

TEACHERS TOPICS

Biochemistry of the Water Soluble Vitamins: A Lecture for First Year Pharmacy Students

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This manuscript describes the lecture on vitamins contained in the core course entitled, "The Biochemical Basis of Drugs and Diseases" (Pharmacy 3050). In the first year, our curriculum is designed to focus on systems and diseases. As such, this course acts in concert with both the Anatomy & Physiology course and the Pathophysiology course to present an integrated view of human diseases and systems. The second year of our curriculum is focused on learning about drugs classes and the mechanism of action. The third year of the curriculum is focused on simulated patients, while the fourth year of the curriculum the students are presented with real patients.

The lecture on vitamins is the last topic of a course focused primarily on metabolism. Because many vitamins play major roles in metabolic cycles, this lecture allows for a brief review of much of the material covered throughout the course. Therefore, vitamin examples are primarily chosen to reinforce major metabolic cycles and also their role in human disease. There is more clinical information that is relevant to vitamins than what is presented in this lecture. However, since first year students in our curriculum have almost no knowledge of therapeutics, the focus of the lecture is on diseases and not clinical practice.

INTRODUCTION

Vitamins are a multibillion dollar industry.¹ They are readily available to the public and are the focus of the most frequently asked questions to pharmacists.² Surprisingly, most pharmacy students receive little training on the many roles of vitamins in nutrition.² Therefore, this lecture attempts to not only reinforce fundamental biochemical and metabolic pathways in the human body, but also to provide pharmacy students with practical information.

The diet is a vast source of important nutrients. These nutrients include several important classes of biomolecules such as: (1) energy yielding components (carbohydrates, lipids, and proteins), (2) essential and

nonessential amino acids, (3) essential fatty acids, (4) minerals, and (5) vitamins. Vitamins are organic substances that must be provided by the diet either because they cannot be biosynthesized or the amount that is provided through biosynthesis is inadequate for maintaining normal health. Vitamins are broadly divided into 2 classes based upon their hydrophobicity. The more hydrophilic vitamins are termed the water-soluble vitamins and are composed of the B-complex vitamins and vitamin C. The more hydrophobic vitamins, referred to as the fat-soluble vitamins, are composed of Vitamins A, D, E, and K. This article focuses primarily on the water-soluble vitamins due to their greater role in the major metabolic cycles, which are the primary focus of this course.

The normal North American diet is sufficient to prevent significant vitamin deficiencies and the related diseases associated with these deficiencies. However, there is increasing concern that slight vitamin deficiencies in a number of water-soluble vitamins (B1, B6, B12, folate and vitamin C) are risk factors for diseases such as

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such as depression, cancer, cardiovascular disease, and neural tube defects. The prevalence of this slight vitamin deficiency is likely related to diet, since most of these vitamins are supplied by fruit and vegetable intake. A recent survey has shown that only 25% of the population meets their daily intake of 5 servings.³ Food preparation is another source of vitamin loss. For example, heating food for more than 2 hours causes more than a 10% loss of most water-soluble vitamins. Refrigeration, freezing, and reheating have all been shown to lead to further loss of vitamins. Exposure to light causes significant loss of riboflavin from foods and the combination of heating and light can almost completely remove this vitamin from food.⁴

Thiamin (Vitamin B₁)

Thiamin functions in the body as thiamin pyrophosphate (TPP) an important enzyme co-factor.⁵ Thiamin reacts with adenosine triphosphate (ATP) to form thiamin pyrophosphate through a reaction mediated by the enzyme thiamin diphosphokinase. Following its production, TPP is incorporated into 2 important enzymes: pyruvate dehydrogenase and α -ketoglutarate dehydrogenase. Pyruvate dehydrogenase is part of a multi-enzyme complex that acts to convert pyruvate generated in glycolysis into acetyl-CoA for entry into the tricarboxylic acid (TCA) cycle. Thiamin pyrophosphate is also used as a co-factor for the enzyme α -ketoglutarate dehydrogenase, which is a key point of regulation in the TCA cycle. α -Ketoglutarate dehydrogenase is involved in the conversion of α -ketoglutarate to succinyl CoA. In the case of both enzymes, TPP assists in decarboxylation of a small ketoacid.

In addition to its uses in metabolism, thiamin may enhance circulation and blood formation. It is required for maintenance of the nervous system and is used in the biosynthesis of the neurotransmitters acetylcholine and γ -hydroxybutyrate (GABA). Thiamin also is used in the production of hydrochloric acid in the stomach and, therefore, has a role in digestion.

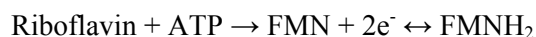
Thiamin is provided in the diet through most grains. A deficiency of thiamin is called beriberi and it is most often observed in Southeast Asia.⁶ The main staple of diet in this part of the world is rice, which does not provide dietary thiamin to as great an extent as other grains. The symptoms of beriberi include limb pain, muscle weakness, and low cardiac output. All symptoms are related to the diminished capacity of the major energy producing pathways that are dependent on TPP as a co-factor.

Mild deficiencies of thiamin are sometimes observed in the elderly and in low-income groups on restricted diets. The earliest symptoms of thiamin deficiency are loss of appetite, constipation, and nausea. Other symptoms such as mental depression, peripheral neuropathy, irritability, and fatigue are related to the role of thiamin in maintaining a healthy nervous system.

The most common form of thiamin deficiency in the United States is alcoholic neuritis. Because alcoholics normally have a poor appetite, overall food consumption is low. In addition, alcoholics are predisposed to developing nutritional deficiencies since alcohol is their primary calorie source. Under the broad umbrella of alcoholic neuritis come two disorders, Wernicke's Syndrome and Korsakoff's Psychosis.⁷ The symptoms of Wernicke's Syndrome (ophthalmoplegia, nystagmus, and ataxia) respond quickly to administration of thiamin, whereas the more severe memory and learning disorders associated with Korsakoff's Psychosis are refractory to thiamin treatment.

Riboflavin (Vitamin B₂)

Riboflavin functions in the body as an enzyme co-factor in many oxidation/reduction reactions and has a central role in energy production and cellular respiration. Riboflavin reacts with ATP to form flavin mononucleotide (FMN). Flavin mononucleotide then reacts with a second molecule of ATP to form a molecule of flavin adenine dinucleotide (FAD). Within cellular metabolism, enzymes such as succinyl dehydrogenase (TCA cycle), AcylCoA Dehydrogenase (β -Oxidation), and Glycerol-3-phosphate Dehydrogenase (Glycerol Phosphate Shuttle) use FAD as a cofactor. The enzyme NADH-CoEnzyme Q oxidoreductase (Complex I, Electron Transport Chain) uses FMN as a co-factor. As enzyme co-factors, FAD and FMN are able to function as electron acceptors. The addition of 2 electrons to FAD results in the formation of a molecule of FADH₂, while the addition of 2 electrons to FMN causes the formation of a molecule of FMNH₂.



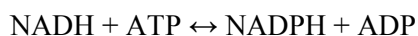
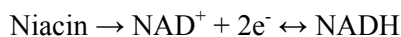
The reduced forms of these enzyme co-factors can donate these electrons to return to their previous fully oxidized forms. It is this ability to act as a conduit for electron transfer reactions that makes FAD and FMN such important enzyme cofactors.

Riboflavin is available from a wide variety of dietary sources such as milk, cheese, meat, eggs, and cereal products. Symptoms associated with riboflavin deficiency include sore throat, dermatitis, anemia, neuropathy

thy, and cataract formation. Riboflavin deficiency is not normally observed in the United States; however, deficiency may be observed as part of a general case of malnutrition or in cases of chronic alcoholism.⁸ The conversion of riboflavin to FMN is required for absorption and transport into many tissues. The conversion has been shown to be inhibited by hypothyroidism and the structurally similar medications chlorpromazine, adriamycin, quinacrine, tetracycline, and tricyclic antidepressants.⁹⁻¹²

Niacin (Vitamin B₃)

The vitamin niacin (nicotinic acid) and its structural analog nicotinamide have identical function due to their facile interconversion in the body. Niacin is converted through a series of reactions to its active form nicotinamide adenine dinucleotide (NAD⁺). NAD⁺ can be converted to a reduced form NADH by gaining 2 electrons through a process similar to FAD reduction. NADH is produced in large quantities by the TCA cycle and β -Oxidation and to a lesser extent by glycolysis. Reduced NADH is returned to its oxidized form under normal cellular conditions by the electron transport chain. This newly reformed NAD⁺ can then return to other metabolic pathways to harvest more electrons. In the body the ratio of NAD⁺/NADH is approximately 1000 demonstrating the primary role of NAD⁺ in supporting cellular oxidation. NADH also can react with ATP to form NADPH. As opposed to the unphosphorylated form, the ratio of NADP⁺/NADPH is only 0.01. This ratio points to the role of NADPH in supporting reductive processes in the body.



Niacin can be found in foods such as meats, breads, and beans. Mild niacin deficiencies have similar symptoms to those observed with riboflavin, which is not surprising due to the similar roles both of these vitamins play in biochemical reactions. Niacin deficiencies are occasionally observed in alcoholics, cases of general malnutrition, and in the elderly on restricted diets. A severe deficiency of niacin is known as pellagra, which is derived from the Italian phrase meaning rough skin. Pellagra is marked by dermatitis and also is notable for causing a blackening of the tongue. Early cases of pellagra were first observed in Europe shortly after the introduction of corn from the voyages of Christopher Columbus. In these cases, poor farmers who were raising corn as animal feed were particularly susceptible. Because niacin in corn is not bioavailable unless treated with a strong

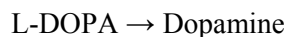
base such as lye, the farmers developed niacin deficiencies. Europeans did not know this processing method until revealed to them by Native-Americans during the early colonial period of North America. However, the connection between pellagra and niacin was not known until the early 20th century. Pellagra was a significant health issue in the United States over the period from 1900-1940 resulting in over 100,000 deaths. Today most diets are supplemented with niacin through enriched flour, which receives its name because of the added niacin.¹³

Niacin can be administered in doses of 2g to 4g to causes a decrease in circulating levels of cholesterol and LDL. While the cholesterol lowering effects of niacin are desirable there are potential side effects from such large doses of this vitamin. The most immediate reaction observed from large doses of niacin is vasodilation resulting in flushing. Over time there may be a reduction in fatty acid mobilization causing a depletion of glycogen and lipid stores in muscle tissue. Long-term exposure may also elevate blood glucose and uric acid levels, suggesting increased risk for patients who are on the borderline for diseases such as diabetes and gout. Prolonged use of high doses of niacin can lead to elevated levels of the serum enzymes alanine aminotransferase and aspartate aminotransferase, which may suggest liver damage.

Pyridoxine (Vitamin B₆)

Pyridoxine is the precursor to the active enzyme co-factor pyridoxal phosphate (PLP). Pyridoxal phosphate is a critical co-factor for enzymes involved in reactions involving many amino acids. The N-terminus of the amino acid forms a covalent bond to PLP, allowing a wide variety of displacement reactions to occur at the alpha carbon. These include decarboxylations, transaminations, and transfers of side chains. Pyridoxine, therefore, plays a central role in the production of many neurotransmitters, such as serotonin, norepinephrine, and histamine. Pyridoxine also is important in the production of heme.

The PLP co-factor in several enzymes is a therapeutic target due to the ability to form irreversible covalent bonds with agents containing a hydrazine moiety, such as carbidopa, isoniazid, and hydralazine.¹⁴ The combination of L-Dopa and carbidopa is a widely used therapy that has a biochemical mechanism involving vitamin B6. The conversion of L-Dopa to dopamine is catalyzed by the PLP-dependent enzyme L-aromatic amino acid decarboxylase (LAAAD).



The enzyme LAAAD is present in both the gut and in the brain. This presents a problem because L-dopa is readily transported across the blood brain barrier while dopamine is not. Therefore, orally administered L-dopa is rendered ineffective in the gut by LAAAD. Co-administration of carbidopa resolves this dilemma by inactivating LAAAD in the gut. Like dopamine, carbidopa cannot cross the blood brain barrier leaving LAAAD in the brain free to carry out the production of dopamine at the site of action. In addition to hydrazine containing drugs, penicillamine, used in the treatment of Wilson's disease, cystinuria, and rheumatoid arthritis reacts with and inactivates pyridoxal phosphate.¹⁵ Patients treated with penicillamine occasionally develop convulsions, which can be prevented by supplementation with vitamin B6.

Pyridoxine is found in foods such as meats, breads, eggs, soybeans, and many vegetables. A deficiency in pyridoxine can cause facial lesions, depression, peripheral neuropathy, and glossitis. The neurological complications can be directly linked to the effects on neurotransmitter production. Mild pyridoxine deficiencies are sometimes observed in young women taking oral contraceptives. Deficiencies have also been observed in patients with gastroenteritis or Crohn's Disease, presumably due to poor absorption. Mild deficiencies are of concern due to a correlation with an increased incidence of breast cancer and to the risk of coronary heart disease.^{16,17} Neurotoxicity has been noted with doses of vitamin B6 in excess of 500 mg/day.

Cobalamin (Vitamin B₁₂)

Cobalamin is vital for cell growth and replication. Its major site of action is at the interface between the folic acid cycle and the active methyl cycle, where cobalamin is a co-factor for the enzyme homocysteine methyltransferase. This enzyme catalyzes the transfer of a methyl group from tetrahydrofolate to homocysteine, forming the amino acid methionine. These 2 cycles impact many other pathways due to the large number of methylation reactions in the body, especially nucleic acid biosynthesis and neurotransmitter biosynthesis.

Cobalamin is found in meats and dairy products. Deficiencies are not common because the liver can store a 6-year supply of cobalamin. In addition, cobalamin is highly conserved by enterohepatic recirculation. Strict vegetarians (vegans) may take up to 20 to 30 years to develop a deficiency, whereas inadequate absorption from the ileum due to ileitis or loss of a glycoprotein that complexes with cobalamin prior

to absorption may take from 2 to 10 years to become symptomatic. The anesthetic nitrous oxide inactivates cobalamin and can cause patients with marginal serum levels to develop deficiencies within a week.¹⁹ While rare, deficiencies in vitamin B12 are severe and manifest in erythrocytes and nervous tissue. In erythrocytes, nucleic acid biosynthesis is slowed. This results in stem cells that are committed to development into erythrocytes possessing twice their normal protein content but lacking the necessary nuclear material for proper cell division. The resulting anemia, termed megaloblastic anemia, can be quite severe. Cobalamin deficiency can also cause permanent damage to the nervous system. The results are swelling of neurons and demyelination of nerve cells, followed by cell death. These progressive manifestations cause a wide range of neurological symptoms including unsteadiness, decreased reflexes, paresthesias of the extremities, and ultimately confusion, memory loss, hallucinations, and psychosis. The neurological symptoms of cobalamin deficiency may be mistaken for multiple sclerosis. In the elderly and in alcoholics, cobalamin deficiency should be considered as a possible cause of dementia even in the absence of anemia. The neurological symptoms associated with cobalamin deficiency are not directly related to irregularities in the formation of erythrocytes.²⁰

Folic Acid (Pteroylglutamic Acid)

Folic acid is derived from the addition of a pteroyl group to the amino acid glutamate. Folic acid can be reduced to form tetrahydrofolate, which is an important acceptor of one-carbon units. Folic acid plays an important role in the conversion of homocysteine to methionine by providing the methyl to the vitamin B₁₂ dependent methyl transferase. Therefore, folic acid levels have a strong influence on the endogenous concentration of homocysteine. Elevated levels of homocysteine are associated with increased risk of coronary heart disease. Supplementation with folic acid has been shown to correct plasma homocysteine levels and decrease the morbidity and mortality from atherosclerotic disease.²¹

Folic acid plays an important role in the biochemistry of amino acids; it is critical for the interconversion of the amino acids serine and glycine and in histidine metabolism. Folic acid also is necessary for the biosynthesis of the nucleic acids thymidine, adenine, guanine, and inosine. Because of its role in amino acid and nucleic acid biosynthesis, folic acid supplementation during pregnancy has been shown to prevent most birth defects involving the brain and spinal cord, known as neural tube defects.²²

Folic acid is found in organ meats such as liver, green leafy vegetables, yeast, and some fruits. Deficiencies in folic acid are most often observed with poor intake or alcoholism but are sometimes seen in pregnancy where there is an increased need for folic acid. The recommended daily allowance for pregnant women is at least twice that for nonpregnant women.²³ Folic acid deficiencies can also be observed from reduced absorption due to diseases in the intestine. Additionally anticonvulsants, such as phenytoin, phenobarbital, and primidone appear to not only to inhibit folic acid absorption, but also to increase catabolism.²⁴ There is also concern that supplementation with folic acid may impact the effectiveness of these anticonvulsants.

The most common effect of folic acid deficiency is megaloblastic anemia. The appearance of this type of anemia is identical to that observed with vitamin B₁₂ deficiency due to the common pathway shared by the folic acid and the active methyl cycles. One major difference between folic acid deficiency and vitamin B₁₂ deficiency is the absence of the neurological symptoms from lowered serum folic acid levels.²⁵

Pantothenic Acid

Pantothenic acid is one of the many B-complex vitamins. Once in the body, pantothenic acid combines with ADP and cysteamine to form coenzyme A (HSCoA). Coenzyme A is involved in a number of cellular pathways, most notably in transferring an acetyl unit from the pyruvate dehydrogenase complex or from β -oxidation to oxaloacetate in the TCA cycle. Coenzyme A is also involved in the biosynthesis of cholesterol, steroid hormones, fatty acids, and porphyrins. Pantothenic acid derives its name from the Greek word meaning "from everywhere", which reflects its ubiquitous nature in foods. Deficiencies in pantothenic acid are difficult to achieve but result in neuromuscular degeneration.²⁶

Biotin

Biotin is an important co-factor for enzymes involved in carboxylation reactions. Biotin aids in these reactions by binding carbon dioxide. An important example of a biotin-containing enzyme is pyruvate carboxylase. This enzyme catalyzes the conversion of pyruvate to oxaloacetate as a preliminary step in gluconeogenesis.

Biotin is found in foods such as liver, egg yolks, milk, fish and nuts. Deficiencies in biotin result in dermatitis, glossitis, muscle pain and anorexia. Deficiency in biotin has been observed in patients ingest-

ing raw eggs over a long period of time. Egg white contains avidin, a protein that binds biotin, strongly preventing its absorption from the intestine.²⁷ Biotin deficiency was observed in early attempts at parenteral nutrition before proper vitamin supplementation became standard practice.²⁸

Choline

Choline has many important biochemical roles in the body. It is incorporated into the formation of lecithin, an important structural phospholipid found most abundantly in mitochondrial membranes. It is also incorporated into platelet-activating factor, an important signaling agent in the clotting cascade. Choline also affects lipid mobilization from the liver. However, its most important role is as a precursor for the neurotransmitter acetylcholine where it plays a critical role in motor coordination.

Choline is found in eggs, peanuts, and liver. Deficiency in choline has not been reported in humans. It is believed that daily needs for choline can be met through biosynthesis and diet.

Carnitine

Carnitine has an important role in the metabolism of fatty acids. Carnitine accepts and donates fatty acids to co-enzyme A. This is an equilibrium process facilitated by cytosolic and mitochondrial enzymes known as carnitine acyl transferases. The attachment of fatty acids to carnitine is critical for their transport from the cytosol to the mitochondria, where they are later metabolized through β -oxidation.

Carnitine is found abundantly in meats and dairy products. Carnitine deficiency is not normally observed in adults unless it is caused by an inherited genetic disorder related to its transport or biosynthesis. Carnitine deficiency causes the storage of lipids in muscle tissue resulting in functional abnormalities in both cardiac and skeletal muscles. However, carnitine deficiencies are not uncommon in preterm or low birth weight infants. These infants generally respond to supplements of carnitine as well as changing to a low-fat, high-carbohydrate diet.²⁹

Ascorbic Acid (Vitamin C)

Ascorbic acid acts as an enzyme co-factor for 2 major biochemical processes. It is important for many hydroxylation reactions, especially those involved in the posttranslational hydroxylation of the amino acid proline in the formation of the structural protein collagen. In the gut, vitamin C is involved in the reduction of iron (III) to iron (II), allowing iron to be absorbed into the bloodstream.

Table 1. Student perception of the most significant part of the lecture, most interesting part of the lecture, and the topic they would like to have greater coverage.

Response	Percentage
Significance (N=28)	
Biochemical function of vitamins	61%
Vitamin deficiencies	18%
Sources of vitamins	11%
Interesting (N=28)	
Biotin and raw eggs	18%
Scurvy and "Limeys"	18%
Alcoholism and vitamins	14%
Increase Focus (N=27)	
Folic acid deficiency	8%
Alcoholism and vitamin deficiencies	8%
Drug interactions and vitamins	8%

Humans are one of the few organisms that are incapable of biosynthesizing ascorbic acid. As such they are highly dependent on obtaining it from their diet. Vitamin C is found in citrus fruits, tomatoes, strawberries, and potatoes. During the Age of Exploration, British sailors were given the nickname "limeys" due to the barrels of limes that were used on long voyages to supply vitamin C to the crew. The sailors were trying to avoid a deficiency in ascorbic acid known as scurvy.³⁰ The symptoms of scurvy are related to inhibited synthesis of collagen. These effects include failure of wounds to heal, defects in tooth formation, and capillary leakage. Anemia caused from failure to adequately absorb iron is also common.³¹ Several factors have been shown to lower serum vitamin C levels including smoking, oral contraceptives, and the use of aspirin.³²⁻³⁴ Patients exposed to any of these conditions long-term should consider taking a vitamin C supplement. However, the use of high doses of vitamin C, a theory popularized by Nobel Laureate Linus Pauling, has not been shown to provide any medical benefit.³⁵ The use of high doses of vitamin C has been shown to cause nausea, diarrhea, and lead to the formation of oxalate kidney stones.^{18,35}

Student Perception of Lecture

Student opinions were obtained using minute papers. Minute papers are given to a group of 25-30 students following each lecture in this course.³⁶ From the results of the minute papers in Table 1, the student's perception of the lecture is apparent. The students believe that the most significant material in the lecture

was the discussion on the major biochemical functions of the vitamins. This is consistent with the intent of the instructor. The students also felt that understanding vitamin deficiencies and the sources of the various vitamins was important. The students were asked what they found interesting about the lecture and not surprisingly they selected various stories as their favorite points. They especially liked the link between biotin, avidin, and eggs, the nautical history of scurvy resulting in the nickname "Limeys" being given to British sailors, and understanding the role of appetite suppression in alcoholism and general vitamin deficiencies. Over the years, students have commented that they enjoy having interesting health related facts to aid them in retaining and understanding the utility of biochemical information. Vitamins provide an enormous number of examples that can be used. Finally, the students were asked to recommend areas that could have increased focus in the future. In general these comments fall into 2 categories. The students are either confused and would like more information to more fully understand the topic, or they are genuinely interested and would like to know more. In this case, 71% of the student responded that they did not see anything else to add. There were a few suggestions for adding additional information on folic acid deficiency, alcoholism, and drug-vitamin interactions. This text incorporates many of these suggestions with the inclusion of the link between folic acid and neural tube defects, discussions of alcoholism throughout the lecture rather than being highly focused around thiamin, and drug interactions involving the vitamins pyridoxine, folic acid, and riboflavin.

CONCLUSIONS

Questions concerning the use of vitamins are common. Vitamins have important roles in many of the major metabolic pathways in the human body. In addition, drug interactions with vitamin and diseases involving vitamin deficiencies are important topics for pharmacy curricula. An understanding of the functional roles of vitamins can contribute to improved understanding of general biochemistry and help pharmacy students become better prepared for their roles as health care educators.

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