Vitamin B12 Deficiency and The Role It Plays in Disease Causing DNA Mutation

Luke Flood
Appalachian State University
Faculty Mentor: Carol Babyak
Appalachian State University

ABSTRACT
A review of how vitamin B12 is used by the human body and the role it plays in DNA mutation is presented. Vitamin B12 is a compound needed for healthy function of the human body but produced downstream of the point of absorption in the digestive system, rendering it unusable. One may become B12 deficient in a variety of ways including lack of intake, disruption of B12 absorption due to disease, or disruption of cellular B12 levels by excess alcohol consumption. Deficient levels of B12 result in hypomethylation of DNA due to improper function of the methionine synthesis pathway in which B12 performs a coenzymatic function. Hypomethylation of DNA may lead to diseases including atherosclerosis, acute myocardial infarction, and a wide variety of cancers.
Vitamins are a large class of compounds necessary for life but not synthesized by the human body, and therefore need to be consumed. A specific vitamin which plays important roles in DNA synthesis is vitamin B12, also called cobalamin. The chemical structure of B12 is the most complex of any of the vitamins and is similar to hemoglobin, as shown in Figure 1. The molecule consists of a cobalt atom covalently bound to six groups in a heavily distorted pseudo octahedral geometry. Four of these groups come from a corrin ring, the fifth comes from a dimethylbenzimidazole group, and the sixth comes from a variable R group which determine the functionality of the B12 molecule. This R group can be any of the following: hydroxy, cyano, methyl, or 5'-deoxyadenosyl. Notably, when a cyano group is attached, B12 is very stable, but can not be utilized as a human vitamin until the cyano group is removed.

Vitamin B12 deficiency is a relatively widespread issue; however, its prevalence cannot be generalized to the entire population as it is vastly different across demographic groups. For example, in the United States and the United Kingdom, approximately 6% of people under the age of 60 are deficient, approximately 20% of people over the age of 60 are deficient, and 11% of all vegans are deficient. Further, 40% of Latin American children and adults, 70% of Kenyan children and Indian adults, and 80% of Indian children have B12 deficiencies. These rates are varied, but it is clear that B12 deficiency is a widespread condition in the world population.

Deficiency in B12 has clear implications in many serious medical conditions. For this reason, understanding B12 sources and mechanisms of absorption are of utmost importance. There are two main classes of vitamin B12 deficiencies: those caused by lack of intake and those caused by improper absorption. This first is seen often in populations with little to no consumption of animal products, such as vegans and vegetarians. The second is perhaps more complicated, with many possible mechanisms for improper B12 absorption which will be elucidated in the body of this review.

Vitamin B12 is a critical nutrient for the body. In this review, I will investigate the ways in which an individual may become B12 deficient.

Figure 1. Structure of Vitamin B12
deficient. With that understanding in place, I will then attempt to show that vitamin B12 deficiency is a direct cause of multiple diseases which are among the leading causes of mortality. Lastly, I will propose solutions to B12 deficiency, and therefore deadly disease.

**Causes of Vitamin B12 Deficiency**

The human body may become deficient of vitamin B12 in two main ways. First, the level of B12 consumed may not be sufficient to meet bodily needs. Second, the intake of B12 may be sufficient, but the pathways by which B12 is absorbed may be interfered with. The result of both of these is essentially the same; B12 levels in the body will be insufficient to meet the demands. However, it is important to understand the differences in these two causes because the two forms of deficiency have different solutions.

**Insufficient Intake of Vitamin B12 as a Cause of Deficiency**

The main sources of B12 are animal products such as meat and dairy. As these foods make up a large portion of many people’s diets, most people who meet their caloric needs also meet and exceed B12 daily minimums. Certain populations, however, either choose not to or are unable to obtain and consume animal products. Adequate consumption of B12 is a serious issue for these people.

People who practice veganism, the omission of all animal products from the diet, provide a useful source of vitamin B12 research. In the 1950s a study was published which used vegans to investigate the way in which B12 was produced by the human colon. In this study, water extraction solutions were made from the stool samples of vitamin B12 deficient vegans. These water extractions were then administered orally to the subject from whom they were taken. The result was a complete curing of B12 deficiency in all subjects. Furthermore, a second study investigated a population of vegans from Iran who had adequate levels of B12 in order to determine how they obtained sufficient B12 without consumption of animal products. It was ultimately determined that their source of B12 was unwashed vegetables they grew themselves in soil fertilized with human manure.

The previous two examples show how the human body on its own is incapable of producing useful vitamin B12. Although bacteria living in the colon of the human digestive system do produce significant amounts of B12, the absorption of B12 occurs in the small intestine, which is prior to the colon. This means that even though B12 is produced in human bodies, B12 cannot be absorbed and utilized in humans except through consumption of B12 containing food. For this reason, it is important that humans consume enough B12 in their foods, estimated as approximately 1 microgram per day.

**Insufficient Utilization of B12 as a Cause of Deficiency**

Vitamin B12 deficiency may still occur even if a healthy level of B12 is consumed. Either the B12 in food is not absorbed efficiently or after it is absorbed it is not utilized properly. Examples of these two scenarios may be seen in those suffering from pernicious anemia (PA) and chronic alcoholics.

In healthy individuals, B12 is absorbed solely from the diet by a complex process of the digestive system. For this to occur, B12 is first bound to the salivary protein haptocorrin, which then releases B12 into the duodenum by proteolysis. The B12 then binds to the intrinsic factor, a glycoprotein, in the proximal ileum. This B12-intrinsic factor is then absorbed into the intestinal mucosal cells by receptor mediated endocytosis. Here B12 is released and subsequently bound to the carrier protein transcobalamin and released into circulation where it enters body cells again by receptor mediated endocytosis. In the cells, B12 separates from its transport protein and performs its function as a coenzyme.

In individuals with PA, a condition affecting approximately 2% of those older than 60, the gastric mucosa atrophies and both chief
cells and parietal cells are lost. Parietal cells are the source of the intrinsic factor used in B12 absorption. Therefore, when significant parietal cell loss occurs, the body is incapable of absorbing adequate amounts of B12. This causes B12 deficiency regardless of the amount of B12 consumed through diet.

This process was quantified by a study in which radioactive cobalt isotopes were used to label B12 samples administered orally to subjects. In these subjects, fecal extractions were analyzed for levels of the isotopically labeled B12. Patients with PA had higher concentrations of labeled B12 in the stool compared to the control group. This shows that the control group absorbed more B12 than patients with PA.

B12 deficiencies may develop even if absorption levels are normal. This is the case with some long term alcoholics who exhibit symptoms of B12 deficiency. Interestingly, circulating levels of B12 are actually higher than those of normal healthy individuals. Upon further examination, it is found that B12 concentrations in liver cells are significantly lower than normal in alcoholics. This has lead to the postulation that alcoholism results in less retention of B12 by peripheral tissues, which causes B12 to accumulate in the circulating plasma. Therefore, even though plasma B12 levels are high, the amount of B12 useful to the cells of the body is very low in people who consume high volumes of alcohol.

Vitamin B12 deficiency has a multitude of causes. Insufficient intake of B12 through food is the simplest reason for B12 deficiency. If healthy levels of B12 are consumed, an individual may still become deficient though disruption of B12 absorption, as is the case with PA, or by inadequate utilization of bodily B12, as is the case in chronic alcoholics. B12 deficiency resulting from any of these causes may result in improper function of the DNA synthesis pathway.

**Mechanism of DNA Synthesis Impairment Caused by B12 Deficiency**

Vitamin B12 is crucial to the methylation of DNA. The methyl group is first provided to the reaction by 5-methyltetrahydrofolate (methyl-THF) as shown in Figure 2. B12 is a coenzyme substrate located on the enzyme methionine synthase (MS). MS catalyzes a reaction in which the methyl group on methyl-THF is transferred to the R group location of B12, forming THF and methyl-B12. This methyl-B12 then transfers the

![Figure 2. Transfer of Methyl group from Methyl-THF to Methionine](image-url)
methyl group to homocysteine, creating methionine (Met) and recreating B12 as shown in Figure 2. Met then combines with adenosine triphosphate (ATP), to form the highly reactive molecule S-adenosylmethionine (SAM) as shown in Figure 3.\textsuperscript{10} SAM then serves as the principle methylating agent of amino acids and nucleotides, including DNA. When SAM is demethylated, the molecule becomes S-Adenosylhomocysteine.\textsuperscript{10} This compound ultimately breaks apart forming adenosine and reforming homocysteine to be reused in the methylating cycle.

Approximately 5-7\% of the cytosines in the human body have a methyl group attached at the 5’ position by the process just explained.\textsuperscript{1} This methylation has been shown to be important to the regulation in the expression of many genes.\textsuperscript{1} Further, hypomethylation of DNA has been shown to contribute to the development of tumors in many organs.\textsuperscript{1}

In addition to these issues in DNA methylation which result from B12 deficiency, issues also arise in the folate metabolism pathways of the body. Although the full extent of its function is beyond the scope of this discussion, folate, or B9, is another critical vitamin to the body. Methyl-THF is one of the main sources of folate to the body, but the folate cannot be utilized until the methyl group is removed.\textsuperscript{5} In the absence of B12, Methyl-THF cannot be demethylated to THF, therefore causing a buildup of Methyl-THF in the plasma, and a shortage of useful Folate.\textsuperscript{5} This Methyl-THF is ultimately secreted and lost from the body. For this reason B12 deficiency and folate deficiency are intrinsically linked, and their symptoms often mask each other. In fact, B12 deficiency can often be treated with folate with significant success as the resultant folate deficiency is often the cause of more immediate symptoms. This, however, only treats the symptom, and not the cause, which is B12 deficiency.

**Examples of Maladies Resulting from B12 Deficiency**

With an understanding established of the
role B12 plays in the body, focus can now turn to the issues that result from B12 deficiency. As discussed above, B12 serves to synthesize methionine, which then combines to make SAM, which methylates cytosines in DNA. Therefore, a deficiency in B12 causes hypomethylation of the DNA. It is mostly this hypomethylation which causes health issues in B12 deficient individuals.

Atherosclerosis

Atherosclerosis and the complications it leads to are one of the leading causes of death in western societies. Significant study has been directed at elucidating the causes of atherosclerosis and ways to prevent it. Atherosclerosis occurs when lipid particles penetrate arterial walls, leading to accumulation of inflammatory and immune cells. Additionally, smooth muscle cells in the artery proliferate. All of this results in the hardening and thickening of the artery around the location of lipid penetration. The close relationship of lipids to this process have historically lead to suspicion that high serum lipid levels are a cause of atherosclerosis. More recently it has been proposed that hypomethylation of the DNA in the cells of the arterial intima are a main cause of the formation of atherosclerosis. This correlation between DNA hypomethylation and atherosclerosis has been clearly shown. For a time it was unclear if hypomethylation was a secondary cause of atherosclerosis, however, a recent study has used mice to demonstrate that DNA hypomethylation occurs before formation of lipid lesions in the artery. Therefore, it is very likely that hypomethylation plays an important primary role in the formation of atherosclerosis.

A common cause of mortality associated with atherosclerosis is acute myocardial infarction (AMI). This occurs when plaque build up from atherosclerosis leads to reduced blood flow to the coronary arteries of the heart, leading to cardiac muscle death. With the relationship between hypomethylation and atherosclerosis established above, it is logical that B12 deficiency may lead to hypomethylation which can then lead to AMI. This has in fact been shown in a case study using subjects from southern asia. In this study, low levels of B12 were shown to correlate to an increased occurrence of AMI.

Cancer

Cancer is a major cause of death world-wide. The causes of cancer have traditionally been attributed largely to natural and man-made chemicals with carcinogenic effects, resulting in mutation of the genome causing unregulated cell growth. In 1983, DNA hypomethylation was the first discovered epigenetic cause of cancerous growth. Since then, all of the major human forms of cancer have been shown to have links to hypomethylation. In fact, this link is so strong that the extent of hypomethylation may be used as an indicator of the extent of cancer progression.

Solutions to B12 Deficiency

It is clear that deficient levels of vitamin B12 can have serious negative consequences. Therefore, it is a worthwhile pursuit to alleviate B12 deficiency. The specifics of this solution depend on the causes of the deficiency. For deficiency induced by lack of dietary intake, simply introducing B12 rich foods should solve this without issue. Similarly, for deficiency associated with alcohol intake, eliminating alcohol in the presence of a B12 rich diet should begin alleviating the deficiency. Although these solutions are simple in theory, there are B12 deficiencies which are difficult to resolve, such as PA discussed above. For cases such as these, supplementation with non oral B12 and folate will be necessary.

Future Work and Conclusion

As described above, a correlation has been established between hypomethylation of DNA and disease such as atherosclerosis and many forms of cancer. The literature is, however, incomplete in understanding fully the
mechanism by which DNA hypomethylation causes disease. Therefore, it would be beneficial for research in this area to continue. Such studies could begin by investigating the genome small animal models to identify concurrent DNA hypomethylation and disease, as has already been shown. With this link established hypotheses could be formulated and tested until likely mechanisms of disease become apparent.

Vitamin B12 is a molecule which the body is unable to produce in useful amounts yet needs for healthy function. This review investigated B12 deficiency and the adverse medical effects it causes. Deficiency may be caused by lack of dietary B12 intake, diseases affecting B12 absorption, or high volumes of alcohol intake. Lack of B12 results in hypomethylation of DNA, which, in turn, can cause atherosclerosis, myocardial infarction, and cancer. There is still research needed to be done to fully elucidate the full extent of the influence of B12 on the development of disease, but it is currently clear that B12 deficiency causes a plethora of harmful medical conditions.

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References


