WATER SOLUBLE VITAMINS

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THIAMINE (VITAMIN B\textsubscript{1})

• Rapidly converted to its active form, *thiamine pyrophosphate (TPP)* in the brain and liver by a specific enzyme, *thiamine diphosphotransferase*

• TPP is formed by the transfer of a pyrophosphate group (PPI) from ATP to thiamine

• TPP serves as a coenzyme in formation or degradation of $\alpha$-ketols by *transketolase* and in the oxidative decarboxylation of $\alpha$-ketoacids
Thiamine
Adenosine triphosphate (ATP)
Thiamine pyrophosphate
• 1) pyruvate + CoA-SH + NAD$^+$, TPP, lipoate, FAD
   → Acetyl CoA + CO$_2$ + NADH
• 2) α-KG + CoA-SH + NAD$^+$, TPP, lipoate, FAD
   → succinyl-CoA + CO$_2$ + NADH + H$^+$
• 3) xylulose 5-P + ribose 5-P ↔ sedoheptulose 7-P + glyceraldehyde 3-P
Reactions that use TPP

• A) *transketolase*

• B) *pyruvate dehydrogenase* and *α-ketoglutarate dehydrogenase*
• In thiamine deficiency, the activity of the two dehydrogenase reaction are decreased, resulting in a decreased production of ATP and thus impaired cellular function
• Reduced acetyl-CoA production from pyruvate leads to decreased acetylcholine production leading to decreased nervous system activity
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• Reduced transketolase activity impairs NADP production by **PPPathway**. This leads to decrease in fatty acid synthesis which can effect myelin sheath synthesis

• **Distribution:** whole grains, legumes, pork, beef, liver, eggs, potatoes

• **Requirement:** about 1.5 mg for normal adult
Deficiency of thiamine

• **Beri-beri**, endemic where polished milled rice is a stable food
• Levels of pyruvate and lactate increase
• Accumulation of these acids is especially notable in the brain
• Impairments also occur in nervous, cardiovascular and gastrointestinal systems
• Individual tires easily, weakness of hands and legs is common, swelling may occur around the ankles

• Disease may be expressed as “wet” or “dry” beriberi

• “wet” beriberi characterized by accumulation of fluid (edema) in legs, chest pain and cardiac palpitations; may terminate in circulatory failure and death
• “dry” beriberi does not involve accumulation of edematous fluid
• Common features of both disorders are peripheral neuritis and muscular weakness
• Other possible developments are mental confusion, anxiety and loss of deep reflexes
• In infants, may produce tachycardia, diarrhea, convulsions and dyspnea; if not treated may develop cyanosis and die of cardiac failure
• **Wernicke-Korsakoff syndrome** is attributed to thiamine deficiency associated with chronic alcoholism

• Due to dietary insufficiency or impaired intestinal absorption of the vitamin

• Symptoms are alleviated (and may even disappear) upon treatment with large doses of thiamine
• **Clinical features** – weakness in the eye muscles, ataxia of the gait, loss of memory and confusion
RIBOFLAVIN (VITAMIN B$_2$)

- Consists of heterocyclic isoalloxazine ring attached to sugar alcohol, ribitol
- Required for intracellular production of FMN and FAD
Structure and biosynthesis of FMN and FAD
• FMN and FAD serve as prosthetic groups of oxidoreductase enzymes – flavoproteins
• Many flavoproteins enzymes contain one or more metals e.g. Mo and Fe as essential cofactors – metalloflavoproteins
• FMN and FAD are each capable of reversibly binding two hydrogen atoms, forming FMNH$_2$ or FADH$_2$
• **Distribution:** beef liver, eggs, milk and green leafy vegetables

• **RDA:** 1.5 mg for normal adults

• **Deficiency:** not associated with a major human diseases; general effects include – dermatitis, cheilosis (fissuring at the corners of the mouth) and glossitis (tongue appearing smooth and purplish)
• Riboflavin decomposes when exposed to visible light. This characteristic can lead riboflavin deficiencies in newborns treated for hyperbilirubinemia by phototherapy
NIACIN (NICOTINIC ACID)

• Provides structural component of the pyridine nucleotide coenzymes, NAD$^+$ and NADP$^+$
Nicotinamide
Structure of NAD$^+$
Structure and biosynthesis of NAD$^+$ and NADP$^+$

![Diagram showing the biosynthesis of NAD$^+$ and NADP$^+$](image_url)
• Function: NAD and NADP serve as coenzymes in oxidation – reduction reactions

• The coenzyme undergoes reduction of pyridine ring by accepting a hydride ion (hydrogen atom plus one electron). The reduced forms of NAD$^+$ and NADP$^+$ are NADH and NADPH
Reduction of $\text{NAD}^+$ to $\text{NADH}$
• Distribution: unrefined grains and cereals, milk, lean meats especially liver
• RDA: a minimum of 13 NE/day
  1 NE = 1 mg of niacin or 60 mg of tryptophan
• Deficiency: **Pellagra**. Symptoms progress through dermatitis, diarrhea and dementia. If untreated - death
• In maize, niacin is present, but in a bound unavailable form, **niacytin**; niacin can be released by pretreatment with alkali

• Sorghum – pellagragenic because of its high leucine content. Apparently excess dietary leucine inhibit *quinolinate phosphoribosyl transferase*, key enzyme in conversion of tryptophan to NAD$^+$

• PLP can potentiate deficiency in niacin
PYRIDOXINE (VITAMIN B₆)

- Consists of three closely related pyridine derivates: pyridoxine, pyridoxal, pyridoxamine
- All three have equal vitamin activity
- Can serve as precursors of biologically active coenzyme, pyridoxal phosphate
• Pyridoxal

• Pyridoxamine
Pyridoxal phosphate
Reaction type

• PLP functions as coenzyme for a large number of enzymes, particularly those that catalyze reactions involving amino acids

• Transamination:
  \[ \text{OAA} + \text{glutamate} \rightleftharpoons \text{aspartate} + \alpha-\text{KG} \]

• Deamination:
  \[ \text{serine} \rightarrow \text{pyruvate} + \text{NH}_3 \]

• Decarboxylation:
  \[ \text{histidine} \rightarrow \text{histamine} + \text{CO}_2 \]

• Condensation:
  \[ \text{glycine} + \text{succinyl CoA} \rightarrow \delta-\text{ALA} \]
• **Distribution:** wheat, corn, egg yolk, liver and muscle meats

• **R.D.A.:** 1.8 – 2.2 mg for normal adult; increased during pregnancy, lactation and with age. Requirement varies depending on amount of dietary protein

• **Deficiency:** Rare. Observed in newborn infants fed formulas low in vitamin B₆

• Observed in alcoholics
• Vitamin B₆ required for the synthesis of sphingolipids, necessary for myelin formation

• These effects thought to explain the irritability, nervousness and depression seen with mild deficiencies, and peripheral neuropathy and convulsions observed with severe deficiencies

• PLP is one of the cofactors required for conversion of tryptophan to NAD
• **Symptoms:** poor growth, anaemia, decreased antibody production, convulsions, as well as renal, hepatic and skin lesions

• **Isoniazid** (isonicotinic acid hydrazide) drug frequently used to treat tuberculosis can induce deficiency by forming a hydrazone with pyridoxal
Biotin

- Is an imidazole derivatives widely distributed in natural foods
- Involved as a cofactor in carboxylation reactions
• Biotin is involved as a cofactor:
• \[ \text{Acetyl-CoA} + \text{HCO}_3^- + \text{ATP} \rightarrow \text{malonyl-CoA} + \text{ADP} + \text{Pi} \]
• \[ \text{Pyruvate} + \text{CO}_2 + \text{ATP} \leftrightarrow \text{Oxaloacetate} + \text{ADP} + \text{Pi} \]
• \[ \text{Propionyl-CoA} + \text{CO}_2 + \text{ATP} \rightarrow \text{methylmalonyl-coenzyme A} \]
• **Distribution:** present in almost all foods; richest sources are liver, milk and egg yolk. Produced by internal flora

• **Requirement:** 100 to 200 μg/day

• **Deficiency:** Rare. Caused by defects in intestine utilization. Raw egg white contains a glycoprotein, avidin, that tightly binds biotin and prevents its absorption from the intestine

• **Symptoms:** dermatitis, glossitis, loss of appetite and nausea
Pantothenic acid

- Formed by combination of pantoic acid and β-alanine
• Required for the structure of coenzyme A and acyl-carrier protein

• **Distribution:** widely distributed. Most important sources – eggs, liver and yeast

• **Requirement:** estimated at 5 to 10 mg/day

• **Deficiency:** not well characterized in humans; however, may include generalized fatigue, sleep disturbances and abdominal distress
FOLIC ACID
• Consists of the base pteridine attached to P-aminobenzoic acid (PABA) and glutamic acid
• Animals are not capable of synthesizing PABA or attaching glutamate to pteroid acid
• Dihydrofolate reductase catalyses a two step reduction of 5,6,7 and 8 positions of pteridine ring to tetrahydrofolate (F4 or THF) - biologically active form
• Sulfanilamide and its derivatives are antibiotics that are structural analogues of PABA. They competitively inhibit bacterial synthesis of THF at PABA incorporation step
• Do not affect human DNA or RNA synthesis
Sulfanilamide and its derivatives competitively inhibit the synthesis of folic acid in microorganisms and, thereby, decrease the synthesis of nucleotides needed for the replication.

Dihydrofolate reductase is competitively inhibited by methotrexate, a folic acid analogue used to effect the remission of acute leukemia in children.
• Function of folic acid:
• THF receives one-carbon fragments from donors such as serine, glycine and histidine and transfers them to intermediates in the synthesis of amino acids, purines and thymine – a pyrimidine found in DNA
• One carbon groups **THF derivares** carried by THF

- CH$_3$ Methyl           N$^5$-Methyl-THF
- CH$_2$ Methylene       N$^5$N$^{10}$-Methylene-THF
- CHO Formyl             N$^5$-Formyl-THF
- N$^{10}$-Formyl-THF
- CHNH Formimino         N$^5$-Formimino-THF
- CH= Methenyl           N$^5$,N$^{10}$-Methenyl-THF
THF and the one-carbon substituted folates

- Tetrahydrofolate (H₄folate)
- N⁵-Methyl H₄folate
- N⁵-Formimino H₄folate
- N⁵⁰-Formyl H₄folate
- N⁵,N⁰-Methylene H₄folate
- N⁵,N⁰-Methenyl H₄folate
Methylation of deoxyuridilate (dUMP) produces deoxythymidylate (dTMP)

- dTMP rather than dUMP is a component of DNA.
- The conversion of uracil residue of dUMP to thymine to form dTMP is essential to the biosynthesis of DNA.
- dUMP is converted to dTMP by thymidylate synthase in a reaction that requires N$_5$N$_{10}$ methyleneTHF and releases 7,8-dihydrofolate.
Synthesis of dTMP from dUMP
• 7,8-DHF is converted back to $N^5N^{10}$ methyleneTHF by the sequential action of dihydrofolate reductase and serine hydroxymethyltransferase

• *Note: A methylene group is more oxidized than a methyl group, and the source of electrons for the reduction of the methylene group to a methyl group is a hydride ion from the tetrahydropyrazine ring of $N^5N^{10}$ methyleneTHF itself
• $\text{N}^5\text{N}^{10}$ methyleneTHF is therefore both a one-carbon donor and an electron donor in the reaction

• Many anti-cancer drugs act directly to inhibit thymidylate synthase, or indirectly, by inhibiting dihydrofolate reductase

• 5-Fluorodeoxyuridylate – a drug metabolite that inhibits thymidylate synthase
• Many dihydrofolate reductase inhibitors have been synthesized including methotrexate, aminopterin and trimethoprim
• Each of these is an analog of folic acid
• *Note: Cancer cells perish by a process termed ”thymineless death”. Normal cells can be affected also, but they usually are growing more slowly and so are less sensitive to the antifolate drug
• Also, normal cells sometimes can be “rescued” by administration of a folate coenzyme sometime after the drug has been administered

• **Distribution:** in green leafy vegetables, liver, whole grain cereals

• **RDA:** 100 μg

• Requirement increases in periods of rapid tissue growth
• During pregnancy and lactation are 800 and 500 μg/day respectively
• Folic acid supplementation before conception and during first trimester has shown to virtually eliminate spina bifida and anencephaly, the most common neural tube defects affecting pregnancies
Spina bifida
Anencephaly
• Inadequate serum levels of folate can also be caused by poor absorption caused by pathology of small intestine, alcoholism or treatment with drugs that are dihydrofolate reductase inhibitors

• **Deficiency**: characterized by growth failure and **megaloblastic anemia**

• **megaloblasts** – large, immature red cell precursors in the bone marrow
COBALAMIN (VITAMIN B$_{12}$)
Cobalamin (vitamin $\text{B}_{12}$)
• Cobalt-corrin complex
• Isolated as cyanocobalamin
• Two coenzyme forms:
  i) 5-deoxyadenosylcobalamin
  ii) methylcobalamin
• **Distribution:** synthesized exclusively by microorganisms; it is not present in plants. Best sources – beef liver, whole milk, eggs, oysters, fresh shrimp, pork and chicken
• Although bacteria in the human colon produce the vitamin, it cannot be utilized, because its absorption must occur in the small intestine

• **RDA:** 3μg/day;
  
  6μg/day during pregnancy and lactation
Function of vitamin $\text{B}_{12}$

- Required for two essential enzymatic reactions:
  - A) synthesis of methionine
  - B) isomerization of methylmalonyl CoA, produced during degradation of some amino acids and fatty acids with odd numbers of carbon atoms
Functions of vitamin B₆

- A) synthesis of methionine
- B) isomerization of methylmalonyl CoA, produced during degradation of some amino acids and fatty acids with odd numbers of carbon atoms
Synthesis of methionine from homocysteine

FIGURE 21-4 Regeneration of methionine from homocysteine: ① methionine synthase (methyltetrahydrofolate homocysteine methyltransferase); ② methionine adenosyltransferase; ③ SAM-dependent methyltransferase; ④ S-adenosylhomocysteine hydrolase.
$\text{HCO}_3^- + \text{ATP} \rightarrow \text{ADP} + \text{P}_i$

- Propionyl-CoA carboxylase (biotin)
- Methylmalonyl-CoA epimerase
- Methylmalonyl-CoA mutase ($B_{12}$)
• Methylmalonyl-CoA mutase+5-deoxyadenosyl-B$_{12}$ → succinyl-CoA

• When the vitamin is deficient, abnormal fatty acids accumulate and become incorporated into cell membranes, including those of the nervous system

• This may account for some of neurologic manifestations of vitamin B$_{12}$ deficiency
Folate trap hypothesis

- Effects of cobalamin deficiency are most pronounced in rapidly dividing cells such as erythropoietic tissue of bone marrow and mucosal cells of intestine. Such tissues need both $N^5-N^{10}$-methylene and $N^{10}$-formyl forms of THF for synthesis of nucleotides required for DNA replication.
- However, in vitamin $B_{12}$ deficiency, $N^5$-methyl form of THF accumulates, whereas the levels of other forms decrease.
- Thus cobalamin deficiency is hypothesized to lead to deficiency of THF forms – **metabolic link between cobalamin and folic acid**.
• Significant amounts (4 – 5 mg) stored in the body
• May take several years for clinical symptoms of vitamin B$_{12}$ deficiency to develop in individuals who have had partial or total gastrectomy
• Deficiency is rarely a result of absence of vitamin in the diet; more common found in patients who fail to absorb the vitamin from the intestine

• Deficiency may occur in individuals who do not eat meat products, eggs, milk or honey and live \textit{exclusively on vegetables} - vegans
Absorption of vitamin B$_{12}$

• Vitamin B$_{12}$ obtained from diet binds to intrinsic factor (IF), a glycoprotein secreted by gastric parietal cells.

• Cobalamin-IF complex travels through the gut and eventually binds to specific receptors on surface of mucosal cells of ileum.

• Bound cobalamin is transported into mucosal cells and subsequently into general circulation, where it is carried by vitamin B$_{12}$ binding proteins.

• Lack of IF prevents absorption of vitamin resulting in pernicious anemia.
• Patients with cobalamin deficiency are usually anemic, but later in the development of the disease they show neuropsychiatric symptoms.
• Disease is treated by giving high-dose vitamin B$_{12}$ orally or intramuscular injection of cyanocobalamin.
• Megaloblastic anemia should not be treated with folic acid alone, but rather with a combination of folate and vitamin B$_{12}$.
ASCORBIC ACID (VITAMIN C)

- Active form is ascorbate acid
- Main function is as a reducing agent in several different reactions
- Has a well-documented role as a coenzyme in hydroxylation reactions
- Is therefore required for maintenance of normal connective tissue as well as for wound healing
- Also facilitates the absorption of dietary iron from the intestine by reducing it to the ferrous state
Ascorbic acid
• Collagen is the most abundant protein in the human body

• A long rigid structure in which three polypeptides (referred to as “α-chains”) are wound around one another in rope-like triple-helix
- Collagen is rich in proline and glycine.
- Glycine is found in every third position of polypeptide chain; thus is a part of repeating sequence Gly-X-Y where X is frequently proline and Y is often hydroxyproline (hyp) or hydroxylysine (hyl).
• Hyp and hyl result from hydroxylation of some of proline and lysine residues after their incorporation into polypeptide chains
• The hydroxylation is thus an example of posttranslational modification
• These hydroxylation reactions require molecular oxygen and a reducing agent such as vitamin C
• Hyp is important in stabilizing the triple-helical structure of collagen because it maximizes interchain hydrogen bond formation

**Prevention of chronic diseases**

• Vitamin C is one of a group of nutrients that includes vitamin E and β-carotene, which are known as antioxidants
• These hydroxylation reactions require molecular oxygen and a reducing agent such as vitamin C
• **Distribution:**
citrus fruits, potatoes, tomatoes and green vegetables

• **RDA:**
60mg/day for adults

• **Deficiency:**
  *scurvy*; characterized by sore, spongy gums, loose teeth, fragile blood vessels, swollen joints and anemia
• No acute toxicity has been observed
• A small portion of the vitamin is oxidized to dehydroascorbic acid and metabolized to oxalate
• The calcium salts of oxalate are one of the major constituents of kidney stones
• Thus chronic massive doses of ascorbic acid should be avoided
Pharmacologic doses of ascorbic acid (1 - 4g/day) appear to decrease the severity or duration of colds slightly but do not alter their frequency significantly.
Legs of 46 year old man with scurvy
Hemorrhage and swollen gums of a patient with scurvy
VITAMIN – LIKE NUTRIENTS

• Perform important metabolic functions; correct some disorders, but not essential under all conditions
• Some can be synthesized, provided precursors are available in the diet
• Included in this group are:
  lipoic acid, choline, ubiquinones (CoQ), myo-inositol
LIPOIC ACID

• Synthesized in adequate amounts in mammals

• 1) pyruvate + CoA-SH + NAD$^+$  TPP,lipoate,FAD
   \[ \rightarrow \text{Acetyl CoA} + \text{CO}_2 + \text{NADH} \]

• 2) $\alpha$-KG + CoA-SH + NAD$^+$  TPP,lipoate,FAD
   \[ \rightarrow \text{succinyl-CoA} + \text{CO}_2 + \text{NADH} + \text{H}^+ \]