The effect of NAD, FAD, and Pyridoxine in immune system, as Potential Therapeutic and Preventive in Cancer and Autoimmunity

Dr. ZelalemKirosBitsue, EmnetAschalewFekede, Dr. ZelalemKirosBitsue,
United States of Africa Health Organization “AHO”,
Department of Immunology, International Campus, Tehran University of Medical SciencesTeklehaimanot General Hospital

Abstract: NAD is the sole substrate for PARP enzymes involved in DNA repair activity in response to DNA strand breaks; thus, NAD is critical for genome stability. Several studies, mostly using in vitro and animal models, suggest a possible role for niacin in cancer prevention. Nevertheless, large studies are needed to investigate the association between niacin deficiency and cancer risk in human populations.

Several clinical trials have explored the cardiovascular benefit of niacin in combination with other lipid-lowering medications.

Riboflavin (in the form, FAD) is required as a cofactor for the key folate-metabolizing enzyme, MTHFR.

Emerging evidence from intervention trials supports a protective role for riboflavin against hypertension in individuals with the MTHFR 677TT genotype. Riboflavin has been evaluated as a potential adjunct therapy in cancer and certain eye disorders.

Oxidative stress can cause opacification of the eye lens, leading to cataracts in older individuals. While the results of several intervention studies demonstrated that a potential benefit of riboflavin in the prevention of cataracts.

High levels of circulating homocysteine are associated with an increased risk of cardiovascular disease. Randomized controlled trialshave demonstrated that supplementation with B vitamins, including vitamin B6, could effectively reduce homocysteine levels.

Growing evidence from experimental and clinical studies suggests that systemic inflammation underlying most chronic diseases may impair vitamin B6 metabolism.

Randomized controlled trials support the use of vitamin B6 to treat morning sickness in pregnant women and suggest a possible benefit in the management of premenstrual syndrome and carpal tunnel syndrome.

In this article, I describe, the current knowledge of the Biological properties of NAD, FAD, and Pyridoxine, Antioxidant activity, the role of NAD, FAD, and Pyridoxine in cancer, and Autoimmunity

Key Words: NAD, FAD, and Pyridoxine, Immunomodulatory, Cancer and Autoimmunity

The Table of Content

1. Introduction

Riboflavin is a water-soluble B vitamin, also known as vitamin B2. In the body, riboflavin is primarily found as an integral component of the coenzymes, flavin adenine dinucleotide (FAD) and flavin mononucleotide (FMN)(1).Coenzymes derived from riboflavin are termed flavocoenzymes, and enzymes that use a flavocoenzyme are called flavoproteins(2).

2. Function

2.1. Oxidation-reduction (redox) reactions

Living organisms derive most of their energy from redox reactions, which are processes that involve the transfer of electrons. Flavocoenzymes participate in redox reactions in numerous metabolic pathways(3).They are critical for the metabolism of carbohydrates, lipids, and proteins. FAD is part of the electron transport (respiratory) chain, which is central to energy production. In conjunction with cytochrome P-450, flavocoenzymes also participate in the metabolism of drugs and toxins(4).

2.2. Antioxidant functions

Glutathione reductase is an FAD-dependent enzyme that participates in the redox cycle of glutathione. The glutathione redox cycle plays a major role in protecting organisms from

reactive oxygen species, such as hydroperoxides. Glutathione reductase (GR) requires FAD to regenerate two molecules of reduced glutathione from oxidized glutathione. Riboflavin deficiency has been associated withincreased oxidative stress(4).Measurement of GR activity in red blood cells is commonly used to assess riboflavin nutritional status(5).The erythrocyte glutathione reductase activation coefficient (EGRac) assay assesses riboflavin status by measuring the activity of GR before and after in vitro reactivation with its prosthetic group FAD; EGRac is calculated as the ratio of FAD-stimulated to unstimulated enzyme activity and indicates the degree of tissue saturation with riboflavin. EGRac is thus a functional measure of riboflavin status and has shown to be effective in reflecting biomarker status from severe deficiency to normal status(6).

Glutathione peroxidases, selenium-containing enzymes, require two molecules of reduced glutathione to break down hydroperoxides. GPx are involved in the glutathione oxidation-reduction (redox) cycle.

Xanthine oxidase, another FAD-dependent enzyme, catalyzes the oxidation of hypoxanthine and xanthine to uric acid. Uric acid is one of the most effective water-soluble antioxidants in the blood. Riboflavin deficiency can result in decreased xanthine oxidase activity, reducing blood uric acid levels(7).

3. **Introduction**

Niacin is a water-soluble vitamin, which is also known as nicotinic acid or vitamin B3. Nicotinamide is the derivative of niacin and used by the body to form the coenzymes nicotinamide adenine dinucleotide (NAD) and nicotinamide adenine dinucleotide phosphate (NADP). The chemical structures of the various forms of niacin are shown. None of the forms are related to the nicotine found in tobacco, although their names are similar(8).

4. Function

4.1. Oxidation-reduction (redox) reactions

Living organisms derive most of their energy from oxidation-reduction (redox) reactions, which are processes involving the transfer of electrons. Over 400 enzymes require the niacin coenzymes, NAD and NADP, mainly to accept or donate electrons for redox reactions(9).NAD functions most often in energy-producing reactions involving the degradation (catabolism) ofcarbohydrates, fats, proteins, and alcohol. NADP functions more often in biosynthetic (anabolic) reactions, such as in thesynthesis of all macromolecules, including fatty acids and cholesterol(8),(10).

4.2. Non-redox reactions

The niacin coenzyme, NAD, is the substrate (reactant) for at least four classes of enzymes that separate the nicotinamide moiety from NAD and transfer ADP-ribose to acceptors.

Mono-ADP-ribosyltransferase enzymes were first discovered in certain bacteria, where they mediate the action of toxins, such as cholera and diptheria. In mammalian cells, these enzymes transfer an ADP-ribose residue from NAD to a specific amino acidof a target protein, with the creation of an ADP-ribosylated protein and the release of nicotinamide. Mono ADP-ribosylation reactions reversibly modify the activity of acceptor proteins, such as G-proteins that bind guanosine-5'-triphosphate (GTP) and act as intermediaries in a number of cell-signaling pathways(11).

Poly-ADP-ribose polymerases (PARPs) are enzymes that catalyze the transfer of polymers of ADP-ribose from NAD to acceptorproteins. PARPs appear to function in DNA repair and stress responses, cell signaling, transcription, regulation, apoptosis, chromatin structure, and cell differentiation, suggesting a role for NAD in cancer prevention(10). At least six different PARPs have been identified, and although their functions are not yet fully understood, their existence indicates a potential for considerable consumption of NAD(12),(13).

A new nomenclature has been proposed for enzymes catalyzing ADP-ribosylation: The PARP family was renamed ARTD, while ARTC designates the mono ADP-ribosyltransferase family (13).

ADP-ribosylcyclases catalyze the formation of cyclic ADP-ribose from ADP-ribose. Cyclic ADP-ribose works within cells to provoke the release of calcium ions from internal storage sites and probably also plays a role in cell signaling(8).

Sirtuins are a class of NAD-dependent deacetylase enzymes that remove acetyl groups from the acetylated lysine residues of target proteins. During the deacetylation process, an ADP-ribose is added to the acetyl group to produce O-acetyl-ADP-ribose. Both acetylation and ADP-ribosylation are known post-translational modifications that affect protein activities. The initial interest in sirtuins followed the discovery that their activation could mimic calorie restriction, which has been shown to increase lifespan in lower organisms. Such a role in mammals is controversial, although sirtuins are energy-sensing regulators involved in signaling pathways that could play important roles in delaying the onset of age-related diseases (e.g.,cardiovascular disease, cancer, dementia, arthritis). To date, the spectrums of their biological functions include gene silencing,DNA damage repair, cell cycle regulation, and cell differentiation(14).

4.3. Tryptophan metabolism

In addition to its synthesis from dietary niacin, NAD can be synthesized from the dietary amino acid tryptophan via the kynurenine pathway. The relative ability to make this conversion varies greatly from mice to humans. The first step is catalyzed by the extrahepatic enzyme indoleamine 2,3-dioxygenase (IDO), which is responsible for the oxidative cleavage of

tryptophan. The chronic stimulation of tryptophan oxidation, mediated by an increased activity of IDO and/or inadequate niacin levels, is observed in a number of diseases, including human immunodeficiency virus (HIV) infection (see HIV/AIDS). In healthy individuals, less than 2% of dietary tryptophan is converted to NAD by this tryptophan oxidation pathway(15).Tryptophan metabolism plays an essential regulatory role by mediating immunological tolerance of the fetus during pregnancy(16).It is now understood that tryptophan oxidation in the placenta drives a physiologic tryptophan depletion that impairs the function of nearby maternal T-lymphocytes and prevents the rejection of the fetus. However, the synthesis of niacin from tryptophan is a fairly inefficient pathway that depends on enzymes requiring vitamin B6 and riboflavin, as well as an enzyme containing heme (iron). On average, 1 milligram (mg) of niacin can be synthesized from the ingestion of 60 mg of tryptophan. The term "niacin equivalent" (NE) is used to describe the contribution to dietary intake of all the forms of niacin that are available to the body. Thus, 60 mg of tryptophan are considered to be 1 mg NE. However, studies of pellagra in the southern US during the early twentieth century indicated that the diets of many individuals who suffered from pellagra contained enough NE to prevent pellagra(17).Challenging the idea that 60 mg of dietary tryptophan are equivalent to 1 mg of niacin. In particular, one study in young men found that the tryptophan content of the diet had no effect on the decrease in red blood cell niacin content that resulted from low dietary niacin(18).

5.Introduction

Vitamin B6 is a water-soluble vitamin that was first isolated in the 1930s. The term vitamin B6 refers to six common forms, namely pyridoxal, pyridoxine (pyridoxol), pyridoxamine, and their phosphorylated forms. The phosphate ester derivative pyridoxal 5'-phosphate (PLP) is the bioactive coenzyme form involved in over 4% of all enzymatic reactions(19),(20),(21).

5.1. Function

Vitamin B6 must be obtained from the diet because humans cannot synthesize it. PLP plays a vital role in the function of over 100 enzymes that catalyze essential chemical reactions in the human body(22).PLP-dependent enzymes have been classified into five structural classes known as Fold Type I-V(23).

- Fold Type I - aspartate aminotransferase family
- Fold Type II - tryptophan synthase family
- Fold Type III - alanine racemase family

- Fold Type IV - D-amino acid aminotransferase family
- Fold Type V - glycogen phosphorylase family

The many biochemical reactions catalyzed by PLP-dependent enzymes are involved in essential biological processes, such as hemoglobin and amino acid biosynthesis, as well as fatty acid metabolism. Of note, PLP also functions as a coenzymefor glycogen phosphorylase, an enzyme that catalyzes the release of glucose from stored glycogen. Much of the PLP in the human body is found in muscle bound to glycogen phosphorylase. PLP is also a coenzyme for reactions that generate glucose from amino acids, a process known as gluconeogenesis(24).

5.2. Nervous system function

In the brain, the PLP-dependent enzyme aromatic L-amino acid decarboxylase catalyzes the synthesis of two major neurotransmitters: serotonin from the amino acid tryptophan and dopamine from L-3, 4-dihydroxyphenylalanine (L-Dopa). Other neurotransmitters, including glycine, D-serine, glutamate, histamine, and γ-aminobutyric acid (GABA), are also synthesized in reactions catalyzed by PLP-dependent enzymes(25).

5.3. Hemoglobin synthesis and function

PLP functions as a coenzyme of 5-aminolevulinic acid synthase, which is involved in the synthesis of heme, an iron-containing component of hemoglobin. Hemoglobin is found in red blood cells and is critical to their ability to transport oxygen throughout the body. Both pyridoxal and PLP are able to bind to the hemoglobin molecule and affect its ability to pick up and release oxygen. However, the impact of this on normal oxygen delivery to tissues is not known(24),(26).Vitamin B6 deficiency may impair hemoglobin synthesis and lead to microcytic anemia(21).

5.4. Tryptophan metabolism

Deficiency in another B vitamin, niacin, is easily prevented by adequate dietary intakes. The dietary requirement for niacin and the niacin coenzyme, nicotinamide adenine dinucleotide (NAD), can be also met, though to a fairly limited extent, by the catabolism of the essential amino acid tryptophan in the tryptophan-kynurenine pathway. Key reactions in this pathway are PLP-dependent; in particular, PLP is the cofactor for the enzyme kynureninase, which catalyzes the conversion of 3-hydroxykynurenine to 3-hydroxyanthranilic acid. A reduction in PLP availability appears to primarily affect kynureninase activity, limiting NAD production and leading to higher concentrations of kynurenine, 3-hydroxykynurenine, and xanthurenic acid in blood and urine(27).Thus, while dietary vitamin B6 restriction impairs NAD synthesis from tryptophan, adequate PLP levels help maintain NAD formation from tryptophan (28).The effect of vitamin B6 inadequacy on immune activation and inflammation may be partly related to the role of PLP in the tryptophan-kynurenine metabolism (see Disease Prevention).

5.4. Hormone function

Steroid hormones, such as estrogen and testosterone, exert their effects in the body by binding to steroid hormone receptorsin the nucleus of target cells. The nuclear receptors themselves bind to specific regulatory sequences in DNA and alter the transcription of target genes. Experimental studies have uncovered a mechanism by which PLP may affect the activity of steroid receptors and decrease their effects on gene expression. It was found that PLP could interact with RIP140/NRIP1, a repressor of nuclear receptors known for its role in reproductive biology(29).Yet, additional research is needed to confirm that this interaction can enhance RIP140/NRIP1 repressive activity on steroid receptor-mediated gene expression. If the activity of steroid receptors for estrogen, progesterone, testosterone, or other steroid hormones can be inhibited by PLP, it is possible that vitamin B6 status may influence one's risk of developing diseases driven by steroid hormones, such as breast and prostatecancers(24).

5.5. Nucleic acid synthesis

The synthesis of nucleic acids from precursors thymidine and purines is dependent on folate coenzymes. The de novo thymidylate (dTMP) biosynthesis pathway involves three enzymes: dihydrofolatereductase (DHFR), serine hydroxymethyltransferase (SHMT), and thymidylate synthase (TYMS). PLP serves as a coenzyme for SHMT, whichcatalyzes the simultaneous conversions of serine to glycine and tetrahydrofolate (THF) to 5, 10-methylene THF. The latter molecule is the one-carbon donor for the generation of dTMP from dUMP by TYMS.

6. Conclusion

Riboflavin is the precursor of the coenzymes, flavin adenine dinucleotide (FAD) and flavin mononucleotide (FMN). They act as electron carriers in a number of oxidation-reduction(redox) reactions involved in energy production and in numerous metabolic pathways. Riboflavin deficiency can affect multiple pathways in the metabolism of vitamin B6, folate, niacin, and iron. Riboflavin has been evaluated as a potential adjunct therapy in cancer and certain eye disorders.

Niacin and its derivative nicotinamide are dietary precursors of nicotinamide adenine dinucleotide (NAD), which can be phosphorylated (NADP) and reduced (NADH and NADPH). NAD functions in oxidation-reduction (redox) reactions and non-redox reactions. Causes of niacin deficiency include inadequate oral intake, poor bioavailability from unlimed grains, defective tryptophan absorption, metabolic disorders, and the long-term use of chemo-therapeutic treatments. NAD is the sole substrate for PARP enzymes involved in DNA repair activity in response to DNA strand breaks; thus, NAD is critical for genome stability. Several studies, mostly using in vitro and animal models, suggest a possible role for niacin in cancer prevention. Nevertheless, large studies are needed to investigate the association between niacin deficiency and cancer risk in human populations.

Vitamin B6 and its derivative pyridoxal 5'-phosphate (PLP) are essential to over 100 enzymes mostly involved in protein metabolism. High levels of circulating homocysteine are associated with an increased risk of cardiovascular disease. Randomized controlled trialshave demonstrated that supplementation with B vitamins, including vitamin B6, could effectively reduce homocysteine levels. However, homocysteine lowering by B vitamins has failed to lower the risk of adverse cardiovascular outcomes in high-risk individuals. Growing evidence from experimental and clinical studies suggests that systemic inflammation underlying most chronic diseases may impair vitamin B6 metabolism.

7. Reference

1. Thiamin R, Niacin V. B6, Folate, Vitamin B12, Pantothenic Acid, Biotin, and Choline. Washington, DC: National Academies Press; 1998.
2. Brody T. Nutritional biochemistry: Academic press; 1998.
3. Manore MM. Effect of physical activity on thiamine, riboflavin, and vitamin B-6 requirements. The American journal of clinical nutrition. 2000;72(2):598s-606s.
4. Powers HJ. Current knowledge concerning optimum nutritional status of riboflavin, niacin and pyridoxine. Proceedings of the Nutrition Society. 1999;58(02):435-40.
5. Ziegler EE, Filer Jr L. Present knowledge of nutrition: International Life Sciences Institute (ILSI Press); 1996.
6. Hoey L, McNulty H, Strain J. Studies of biomarker responses to intervention with riboflavin: a systematic review. The American journal of clinical nutrition. 2009:ajcn. 27230B.
7. Böhles H. Antioxidative vitamins in prematurely and maturely born infants. International journal for vitamin and nutrition research Internationale Zeitschrift fur Vitamin- und Ernahrungsforschung Journal international de vitaminologie et de nutrition. 1996;67(5):321-8.
8. Brody T. Nutrients that resist or escape digestion. Nutritional Biochemistry 2nd edition, Academic Press, San Diego. 1999:53-61.
9. Erdman Jr JW, MacDonald IA, Zeisel SH. Present knowledge in nutrition: John Wiley & Sons; 2012.
10. Shils ME, Shike M. Modern nutrition in health and disease: Lippincott Williams & Wilkins; 2006.
11. Di Girolamo M, Dani N, Stilla A, Corda D. Physiological relevance of the endogenous mono (ADP-ribosyl) ation of cellular proteins. FEBS journal. 2005;272(18):4565-75.
12. CH O, OC O, HO O. Discovering new ADP-ribose polymer cycles: protecting the genome and more. 1999.
13. Hottiger MO, Hassa PO, Lüscher B, Schüler H, Koch-Nolte F. Toward a unified nomenclature for mammalian ADP-ribosyltransferases. Trends in biochemical sciences. 2010;35(4):208-19.
14. Morris BJ. Seven sirtuins for seven deadly diseases ofaging. Free Radical Biology and Medicine. 2013;56:133-71.
15. Murray MF. Tryptophan depletion and HIV Tryptophan depletion and HIV infection: a metabolic link to pathogenesis. The Lancet infectious diseases. 2003;3(10):644-52.
16. Hao K, Zhou Q, Chen W, Jia W, Zheng J, Kang J, et al. Possible role of the 'IDO-AhR axis' in maternal-foetal tolerance. Cell biology international. 2013;37(2):105-8.

17. Jacob RA, Gretz DM, Taylor PC, James SJ, Pogribny IP, Miller BJ, et al. Moderate folate depletion increases plasma homocysteine and decreases lymphocyte DNA methylation in postmenopausal women. The Journal of nutrition. 1998;128(7):1204-12.
18. Fu CS, Swendseid ME, Jacob RA, McKee RW. Biochemical markers for assessment of niacin status in young men: levels of erythrocyte niacin coenzymes and plasma tryptophan. The Journal of nutrition. 1989;119(12):1949-55.
19. Dakshinamurti K, Dakshinamurti S. Neonatal epileptic encephalopathy: role of vitamin B-6 vitamers in diagnosis and therapy. The American journal of clinical nutrition. 2014;100(2):505-6.
20. Galluzzi L, Vacchelli E, Michels J, Garcia P, Kepp O, Senovilla L, et al. Effects of vitamin B6 metabolism on oncogenesis, tumor progression and therapeutic responses. Oncogene. 2013;32(42):4995-5004.
21. Bailey L, Moyers S, Gregory J, Bowman B, Russell R. Present knowledge in nutrition. Washington, DC: Internation Life Sciences Institute. 2001.
22. Chandra RK. Nutrition, immunity, and infection: present knowledge and future directions. The Lancet. 1983;321(8326):688-91.
23. Eliot AC, Kirsch JF. Pyridoxal phosphate enzymes: mechanistic, structural, and evolutionary considerations. Annual review of biochemistry. 2004;73(1):383-415.
24. Shils M, Shike M. Nutritional support of the cancer patient. Modern Nutrition in Health and Disease Philadelphia. 1999.
25. Clayton PT. B6-responsive disorders: a model of vitamin dependency. Journal of inherited metabolic disease. 2006;29(2-3):317-26.
26. Schnackerz K, Benesch R, Kwong S, Benesch R, Helmreich E. Specific receptor sites for pyridoxal 5'-phosphate and pyridoxal 5'-deoxymethylenephosphonate at the alpha and beta NH2-terminal regions of hemoglobin. Journal of Biological Chemistry. 1983;258(2):872-5.
27. Rios-Avila L, Nijhout HF, Reed MC, Sitren HS, Gregory JF. A mathematical model of tryptophan metabolism via the kynurenine pathway provides insights into the effects of vitamin B-6 deficiency, tryptophan loading, and induction of tryptophan 2, 3-dioxygenase on tryptophan metabolites. The Journal of nutrition. 2013;143(9):1509-19.
28. Oxenkrug G. Insulin resistance and dysregulation of tryptophan–kynurenine and kynurenine–nicotinamide adenine dinucleotide metabolic pathways. Molecular neurobiology. 2013;48(2):294-301.
29. Huq MM, Tsai N-P, Lin Y-P, Higgins L, Wei L-N. Vitamin B6 conjugation to nuclear corepressor RIP140 and its role in gene regulation. Nature chemical biology. 2007;3(3):161-5.