INE | INSTITUTE OF NUTRITIONAL ENDOCRINOLOGY

Micronutrients: Vitamin B12

Dr. Ritamarie Loscalzo

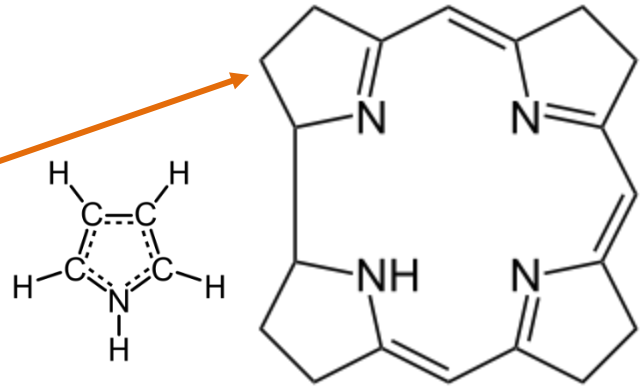
Medical Disclaimer: The information in this presentation is not intended to replace a one-on-one relationship with a qualified health care professional and is not intended as medical advice. It is intended as a sharing of knowledge and information from the research and experience of Dr. Ritamarie Loscalzo, drritamarie.com, and the experts who have contributed. We encourage you to make your own health care decisions based upon your research and in partnership with a qualified health care professional. This presentation is provided for informational purposes only and no guarantees, promises, representations or warranties of any kind regarding specific or general benefits, have been or will be made by Dr. Ritamarie Loscalzo, her affiliates or their officers, principals, representatives, agents or employees. Dr. Ritamarie Loscalzo is not responsible for, and shall have no liability for any success or failure, acts and/or omissions, the appropriateness of the participant's decisions, or the use of or reliance on this information.

Vitamin B12 General Info

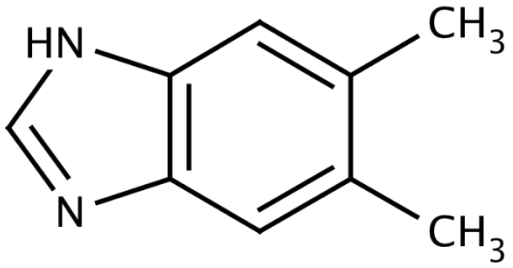
- ✓ Water-soluble vitamin
- ✓ The largest and most complex chemical structure of all the vitamins
- ✓ Contains a metal ion – cobalt – thus cobalamin
- ✓ Group of molecules called corrinoids
- ✓ Methylcobalamin and 5-deoxyadenosylcobalamin are the forms of vitamin B12 used in the human body
- ✓ Cofactor for two enzymes
  - ➢ Methionine synthase
  - ➢ L-methylmalonyl-coenzyme A mutase

Vitamin B12 Structure

Core is Corrin Ring



- 4 reduced pyrolle rings
- Cobalt at center
- Nucleotide 5,6-dimethylbenzimidazole
- One of the following
  - CN – cyanide (cyanocobalamin)
  - OH – hydroxyl (hydroxycobalamin)
  - H_2O – water (aquocobalamin)
  - NO_2 – nitric acid (nitritocobalamin)
  - 5’-deoxyadenosyl (adenosylcobalamin)
  - $-CH_3$ – methyl (methylcobalamin)

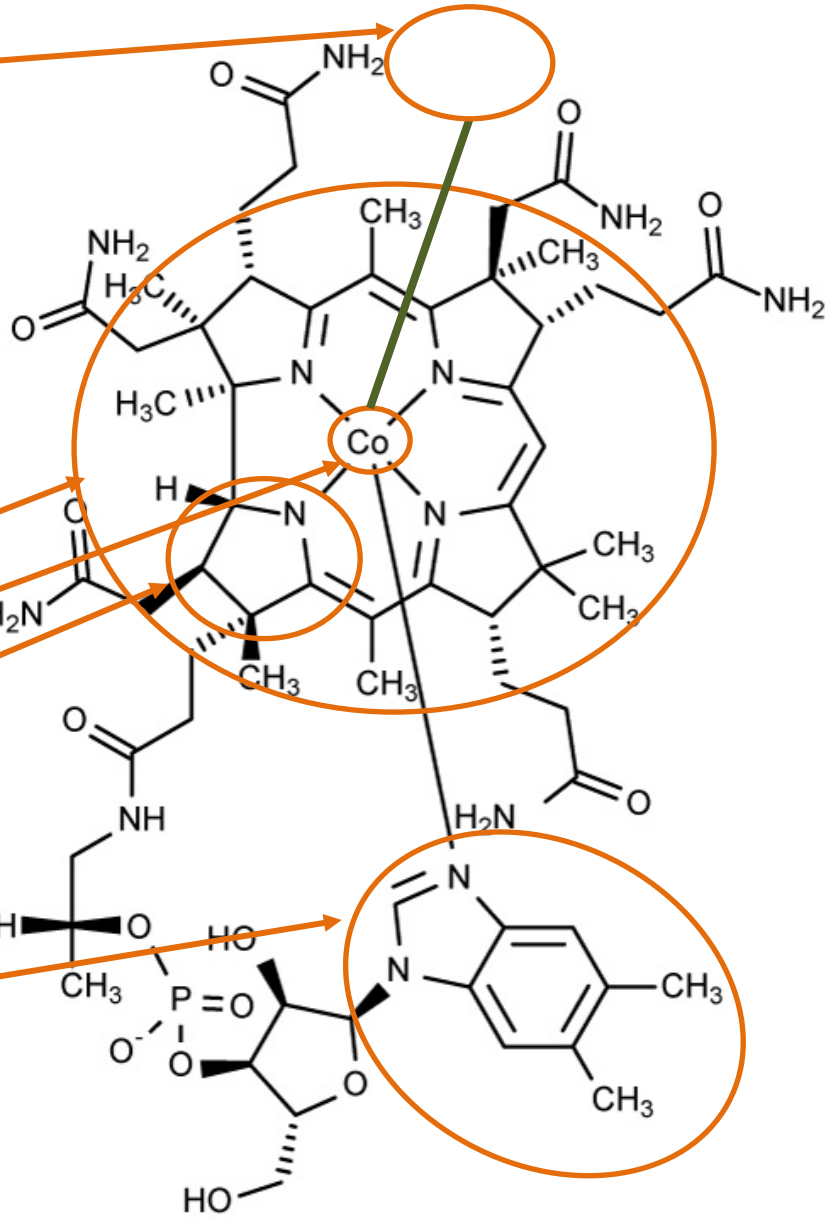


Vitamin B12 Structure Up-close

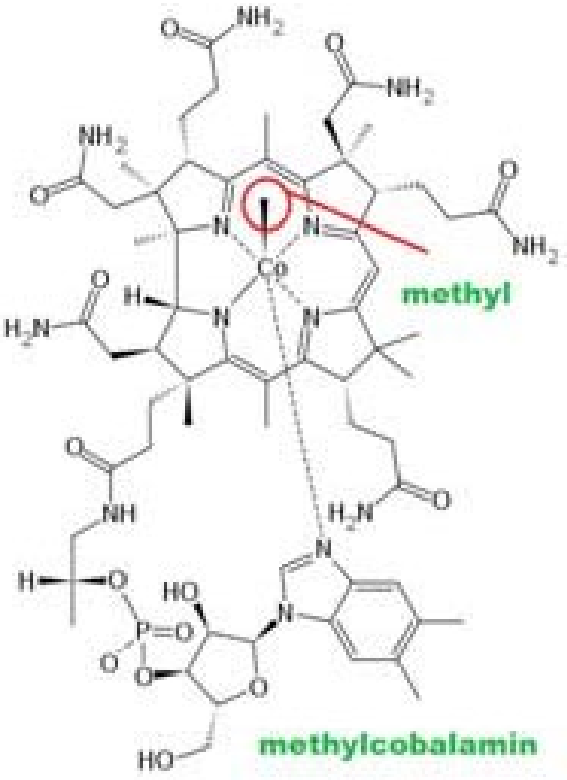
One of the following:

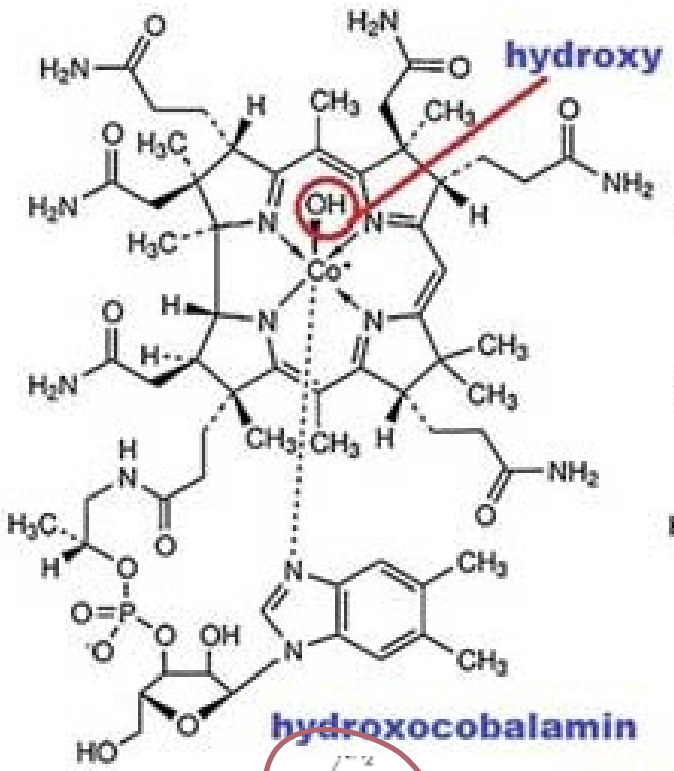
- CN – cyanide (cyanocobalamin)
- OH – hydroxyl (hydroxycobalamin)
- H_2O – water (aquocobalamin)
- NO_2 – nitric acid (Nitritocobalamin)
- 5'-deoxyadenosyl (adenosylcobalamin)
- $-CH_3$ – methyl (methylcobalamin)

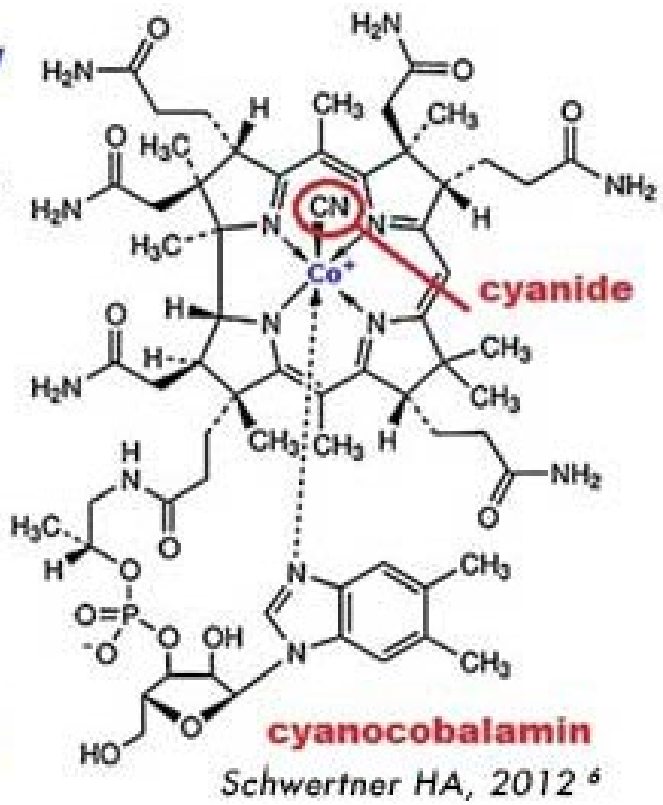
- Corrin Ring
- Cobalt
- Pyrolle
- 5,6-dimethylbenzimidazole

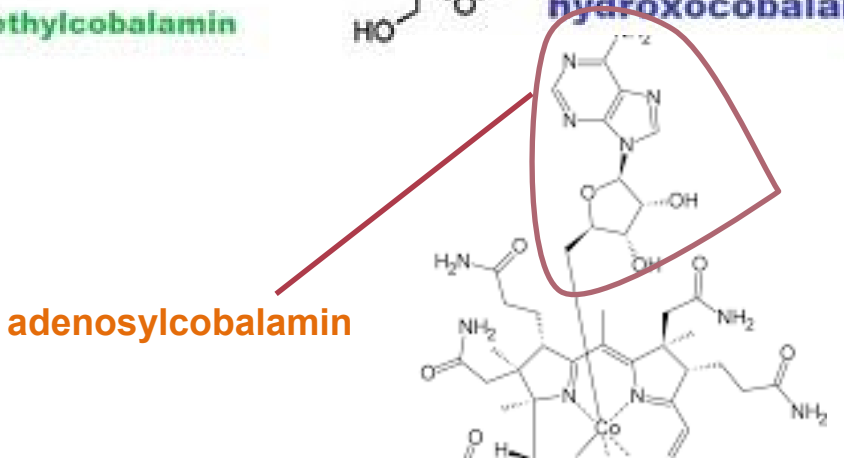


Vitamin B12 Structures Compared









Vitamin B12 Absorption and Storage

✓ Stomach acid needed for absorption:

- Bound to proteins and is released from the proteins by the action of a high concentration of hydrochloric acid
- Results in the free form of the vitamin, which is immediately bound to a mixture of glycoproteins secreted by the stomach and salivary glands (R-proteins, aka cobalophilins or haptocorrins)
- R-proteins protect vitamin B12 from chemical denaturation in stomach

✓ Binds to Intrinsic Factor (IF) in duodenum **(IF created by stomach):**

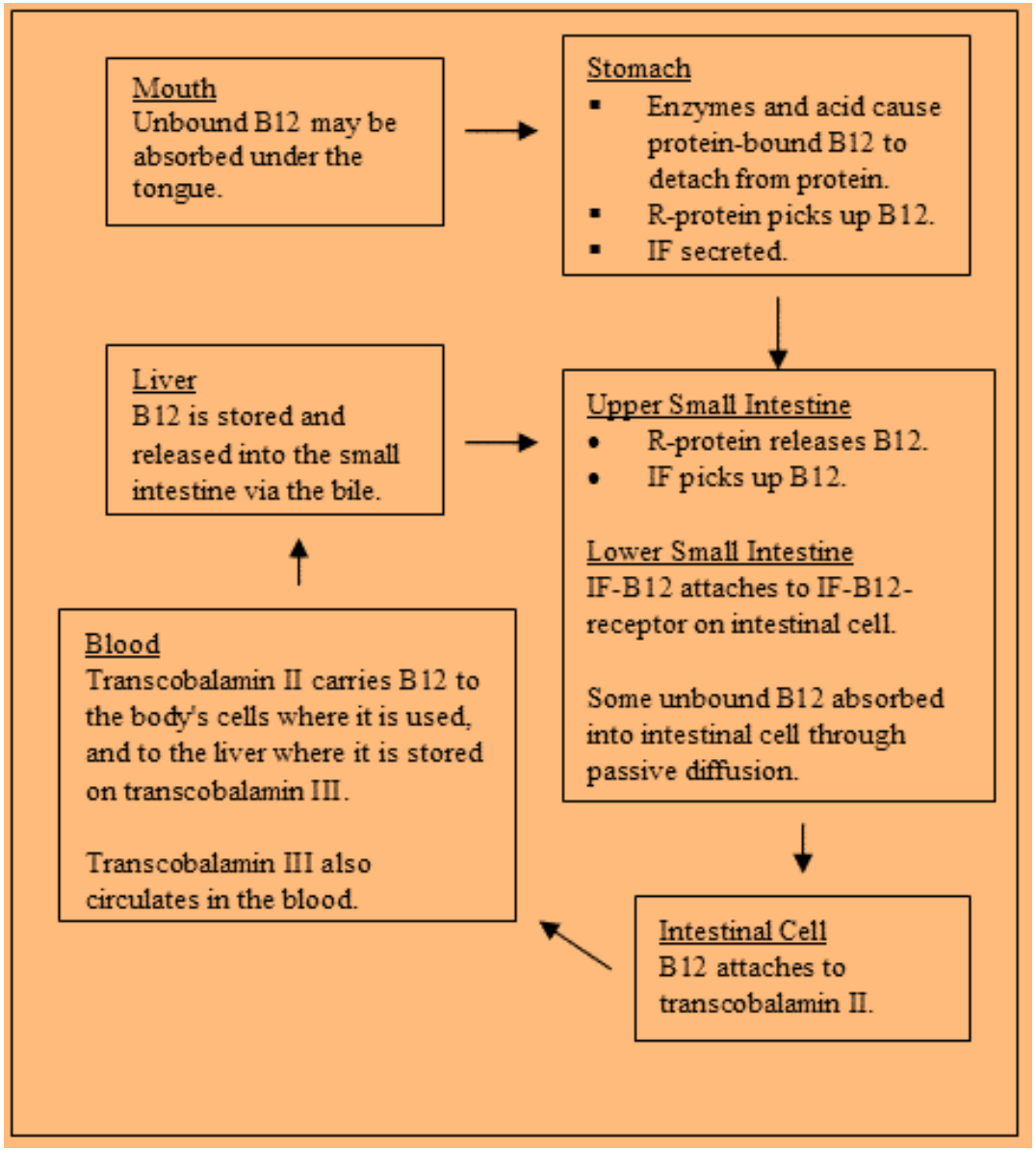
- Pancreatic proteases hydrolyze R-Proteins and free cobalamin is released
- IF binds B12 and carries to ileum for absorption via receptors (cubulins) – mostly distal 1/3
- 1 to 3% of intake can be absorbed by passive diffusion

✓ Overall absorption from diet: 11 to 65% (average 50%)

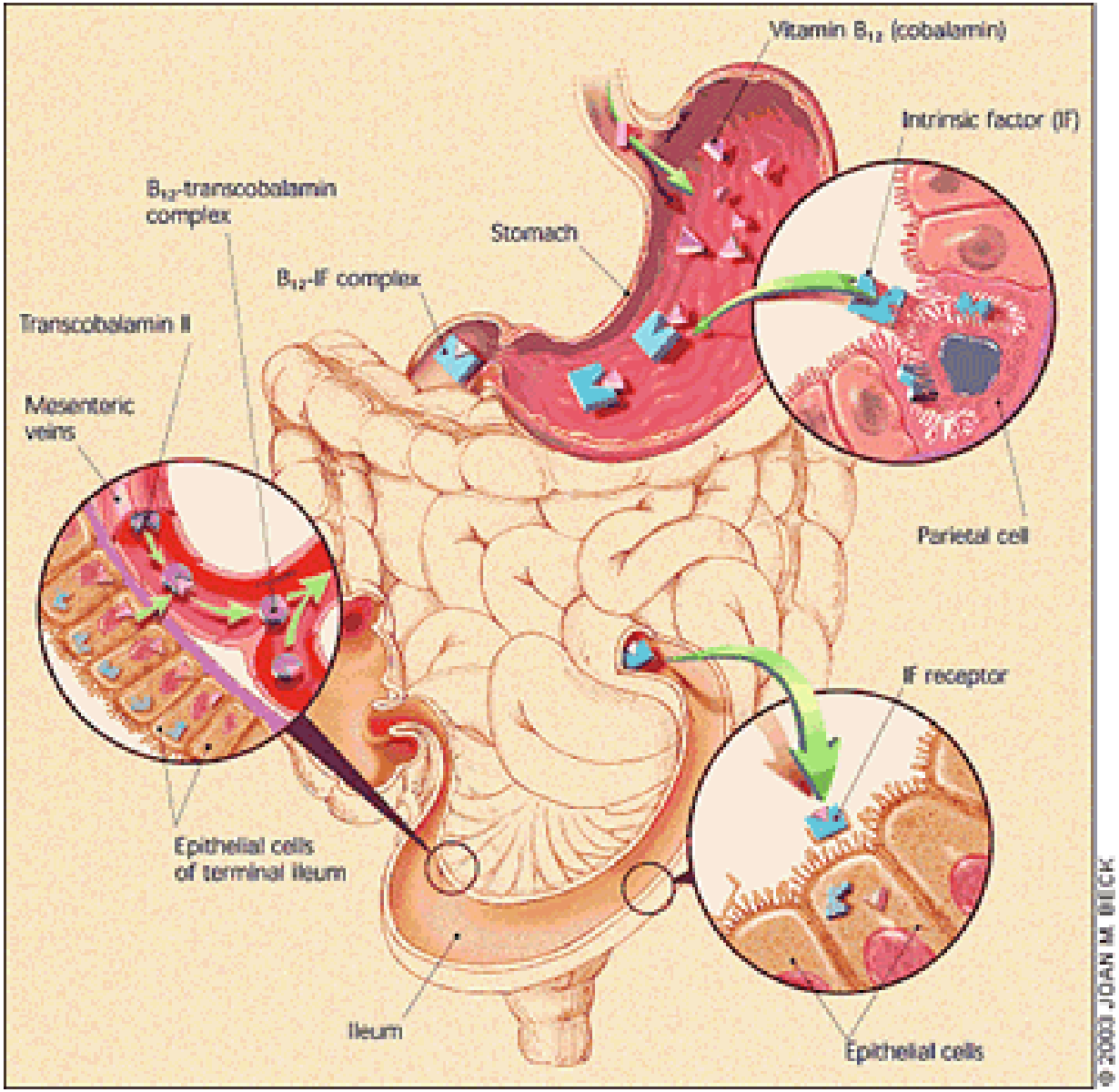
Vitamin B12 Transport and Storage

- ✓ Appears in blood **3 to 4 hours after ingestion**
- ✓ Transported by **transcobalamin** (TCI, TCII, TCIII)
- ✓ TCII is main protein that carries newly absorbed B12 to tissues
- ✓ **80% of B12 is carried by TCI**, considered a circulating storage form
- ✓ ***All cells have TCII-cobalamin receptors***
- ✓ 2 to 4 mg stored in liver; small amounts stored in muscle, bone, kidneys, heart, brain and spleen, mostly as **adenosylcobalamin**
- ✓ About **1.4 mcg per day of liver stores are secreted into bile** to provide B12 even in absence of dietary intake

Vitamin B12 Absorption Flowchart

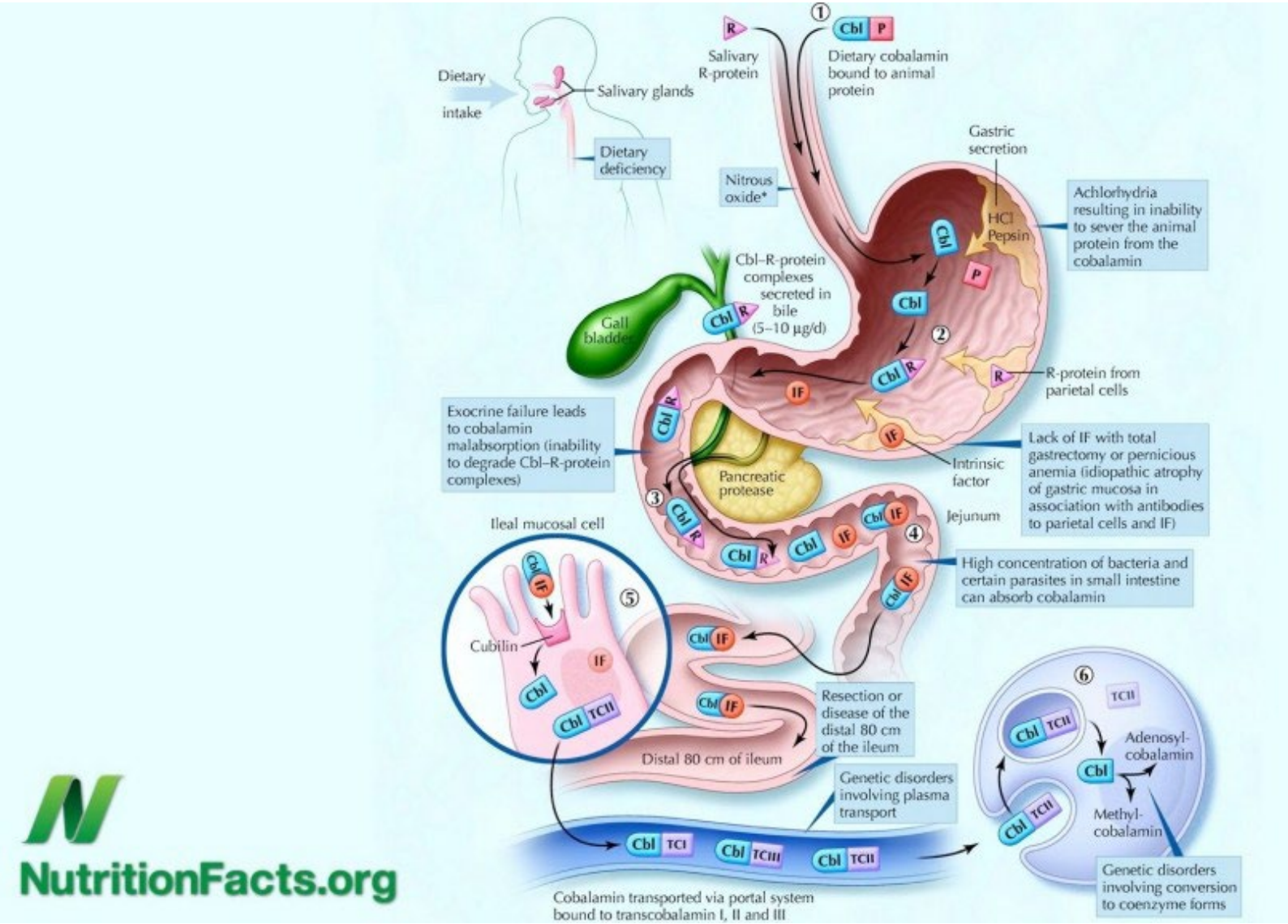


Absorption of Vitamin B12



Vitamin B12 Absorption Video

https://drritamarie.com/CheapestSourceOfVitaminB12



Metabolism of Vitamin B12

- ✓ **Hydroxycobalamin**
  - ➢ Cytosolic methylation to methylcobalamin
  - ➢ Reduction and reaction with ATP to produce adenosylcobalamin
- ✓ **Methylcobalamin**
  - ➢ Active cofactor in methylation pathways
- ✓ **Adenosylcobalamin**
  - ➢ Active cofactor for formation of succinyl CoA
- ✓ **Cyanocobalamin**
  - ➢ "Expensive" and "messy" conversion to methyl

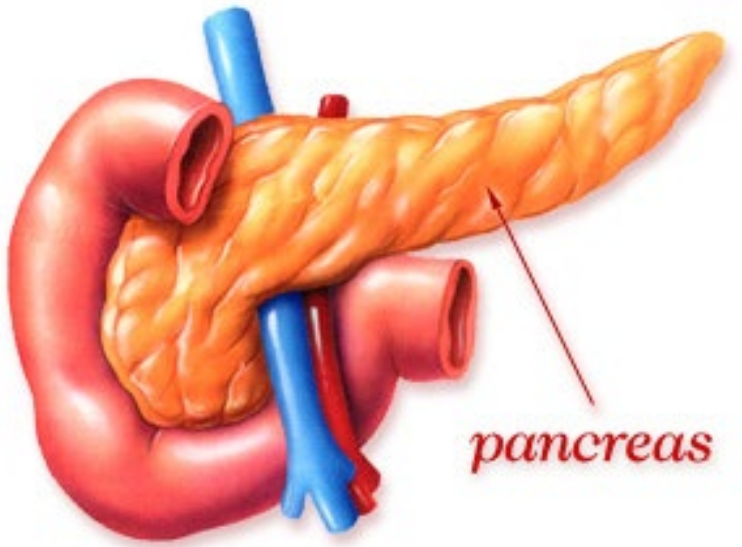
Influences on Vitamin B12 Absorption

Decreases Absorption:

- ✓ **Age:** Many people over the age of 50 lose the ability to absorb B12 from foods
- ✓ **Gastrointestinal surgery:** i.e., Weight loss surgery
- ✓ **Digestive disorders:** Celiac disease or Crohn's
- ✓ **H. pylori infection and ulcers**
- ✓ **Pancreatic insufficiency**

Increases Absorption:

- ✓ **Adequate stomach acid**
- ✓ **Intrinsic factor**
- ✓ **Large supplemental doses**
- ✓ **Good pancreatic function**

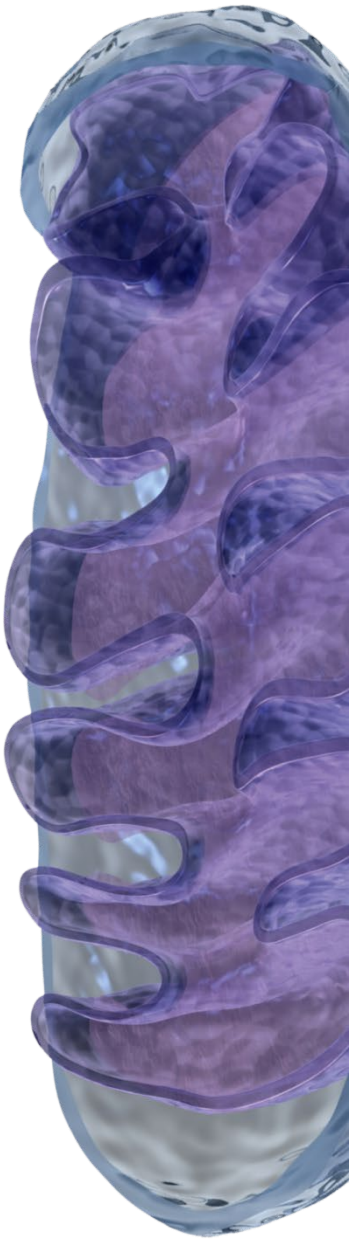


Functions of Vitamin B12

- ✓ **Energy** production
- ✓ Reduces homocysteine
- ✓ Helps **protect nerve cells**
- ✓ Supports **replenishment of the myelin sheath** and maintenance of the central nervous system
- ✓ Important for **protein metabolism**
- ✓ Supports the production and repair of DNA and RNA
- ✓ **Works with B9/folate:**
  - ➢ Maturation of red blood cells
  - ➢ Helps iron work more efficiently
  - ➢ Produce S-adenosylmethionine (SAMe)

Active Co-Enzyme Vitamin B12 Forms and Functions

- ✓ **Methylcobalamin** (60 - 80% of B12 in blood)
  - ➢ Used by the enzyme methionine synthase to turn homocysteine (HCY) into methionine
  - ➢ Methionine is further converted to the most important methyl donor, S-adenosylmethionine (SAM, aka SAMe)
- ✓ **5'-deoxyadenosylcobalamin** (up to 20% of B12 in blood)
  - ➢ Used by the enzyme L-methylmalonyl-CoA mutase to convert methylmalonyl-CoA to succinyl-CoA (magnesium and biotin required to produce L-methylmalonyl-CoA)
  - ➢ Occurs in mitochondria
  - ➢ Used by the enzyme leucine aminomutase to convert B-leucine into L-leucine and vice-versa
  - ➢ B12 deficiency leads to a build-up of methylmalonic acid



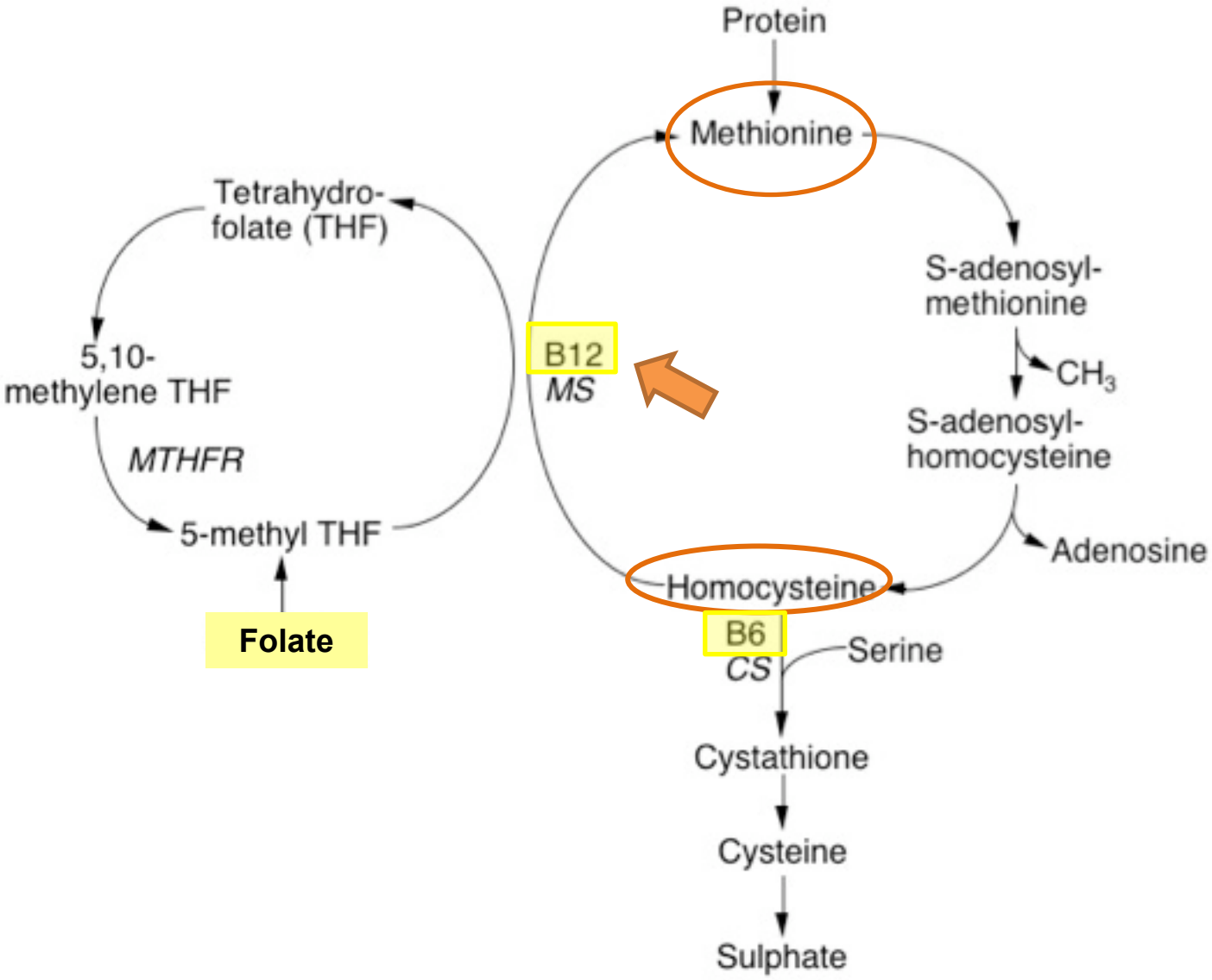
Vitamin B12 and Homocysteine

- ✓ Homocysteine is a **nerve and vessel toxin**
- ✓ Methionine is an essential amino acid
- ✓ Some **methionine is turned into homocysteine**
- ✓ The **body normally converts homocysteine to other molecules**, one of which is back into methionine
- ✓ If this pathway is blocked, homocysteine increases
- ✓ **Methylcobalamin (B12)** is needed by methionine synthase to convert homocysteine into methionine
- ✓ Thus, in B12 deficiency, homocysteine levels increase, and so does risk of cardiovascular and nerve damage

Homocysteine and Heart Disease

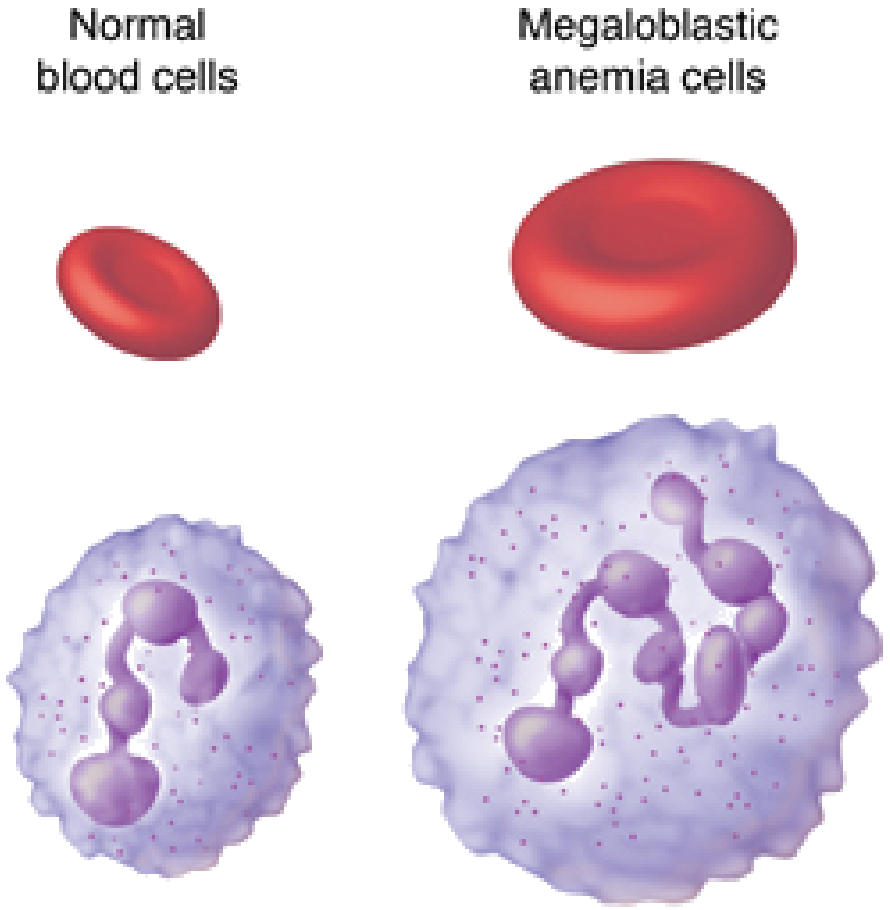
- ✓ Homocysteine is thought to cause **cardiovascular disease** by oxidative stress and vessel wall damage
- ✓ With high homocysteine, **risk of developing coronary artery disease doubles**
- ✓ 2.5 times increased risk of **stroke**
- ✓ Risk factor for cardiovascular disease, blood clotting abnormalities, and atherosclerosis
- ✓ **Related to deficiencies** of **vitamin B6 and Folate (B9)** in addition to vitamin B12

Vitamin B12 Homocysteine Pathway



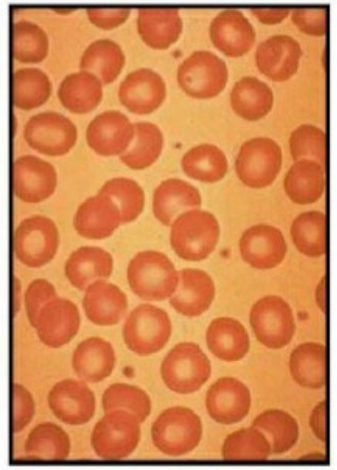
Vitamin B12 and Anemia

- ✓ **Macrocytic anemia:** Mean Corpuscular Volume (MCV) increased
- ✓ **Megaloblastic anemia:** Abnormally large red blood cells are observed under a microscope

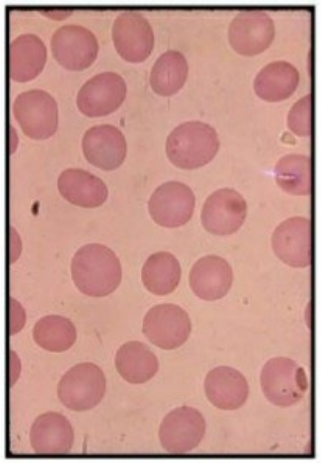


Folate, Vitamin B12 and Megaloblastic Anemia

- ✓ Folate needed to turn **uracil into thymidine**, an essential building block of DNA
- ✓ **DNA needed for new red blood cell production and division**
- ✓ In creating methylcobalamin, a methyl group is donated from folate and folate converts from the **5-MTHF form to the THF form** needed to make DNA
- ✓ Without adequate B12, the methyl groups become "trapped" (known as the methyl-folate trap) and the **THF form of folate is not available for DNA synthesis and repair**
- ✓ Without THF form of folate, thymidine is not produced, and DNA is not available to facilitate the maturation of red blood cells, leading to **megaloblastic anemia**

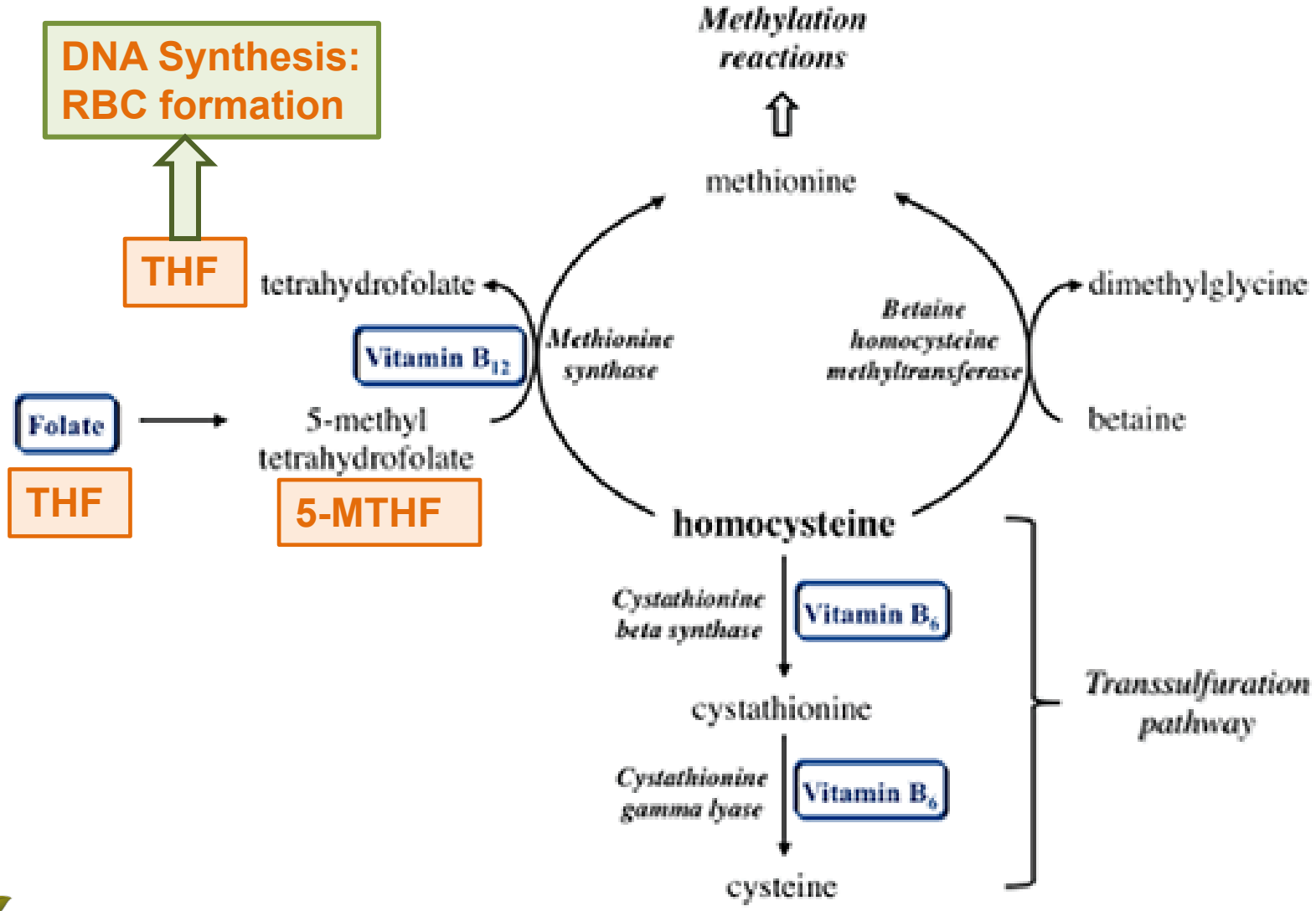


Normal



Megaloblastic Changes

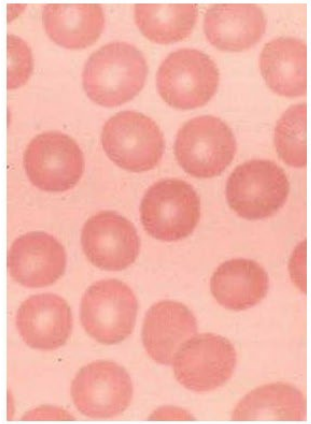
Vitamin B12, Folate, and DNA



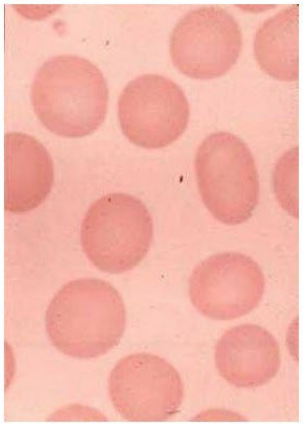
Role of B12 and Folate in Megaloblastic Anemia

DNA needed for new red blood cell production and division

- ✓ Without THF form of folate, DNA is not available to facilitate the maturation of red blood cells, leading to **megaloblastic anemia**
- ✓ Without adequate folate, **new red blood cells** (which start out as large cells called reticulocytes) **divide slowly**, as they are dependent on DNA for division
- ✓ Only RNA, not DNA, is **needed to produce the hemoglobin** found in the red blood cells
- ✓ Since **RNA does not require thymidine**, and hemoglobin is only dependent on RNA, it's produced at a normal rate which causes the production of **large red blood cells known as macrocytes**
- ✓ The result is macrocytic anemia



normal red cells



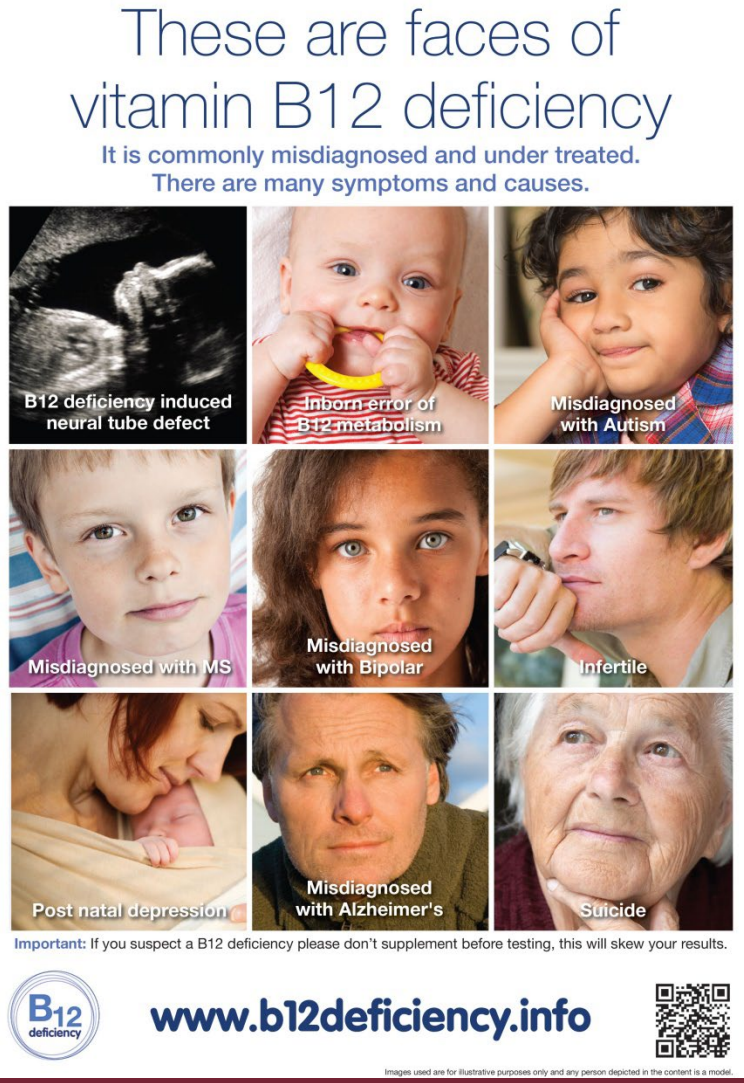
macrocytic red cells

Masking of B12 Deficiency by Folate and Iron

- ✓ If the diet contains large amounts of folate (i.e., lots of uncooked greens), **adequate THF regeneration occurs to produce DNA, even in the absence of B12**
- ✓ **High intake of folate (>800 mcg) can "mask" a B12 deficiency** since megaloblastic anemia is avoided leading to possible nerve damage as deficiency goes unnoticed
- ✓ B12 deficiency can make itself worse because it can **prevent the production of the intestinal cells needed to absorb B12**, which are rapidly dying and need to be replaced using DNA
- ✓ **Iron deficiency leads to small red blood cells** so the MCV looks normal when megaloblastic anemia is present

B12 Deficiency Without Megaloblastic Anemia

- ✓ **Megaloblastic, aka macrocytic, anemia** is indicative of a B12 and/or folate deficiency
- ✓ **Neurological disorders due to B12 deficiency** often occur in the absence of a macrocytic anemia (sometimes due to "masking" by adequate folate)



B12 Deficiency Without Megaloblastic Anemia: Cases

- ✓ Lindenbaum examined 141 cases of neurological problems due to B12 deficiency. 40 (28%) had no macrocytic anemia (iron deficiency may have contributed to a lack in 6 patients, and folate therapy could account for 2 others).
  - ➢ **Labs:**
    - Very high serum MMA
    - High homocysteine
    - Borderline (and sometimes normal) serum B12 levels
  - ➢ **Symptoms:**
    - Sensory loss
    - Ataxia
    - Dementia
    - Psychiatric disorders

- ✓ 2011 study from Korea, 35 patients with vitamin B12 deficiency, most with neurological symptoms; none had anemia.

Vitamin B12 and Pernicious Anemia

- ✓ **Cause:** Stomach cells unable to make intrinsic factor
- ✓ **Symptoms:**
  - Weakness
  - Pale skin
  - Diarrhea
  - Weight loss
  - Fever
  - Numbness or tingling sensation in the hands and feet
  - Loss of balance
  - Confusion, memory loss, and moodiness
- ✓ **Solution:** B12 in high doses – ideally injections

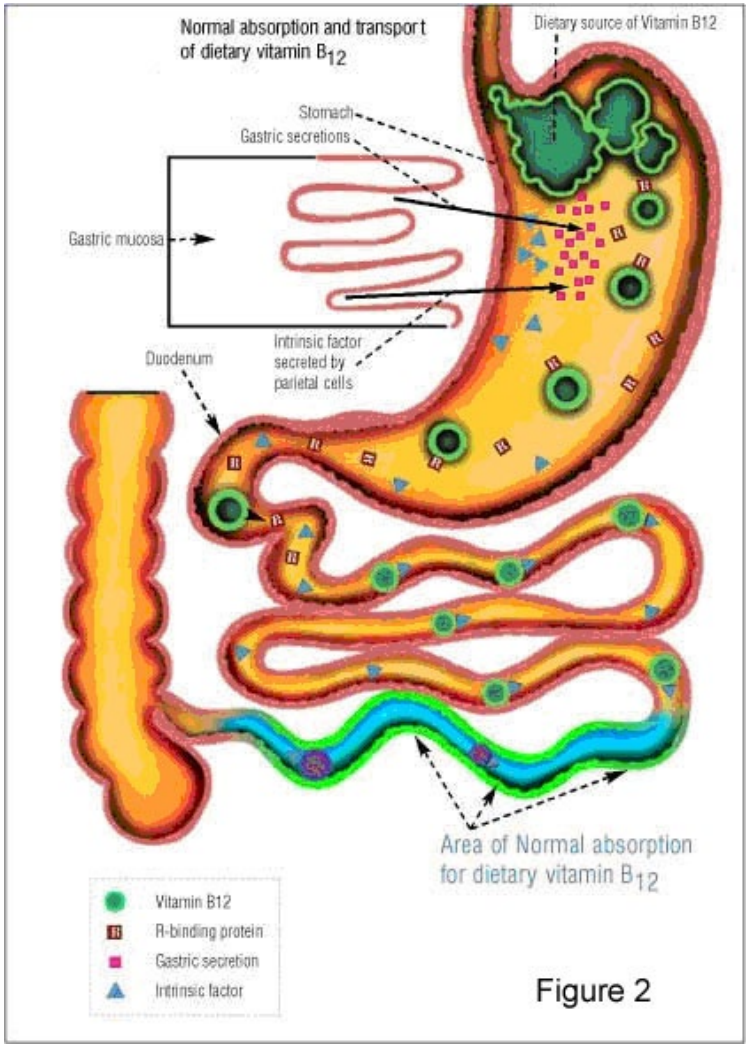
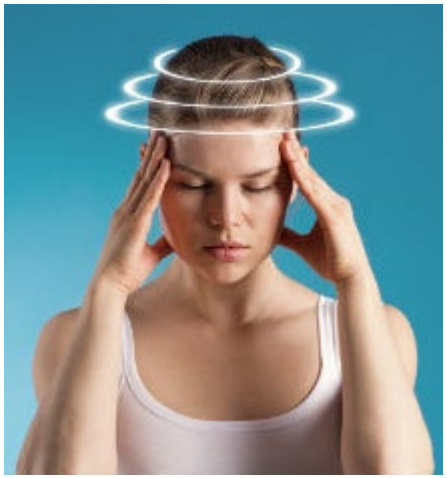


Figure 2

Symptoms of Vitamin B12 Deficiency

- ✓ Fatigue
- ✓ Weakness
- ✓ Shortness of breath
- ✓ Skin pallor
- ✓ Loss of balance
- ✓ Palpitations
- ✓ Insomnia
- ✓ Abnormal gait
- ✓ Loss of concentration
- ✓ Disorientation
- ✓ Memory loss
- ✓ Diarrhea
- ✓ Nervousness
- ✓ Numbness and tingling in fingers and toes
- ✓ Anemia and pernicious anemia
- ✓ Swelling of myelin fibers
- ✓ Possible dementia



Severe deficiency can cause irreversible nerve damage

Vitamin B12 Deficiency Risk Factors

✓ Impaired stomach function

- H. pylori or ulcers: damage to stomach cells that make intrinsic factor
- Atrophic gastritis

✓ Intestinal malabsorption

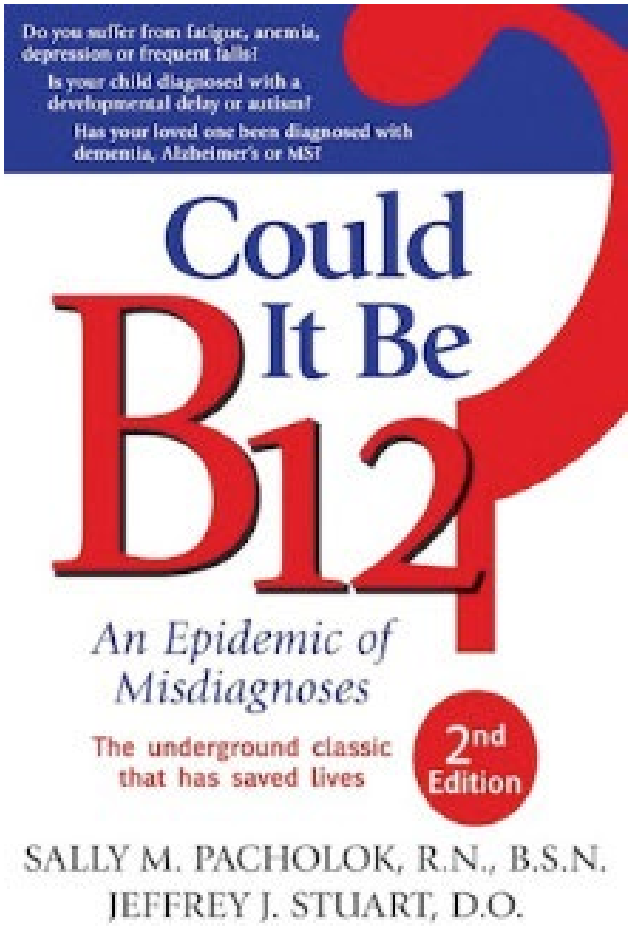
- Crohn's disease and other inflammatory bowel diseases
- Pancreatic disease
- Bariatric surgery
- Medications

✓ Specific populations

- Vegan and vegetarians
- The elderly

✓ Specific conditions

- Eating disorders
- HIV
- Diabetes



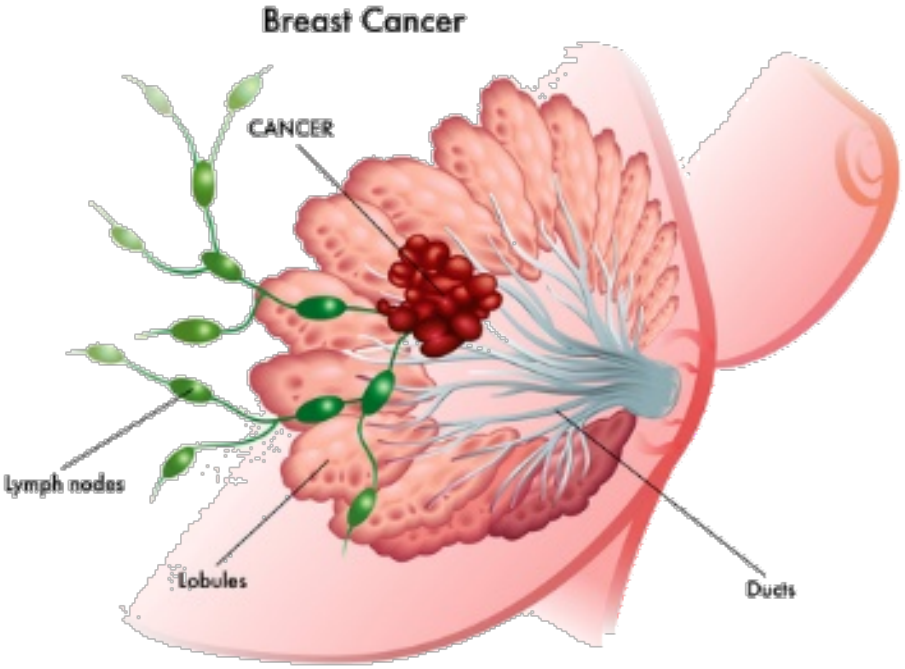
B12 and Fatigue

- ✓ Most common symptom of B12 deficiency
- ✓ Chronic fatigue syndrome (CFS) and fibromyalgia
  - ➢ B12 is nitric oxide scavenger, and high levels of nitric oxide and peroxynitrite linked to fibromyalgia and CFS
  - ➢ Benefit from B12 shots

Pall ML. Elevated, sustained peroxynitrite level as the cause of chronic fatigue syndrome. Medical Hypotheses 2000;54:115-125. Pall ML.

B12 and Breast Cancer

- ✓ B12 and folate decrease risk of breast cancer
  - ➢ Deficiency leads to impaired DNA synthesis and repair
- ✓ **Postmenopausal women**
  - ➢ Shown to have lower B12 levels
  - ➢ Increased risk of breast cancer
- ✓ **Women with breast cancer**
  - ➢ Tend to have lower vitamin B12 levels than women without breast cancer



Wu K, Helzlsouer KJ, Comstock GW, Hoffman SC, Nadeau MR, Selhub J. A prospective study on folate, B_{12}, and pyridoxal 5'-phosphate (B6) and breast cancer. Cancer Epidemiol Biomarkers Prev. 1999;8(3):209-217.

B12 and Male Fertility

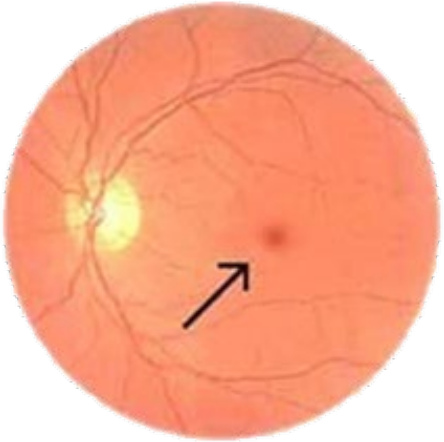
- ✓ **B12 supplements**
  - ➢ Improve sperm count
  - ➢ Improve sperm motility
- ✓ **Infertility**
  - ➢ Deficiencies in B12 lead to genetic damage of the sperm cells
- ✓ **Erectile dysfunction**
  - ➢ Insufficient B12 can damage the nerves within the penis

"Sandler B, Faragher B. (1984). Treatment of oligospermia with vitamin B12. Infertility, Volume 7:133–8."

Vitamin B12 and Macular Degeneration

✓ B12 deficiency

- ➢ Renders nerves, including the optic nerve, more brittle
- ➢ Can cause damage to the optic nerve, resulting in decreased central vision



✓ Vitamin B12 supplementation

- ➢ 1,000 mcg of vitamin B12 along with 2500 mcg of folic acid and 500 mg of vitamin B6 daily reduced risk of developing loss of vision

Christen WG, Glynn RJ, Chew EY, Albert CM, Manson JE. Folic acid, pyridoxine, and cyanocobalamin combination treatment and age-related macular degeneration in women: The Women's Antioxidant and Folic Acid Cardiovascular Study. Arch Intern Med.2009;169(4):335-41.

Impact of Vitamin B12 Excess

- ✓ Well tolerated in general
- ✓ Rarely, excess B12 supplementation can cause some **numbness or tingling in the arm, hands and face**
- ✓ According to the NIH, people with an **hereditary eye disease called Leber's Disease** can have damage to the optic nerve from excess vitamin B12
- ✓ Some studies looked at **increased risk of prostate cancer**
  - ➢ They are retrospective
  - ➢ Look at blood levels
  - ➢ Are without specific ranges
  - ➢ Used cyanocobalamin and synthetic folic acid

- ✓ **High-dose supplementation or injections** can cause diarrhea, itching, blood clots, and allergic reactions in some people
- ✓ People who are **allergic to cobalt** should also avoid taking cobalamin

http://www.drritamarie.com/go/PMID15499634

Vitamin B12 Interactions with Other Nutrients

- ✓ **Folate:** previously covered
  - ➢ Red blood cell production
  - ➢ Helps iron work better
  - ➢ Produces S-adenosylmethionine (SAMe)
- ✓ **B6 and B9 (Folate)**
  - ➢ Controls level of homocysteine
- ✓ **Iodine**
  - ➢ Improved thyroid function leads to riboflavin (vitamin B2) activation to coenzyme form "FAD" which is needed for proper *methylation*
- ✓ **Magnesium and biotin**
  - ➢ Required to produce L-methylmalonyl-CoA

Things That Deplete Vitamin B12

Anti-seizure medications

- Phenytoin (Dilantin)
- Phenobarbital
- Primidone (Mysoline)

Chemotherapy

- Particularly methotrexate
- Doxorubicin

Colchicine

(Gout medication)

Bile acid sequestrants

(Cholesterol lowering medication)

- Colestipol (Colestid)
- Cholestyramine (Questran)
- Colsevelam (Welchol)

H2 blockers

(Reduce stomach acid)

- Cimetidine (Tagamet)
- Famotidine (Pepcid AC)
- Ranitidine (Zantac)

Metformin (Glucophage)

- Blood sugar lowering medication for diabetes and insulin resistance

Proton pump inhibitors

(Used to reduce stomach acid)

- Esomeprazole (Nexium)
- Lansprazole (Prevacid)
- Omeprazole (Prilosec)
- Rabeprazole (Aciphex)

Antibiotics

- Tetracycline

Vitamin B12 RDA and Adequate Intake

✓ Pediatric

- **Newborns to 6 months:** 0.4 mcg (adequate intake)
- **Infants 6 months to 1 year:** 0.5 mcg (adequate intake)
- **Children 1 to 3 years:** 0.9 mcg (RDA)
- **Children 4 to 8 years:** 1.2 mcg (RDA)
- **Children 9 to 13 years:** 1.8 mcg (RDA)
- **Teens 14 to 18 years:** 2.4 mcg (RDA)

✓ Adult

- **19 years and older:** 2.4 mcg (RDA)
- **Pregnant women:** 2.6 mcg (RDA)
- **Breastfeeding women:** 2.8 mcg (RDA)

Because 10% to 30% of older people may not absorb B12 from food very well, people over 50 may need to meet their daily requirement through supplements.

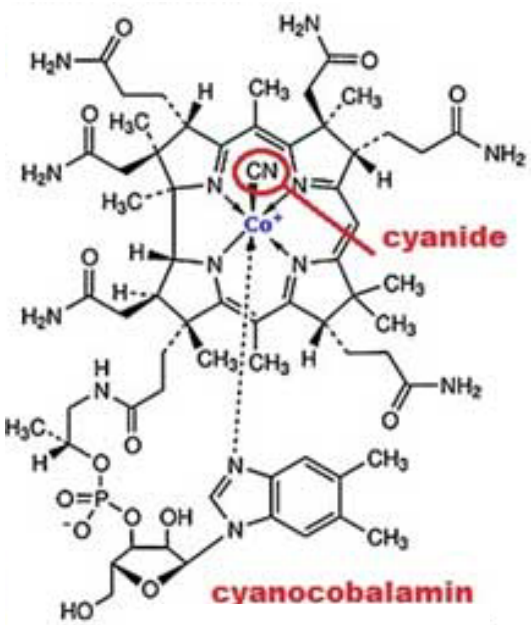
Food Sources of Vitamin B12

- ✓ Vitamin B12 is found only in animal foods
- ✓ **Microorganisms,** and especially bacteria and fungi, are the only organisms definitively known to produce vitamin B12
- ✓ **Germ-phobia and triple washing of produce** eliminates these organisms
- ✓ Nutritional yeast grown on a molasses medium is an example of a food-based, quasi-supplement that would provide a vegan source of vitamin B12
- ✓ Some reports of B12 in herbs and microalgae – likely B12 analogues not the "real" thing

Food Forms of Vitamin B12

- ✓ **Hydroxycobalamin:** Poultry, fish, shellfish, eggs, dairy

- ✓ **Adenosylcobalamin:** Poultry, fish, shellfish, eggs
- ✓ **Methylcobalamin:** Dairy
- ✓ **Cyanocobalamin:** Not naturally occurring in food; cyanide in tobacco can cause formation



Food Sources of Vitamin B12

Food	Serving Size	Cals	Amount (mcg)	DRI/DV (%)
Sardines	3.20 oz	188.7	8.11	338
Salmon	4 oz	157.6	5.67	236
Tuna	4 oz	147.4	2.66	111
Cod	4 oz	96.4	2.62	109
Lamb	4 oz	310.4	2.51	105
Scallops	4 oz	125.9	2.44	102
Shrimp	4 oz	134.9	1.88	78
Beef	4 oz	175.0	1.44	60
Yogurt	1 cup	149.4	0.91	38
Cow's milk	4 oz	74.4	0.55	23
Eggs	1 each	77.5	0.55	23
Turkey	4 oz	166.7	0.42	18
Chicken	4 oz	187.1	0.39	16
Cheese	1 oz	114.2	0.24	10
Mushrooms, Crimini	1 cup	15.8	0.07	3

http://www.drritamarie.com/go/WHFVitaminB12Foods

Herbs Reported to Be High in Vitamin B12

Alfalfa

Dandelion

Hawthorn berries

Hops

Bladderwrack

White oak bark

Reports are unreferenced and anecdotal at best.

Likely, these are B12 analogues.

http://www.drritamarie.com/go/HerbsEqualTonsOfBVitaminsToo

Assessing Status of Vitamin B12

✓ Clinical Assessment

- Signs and symptoms
- Food journals and diet history

✓ Blood Testing

- **Serum B12 test:** not accurate, reflects only current, not active forms
- **Mean corpuscular volume (MCV)** (functional): good indicator but many interfering factors
- **Homocysteine:** good indicator of B12, folate, and/or vitamin B6
- **Methylmalonic acid (MMA), serum:** good indicator but not as good as urine MMA

✓ Urine

- **Methylmalonic acid (MMA), urine:** Norman Clinical Labs – gold standard (www.B12.com)
- **Schilling test:** multiple stages with supplementation – invasive, requires injection of radioactive B12 and 24-hour collection

Good comparison of testing methods at: http://www.drritamarie.com/go/B12TestComparison

B12 and Methylmalonic Acid

- ✓ **Mechanism:**
  - ➢ Adenosylcobalamin: cofactor for conversion of methylmalonyl-CoA to succinyl-CoA
  - ➢ When B12 is not available, methylmalonyl-CoA levels increase
  - ➢ Methylmalonyl-CoA is then converted to methylmalonic acid (MMA) which then accumulates in the blood and urine
  - ➢ Since B12 is the only coenzyme required in this pathway, MMA levels are the best indicators of a B12 deficiency

- ✓ **High MMA levels can also (but rarely) be caused by:**
  - ➢ Genetic defects, kidney failure, low blood volume, gut bacteria changes, pregnancy, and thyroid disease
- ✓ **Normal serum MMA: .07 to .27 µmol/l**
  - ➢ Patients with serum MMA levels in the range of .76 to 187 µmol/l had neurological problems
- ✓ **Normal urine MMA: 0.0 to 3.5**
  - ➢ Test developed by Eric J. Norman, Ph.D., in collaboration with the late M. Drue Denton, M.D., and co-workers at the Hematology Division of the University of Cincinnati College of Medicine, USA

Yamauchi H, Omine M, Tsukamoto N, et al. Urinary methylmalonic acid excretion and clinical features in megaloblastic anemia due to vitamin B12 deficiency. Jpn J Clin Hematol 1989; 30:835-839.

Vitamin B12 Supplement Forms

- ✓ **Cyanocobalamin:**
  Least effective, not easily converted to methyl or adenosyl
- ✓ **Methylcobalamin:**
  Metabolically active
- ✓ **Adenosylcobalamin:**
  Metabolically active
- ✓ **Hydroxycobalamin:**
  Easily converted to methyl and adenosyl

Caution with high dose methylcobalamin and certain genetic SNPs, like COM-T – can lead to methyl trapping

Vitamin B12 Supplementation: Methods Administration

- ✓ Intramuscular
- ✓ Intranasal
- ✓ Topical patch
- ✓ Topical cream
- ✓ Liposomal
- ✓ Sublingual drops
- ✓ Sublingual lozenges
- ✓ Capsules
- ✓ Tablets
- ✓ Soft gels
- ✓ Lozenges
- ✓ Gum

Take with water after eating. Doses range from 150 mcg up to 15 gm

References

- ***Advanced Nutrition and Human Metaboli***sm – Gropper, Smith and Groff.
- Scalabrino G. The neurotrophic action of cobalamin (vitamin B12) revisited. http://www.drritamarie.com/go/PMID11272999
- Seetharam B, Li N. Transcobalamin II and its cell surface receptor. http://www.drritamarie.com/go/PMID10714245
- Hackam DG, Peterson JC, Spence JD. ***What level of plasma homocysteine should be treated?*** Effects of vitamin therapy on progression of carotid atherosclerosis in patients with homocysteine levels above and below 14 micromol/L. http://www.drritamarie.com/go/PMID10678280
- Guyton AC, Hall JE. *Textbook of Medical Physiology*, 9th ed.
- Murray RK, Granner DK, Mayes PA, Rodwell VW. ***Harper's Biochemistr***y, 24th ed.
- ***Linus Pauling Institute -*** http://www.drritamarie.com/go/LPIVitaminB12

