to be a symbiosis between the staple crop of choice and B_{12} producing bacteria.[107,108] Tweaking the relevant genes of the microbiome genome may improve B_{12} levels.

Comprehensive knowledge of the epigenome and its correlation with disease onset, developing a better diagnostic method for B_{12} deficiency, and establishing further mitigation and prevention strategies, while critically comparing B_{12} sources, may save some of the future generations of vegans from potentially developing cancer and other major diseases.

8. Conclusion

A growing body of research has shown that insufficient intake of B_{12} may have a role in carcinogenesis. Evidence indicates that a vegan diet can lead to a B_{12} deficiency and could possibly inadvertently lead to cancer. This is due to the critical role B_{12} plays in regulating crucial physiological processes, such as DNA synthesis and DNA methylation. A deficiency in B_{12} henceforth leads to base-substitution anomalies and aberrant methylation patterns, leading to genomic instability and abnormal gene expression. Both have been implicated in the development of multiple forms of cancer. Due to the detrimental effects of a B_{12} deficiency, individuals who partake in a strict vegan diet need to ensure adequate intake through supplementation and/or the consumption of fortified products. However, in light of the existing evidence, the complex correlation between B_{12} and cancer needs further in-depth studies. This is in part due to the multifactorial nature of cancer, the role of B_{12} in MMA conversion, and the interconnected nature of B_{12} and the other B vitamins.

Conflict of Interest

The authors declare no conflict of interest.

Data Availability Statement

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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