Chapter 11: 1-Carbon Metabolism Micronutrients

Three B vitamins are involved in what is known as 1-carbon metabolism. This is the movement of a 1-carbon unit, usually a methyl group \((\text{CH}_3)\) from one compound to another. It is similar to the movement of the amino group that occurs in transamination. As shown in the figure below, folate, vitamin B\(_{12}\), and vitamin B\(_6\) are the B vitamins involved in 1-carbon metabolism.

![Figure 11.1 1-carbon metabolism depiction. 5-methyl tetrahydrofolate (THF) donates a methyl group to cobalamin forming methylcobalamin. Methylcobalamin donates a methyl group to homocysteine, forming methionine (amino acid). Alternatively, vitamin B\(_6\) can be utilized to convert homocysteine into cysteine.](image)

Vitamin B\(_6\) has been covered already in the previous chapter, so this chapter is going to focus on folate and vitamin B\(_{12}\). We will examine this figure in pieces, so that hopefully by the time this chapter is completed, you will understand the role of all these vitamins in 1-carbon metabolism.

Sections:
- 11.1 Folate & Folic Acid
- 11.2 Vitamin B\(_{12}\)
- 11.3 B Vitamins, Homocysteine, & Cardiovascular Disease

### 11.1 Folate & Folic Acid

Folate is a B vitamin that exists in either its reduced form (folate) or oxidized form (folic acid). When folate is used in this section, we are referring to the reduced form, not the vitamin itself. Another key distinction between the 2 terms is that folic acid refers to the synthetic form, while folate refers to the natural form. Folic acid is only found in certain foods because they have been fortified with it, not because they produce it. The structure of folic acid is shown below.
Another key difference between folate and folic acid is the number of glutamates in their tails. Notice that glutamate is boxed in the structure of folic acid above. Folic acid always exists as a monoglutamate, meaning it only contains one glutamate. On the other hand, about 90% of the folate found in foods are polyglutamates, meaning there is more than one glutamate in their tail. Folic acid is more stable than folate, which can be destroyed by heat, oxidation, and light\(^2\). Table 11.11 summarizes the key differences between folate and folic acid.

<table>
<thead>
<tr>
<th>Folate</th>
<th>Folic Acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reduced Form</td>
<td>Oxidized Form</td>
</tr>
<tr>
<td>Natural</td>
<td>Synthetic</td>
</tr>
<tr>
<td>Polyglutamate</td>
<td>Monoglutamate</td>
</tr>
<tr>
<td></td>
<td>More Stable</td>
</tr>
</tbody>
</table>

The bioavailability of folate was believed to be much lower than folic acid\(^3\). To account for these differences, the DRI committee created dietary folate equivalents (DFEs) to set the RDAs\(^4\). DFEs are defined as follows:

1 DFE = 1 ug food folate = 0.6 ug food folic acid = 0.5 ug folic acid on an empty stomach

Or

1 DFE = ug food folate + (ug folic acid X 1.7)

The 1.7 comes from research suggesting that folic acid from food was 85% bioavailable, compared to 50% for folate (85%/50% = 1.7)\(^4\).
Before folate (polyglutamates) can be taken up into the enterocyte, the extra glutamates must be cleaved prior to uptake into the enterocyte by the reduced folate transporter (RFT, aka reduced folate carrier)\(^5\,^7\). Folic acid, because it is a monoglutamate, requires no cleavage for uptake before it is taken up through the RFT. Once inside the enterocyte, the monoglutamate form is methylated (notice the addition of CH\(_3\) to the lower monoglutamate) and transported into circulation through a yet unknown carrier\(^5\). This series of events is depicted in the figure below.

![Figure 11.12 The uptake and absorption of folate and folic acid (orange boxes represent glutamate)](image)

Thus, the methylated monoglutamate form is the circulating form. This is transported to the liver where it is converted back to the polyglutamate form for storage. Folate is excreted in both the urine and feces\(^5\).

For more information on folate, see the Required Web Link below.

**Required Web Link**
- [Folate Fact Sheet](#)

Subsections:
- 11.11 Folate Functions
- 11.12 Folate Deficiency & Toxicity
**References & Links**

**Links**
Folate Fact Sheet - [https://ods.od.nih.gov/factsheets/Folate-HealthProfessional/](https://ods.od.nih.gov/factsheets/Folate-HealthProfessional/)

### 11.11 Folate Functions

The major function of folate is that it participates in 1-carbon metabolism. As described earlier, this is the transfer of 1-carbon units from one compound to another. The cofactor form of folate is **tetrahydrofolate (THF)**. As is shown in Figure 11.111, in order for THF to be formed, a methyl group is transferred to cobalamin (vitamin B_{12}) forming methyl-cobalamin. You can see this on the left side of the figure below.
There are 2 major functions of THF:\(^1\):

1. **DNA Synthesis** – THF is required for the synthesis of DNA bases (purines and pyrimidines)\(^1\).
2. **Amino Acid Metabolism** – THF is a cofactor for enzymes that metabolize histidine, serine, glycine, and methionine\(^1\).

**References & Links**

### 11.12 Folate Deficiency & Toxicity

Folate deficiency is a vitamin deficiency that affects some Americans. The hallmark symptom of folate deficiency is megaloblastic (a.k.a. macrocytic) anemia. **Megaloblastic anemia**, as the name suggests, is characterized by large, nucleated, immature red blood cells. This occurs because folate is needed for DNA synthesis. Without it, red blood cells are not able to divide properly\(^1\). As a result, fewer and poorer functioning red blood cells are produced that cannot carry oxygen as efficiently as normal red blood cells\(^2\).

A maternal folate deficiency can lead to neural tube defects in infants. The neural tube is the embryonic structure that gives rise to the brain and spinal cord. The exact cause of neural tube defects is unknown, but folate supplementation has been shown to decrease the incidence of neural tube defects\(^3\). The most common of these neural tube defects is spina bifida (1 out of 2500 babies born in the United States), which is a failure of the neural tube to close and the spinal cord and its fluid protrude out the infant's back, as shown in Figure 11.121\(^4,5\).

![Spina Bifida (Open Defect) Image](Image)

*Figure 11.121 Spina bifida*\(^6\)
The neural tube closes 21-28 days after conception\(^1\), and with 50% of pregnancies estimated to be unplanned, many women aren't aware they are pregnant during this period\(^1,2\). Thus, it is recommended that women of childbearing age consume 400 ug of folic acid daily\(^1\). In addition, in 1998 the FDA mandated that all refined cereals and grains be fortified with 140 ug folic acid /100 grams of product\(^7\). As you can see in Figure 11.122, spina bifida prevalence rates declined during the optional fortification years and declined further once fortification became mandatory in the United States.

![Figure 11.122 Neural tube defect prevalence 1995-2011\(^8\)](image)

However, more recent research has found that folic acid supplementation begun before conception reduced the occurrence and severity of neural tube defects\(^9\).

The following link is an interesting account of the history that led up to the folic acid fortification. It is debatable whether folic acid fortification was fully responsible for the decrease in spina bifida rates shown above, but the rates are lower than they were pre-fortification.

**Web Link**
Folic Acid Fortification: Fact and Folly

Folate/Folic acid is not toxic, but it can mask a vitamin B\(_{12}\) deficiency and prevent its diagnosis. This effect will be discussed further in the vitamin B\(_{12}\) deficiency section.
References & Links
8. http://www.cdc.gov/mmwr/preview/mmwrhtml/mm6401a2.htm

Link
Folic Acid Fortification: Fact and Folly - http://www.fda.gov/AboutFDA/WhatWeDo/History/ProductRegulation/SelectionsFromFDLIUpdateseriesonFDAHistory/ucm091883.htm

11.2 Vitamin B₁₂

Vitamin B₁₂ is unique among vitamins in that it contains an element (cobalt) and is found almost exclusively in animal products. Vitamin B₁₂’s scientific name is cobalamin, which is a reference to that fact that it contains cobalt. Neither plants nor animals can synthesize vitamin B₁₂. Instead, vitamin B₁₂ in animal products is produced by microorganisms within the animal itself. Animals consume the microorganisms in soil while eating and grazing. Additionally, bacteria in the stomachs of ruminant animals, like cows and sheep, can produce vitamin B₁₂. Some plant products, such as fermented soy products (tempeh, miso) and the sea algae supplement, spirulina, are advertised as being good sources of B₁₂. However, fermented soy products are not a reliable vitamin B₁₂ source, and spirulina contains a pseudovitamin B₁₂ compound that is not bioavailable. For vegans, supplements, nutritional yeast, and fortified products like fortified soy milk can help them meet their vitamin B₁₂ needs.

The uptake, absorption, and transport of vitamin B₁₂ is a complex process. The following descriptions explain, and figures illustrate, this process.
Vitamin B$_{12}$ is normally bound to protein in food. Salivary glands in the mouth produce haptocorrin (formerly known as R protein), which travels with the food into the stomach. In the stomach, acid converts pepsinogen into pepsin, which breaks the B$_{12}$ free from its protein. In addition, vitamin B$_{12}$ intrinsic factor is released from the parietal cells$^{1-7}$. Vitamin B$_{12}$ intrinsic factor (sometimes referred to simply as intrinsic factor) is a protein-like compound that will aid in B$_{12}$ absorption as will see in a moment.

As pepsin frees B$_{12}$ from protein, haptocorrin binds to the newly freed vitamin B$_{12}$ (haptocorrin + B$_{12}$). Intrinsic factor escapes digestion and, along with haptocorrin + B$_{12}$, exits the stomach and enters the duodenum$^{1,7}$.

Figure 11.22 Vitamin B$_{12}$ in the stomach part 1$^{7,8}$

Figure 11.23 Vitamin B$_{12}$ in the stomach part 2$^{7,8}$
In the duodenum, pancreatic proteases break down haptocorrin, and again vitamin B\textsubscript{12} is freed. Intrinsic factor then binds vitamin B\textsubscript{12} (intrinsic factor + B\textsubscript{12}); intrinsic factor + B\textsubscript{12} continues into the ileum to prepare for absorption\textsuperscript{1,7}.

Figure 11.24 Vitamin B\textsubscript{12} in the duodenum\textsuperscript{7,8}

In the ileum, intrinsic factor + B\textsubscript{12} is believed to be endocytosed into the enterocyte. Intrinsic factor is broken down in the enterocyte, freeing vitamin B\textsubscript{12}. The free vitamin B\textsubscript{12} is then bound to transcobalamin II (TC II + B\textsubscript{12}); TC II + B\textsubscript{12} moves into circulation\textsuperscript{7}.

Figure 11.25 Vitamin B\textsubscript{12} absorption\textsuperscript{8,9}
The liver is the primary storage site for vitamin B$_{12}$. Unlike most other water-soluble vitamins, the liver is able to maintain significant stores of vitamin B$_{12}$. Uptake into the liver occurs through the binding of TC II + B$_{12}$ to the TC II Receptor and the endocytosis of both the compound and the receptor. Vitamin B$_{12}$ is once again freed after degradation of TC II. Vitamin B$_{12}$ is primarily stored in the liver as adenosylcobalamin.$^{5,7}$

Figure 11.26 Hepatic uptake and storage of vitamin B$_{12}$$^{8}$

The overall bioavailability of vitamin B$_{12}$ is believed to be approximately 50%.$^{3}$ Sublingual supplements of vitamin B$_{12}$ have been found to be equally efficacious as oral supplements.$^{6}$ Excretion occurs mostly through bile, with little loss in urine.$^{5}$

The Required Web Link below provides more information on vitamin B$_{12}$.

**Required Web Link**

[Vitamin B12 Fact Sheet](#)

Subsections:
- 11.21 Vitamin B$_{12}$ Functions
- 11.22 Vitamin B$_{12}$ Deficiency & Toxicity

**References & Links**
8. [http://commons.wikimedia.org/wiki/File:Illu_small_intestine_catal%C3%A0.png](http://commons.wikimedia.org/wiki/File:Illu_small_intestine_catal%C3%A0.png)

**Links**

### 11.21 Vitamin B₁₂ Functions

Vitamin B₁₂ is a cofactor for 2 enzymes:

1. Methionine synthase
2. Methylmalonyl mutase

#### Methionine Synthase

Methionine synthase is an important enzyme in 1-carbon metabolism that uses methylcobalamin as its cofactor and converts homocysteine to methionine by adding a methyl group. Methionine then is converted to other compounds that serve as methyl donors, as shown below⁴.

![Methionine Synthase Diagram](https://commons.wikimedia.org/wiki/File:Illu_small_intestine_catal%C3%A0.png)

*Figure 11.211 1-carbon metabolism*
**Methymalonyl Mutase**
Methymalonyl mutase is important in the breakdown of odd chain fatty acids (one containing 5, 7, 9 carbons, etc.). Odd chain fatty acids are less common than even chain fatty acids, but this enzyme is required to properly handle these less common fatty acids\(^1\).

**Demyelination**
In addition to its role as a cofactor for enzymes, vitamin B\(_{12}\) is also important for preventing degradation of the myelin sheath that surrounds neurons, as shown below.

![Myelin Sheath](image)

Figure 11.212 Vitamin B\(_{12}\) is needed to maintain the myelin sheath that surrounds neurons\(^2\)

The mechanism through which vitamin B\(_{12}\) prevents demyelination is not known\(^3\).

**References & Links**

**11.22 Vitamin B\(_{12}\) Deficiency & Toxicity**

There are 2 primary symptoms of vitamin B\(_{12}\) deficiency:
- 1. Megaloblastic (Macrocytic) Anemia
- 2. Neurological Abnormalities

**Megaloblastic (Macrocytic) Anemia**
This is the same type of anemia that occurs in folate deficiency, and is also characterized by fewer, enlarged, immature red blood cells. In vitamin B\(_{12}\) deficiency, this occurs because there is not enough cobalamin to generate THF (illustrated in Figure 11.211). Thus, THF is not available for normal DNA synthesis and the red blood cells do not divide correctly.
Neurological Abnormalities

Vitamin B₁₂ deficiency also results in nerve degeneration and abnormalities that can often precede the development of anemia. These include a decline in mental function, and burning, tingling, and numbness of legs. These symptoms can continue to worsen and deficiency can be fatal¹.

The most common cause of vitamin B₁₂ deficiency is pernicious anemia, a condition of inadequate intrinsic factor production that causes poor vitamin B₁₂ absorption. This condition is common in people over the age of 50 because they have the condition atrophic gastritis². Atrophic gastritis is a chronic inflammatory condition that leads to the loss of gastric glands in the stomach, as shown in the figure in the following Required Web Link.

Required Web Link
Atrophic Gastritis

The loss of gastric glands leads to decreased intrinsic factor production. It is estimated that ~6% of individuals age 60 and over are vitamin B₁₂ deficient, with 20% having marginal status³. In addition to the elderly, vegans are also at risk for vitamin B₁₂ deficiency because they do not consume animal products. However, the deficiency may take years to develop in adults because of stores and recycling of vitamin B₁₂². Deficiency has the potential to occur much quicker in infants or young children on vegan diets because they do not have adequate B₁₂ stores like adults⁴.

Folate/Folic Acid masking vitamin B₁₂ deficiency

As mentioned above, folate and vitamin B₁₂ lead to the same megaloblastic (macrocytic) anemia. If high levels of folate or folic acid (most of the concern is with folic acid since it is fortified in foods and commonly taken in supplements) is given during vitamin B₁₂ deficiency, it can correct this anemia. This is referred to as masking because it does not rectify the deficiency, but it "cures" this symptom. This is problematic because it does not correct the more serious neurological problems that can result from vitamin B₁₂ deficiency. There are some people who are concerned about the fortification of cereals and grains with folic acid because people who are B₁₂ deficient might not develop megaloblastic anemia, which makes a vitamin B₁₂ deficiency harder to diagnose².

No toxicity of vitamin B₁₂ has been reported.
References & Links

Links

11.3 B Vitamins, Homocysteine & Cardiovascular Disease

Homocysteine is a sulfur containing, non-proteinogenic (not used for making proteins) amino acid.

Elevated circulating homocysteine levels have been found in people with cardiovascular disease. Folate, vitamin B₆, and vitamin B₁₂ contribute to the conversion of homocysteine to methionine by providing methyl groups, thereby decreasing homocysteine levels, as illustrated in the figure below. Thus, based on these facts, it was hypothesized that intake of these B vitamins may decrease the risk of cardiovascular disease.

![1-carbon metabolism diagram](image)

Research has found that intake of these B vitamins does decrease circulating homocysteine levels. However, most studies have NOT found that it results in improved cardiovascular disease outcomes¹-³. It is debated why B vitamin intake has not resulted in improved outcomes. Some think it is because the studies have not focused on individuals with elevated homocysteine levels¹, while others believe that homocysteine is simply a biomarker or indicator of cardiovascular disease, not a causative or contributing factor to cardiovascular disease development². More research needs to be done.
References & Links