Quantitative Biology 2015, 3(4): 193–198
DOI 10.1007/s40484-015-0055-9

Mini Review

NAD^+ and its precursors in human longevity

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Received September 18, 2015; Revised November 13, 2015; Accepted November 18, 2015

Aging is a complex issue due to its nature in progressive physiological and functional decay. As better medicine, technology, and living conditions became accessible to many people, the longevity of human beings increased during the past centuries. Recent research established vital roles for NAD^+ and its precursors in protecting and maintaining the redox homeostasis in cells, which might be applicable therapeutically to prevent cell degeneration. Notably, the contribution of NAD^+ metabolites to lifespan extension in model systems indicates that the potential beneficial effects of NAD^+ precursors. In this mini review, by introducing the background of NAD^+-consuming enzymes in "caloric restriction", we focus on NAD^+ and its precursors in diet, with further emphasis on its association with health and diseases. We also provide insights in future utilization of NAD^+ and its precursors as nutrition supplement for lifespan extension.

Keywords: longevity; sirtuins; NAD^+; NAD^+ synthesis; NAD^+ precursor; nicotinamide riboside

INTRODUCTION

In the early 20th century, nicotinamide adenine dinucleotide (NAD^+) coenzyme was initially found in fractions passed through dialysis in reconstitution of cell-free glucose fermentation [1]. Acting as the hydride acceptor, NAD^+ is reduced to NADH in the process of glucose fermentation and respiration. In addition, NADH functions as the hydride donor to regenerate NAD^+. For example, the reduction of acetaldehyde to ethanol by alcohol dehydrogenase requires NADH oxidation to NAD^+. Similarly, numerous hydride transfer enzymes or oxidoreductases interconvert NAD^+ and NADH to reduce or oxidize other substrates. Moreover NAD^+ is not only a hydride-transfer coenzyme, but also a substrate for NAD^+-consuming enzymes that break down NAD^+ into smaller form of metabolites, usually its precursors. NAD^+'s phosphorylated form, nicotinamide adenine dinucleotide phosphate ($NADP^+$), also has similar roles as NAD^+ in intracellular metabolism and energy production as hydride-accepting coenzymes in cell.

Later in the mid-20th century, a non-pharmacological intervention called "caloric restriction" was proposed, as a way to increase lifespan and protect against metabolic diseases [2]. Among the diverse mechanisms by which caloric restriction might act, Silent information regulator 2/Silent information regulator T1 (Sir2/SIRT1) has been the focus of much attention. The hypothesis addressed Sir2/SIRT1 as a key mediator of the beneficial effects of caloric restriction. In caloric restriction, there is an increase in respiration thus as a result increase the amount of NAD^+ pool in cell. Under the stresses such as DNA damage and inflammation, NAD^+-consuming enzymatic activities are induced [3]. Many of these stresses are accompanied by specifically induced biosynthetic pathways, along with the increase and/or reduction in the concentration of NAD^+ pool to promote regulatory functions.

NAD^+ consumption is linked intrinsically to intracellular and intercellular signaling reactions that control downstream gene expressions, including aging and cell death. In this mini review, we provide a detailed overview of NAD^+ metabolism with an emphasis on the potential NAD^+-boosting therapies to maintain health and treat diseases.

CALORIC RESTRICTION AND LONGEVITY

More than 40 years ago, scientists have realized that

dietary habit might effect on longevity [2]. A major cornerstone is called "caloric restriction" (CR), i.e., a normally 20%–40% reduced caloric intake compared with an *ad libitum* diet within the essential demand of nutrients, which may increase lifespan and protect against the deterioration of biological functions [3]. In a simple word, "eat less, live longer". CR was initially proposed by the findings in rats [2] and remains the most consistent non-pharmacological intervention in the aging field. During recent decades, multiple evidences supporting that caloric restriction increases the maximal longevity have been reported in model organisms such as yeasts, worms, flies, fish and mice [4], even in rhesus monkeys [5], indicating that even in the whole evolutionary scale, the effects of CR still make sense.

Despite that the mechanism of CR is still a matter of debate, the hypothesis that CR impacts Sir2/SIRT1 activity might be the most convincing one, i.e., the metabolic shift from fermentation to mitochondrial respiration occurring in response to CR results in increased NAD^+ intracellular levels [3], which would in turn increases Sir2 activity. Sir2 and SIRT1 are members of the sirtuin family characterized by a sirtuin core domain [6]. NAD^+ acts as an activator while NADH acts as a competitive inhibitor of Sir2 deacetylase, further triggering the downstream physiological reactions [6]: human sirtuins function as intracellular regulatory proteins with mono-ADP-ribosyltransferase activity, reacting to stressors and inflammation [7]; yeast sirtuin proteins are known as histone deacetylase to regulate epigenetic gene silencing and suppress recombination of rDNA [8]. In all, CR produces a signal in cell that boosts the level of NAD^+, and then the activation of Sir2/SIRT1 by NAD^+ further regulates cell-fate related gene expression.

In addition, there is also compelling evidence supporting that mitochondrial activity is positively correlated to lifespan [9]. However, a contradictory evidence challenging this prediction that CR can efficiently increase lifespan in several respiratory deficient yeast strains [10]. A conciliatory view would be that mitochondrial respiration might be associated with proper longevity, but how mitochondrial respiration might affect NAD^+/ NADH levels is still largely unknown. After all, NAD^+/ NADH levels are determined by complex networks of reactions [11] (as shown in Figure 1). Whether NAD^+ acts as a true metabolic sensor in redox balance and how its compartmentalization affects lifespan is worth further researching.

NAD^+ AND ITS PRECURSORS IN DIET

NAD^+'s dietary sources, which is commonly called niacin or vitamin B3, include tryptophan (Trp), nicotinic acid (Na), nicotinamide (Nam), and the newly identified NAD^+ precursor, nicotinamide riboside (NR) (Figure 2). Especially, NR is a natural product, the so-called hidden vitamin found in cow's milk [12]. Trp is converted to NAD^+ through a *de novo* pathway [13], while Na, Nam, NR are considered as "salvageable precursors" to rebuild NAD^+, respectively. Interestingly, bacterium called *Haemophilus influenza* can only grow on medium supplied with NR, but cannot grow on Na, Nam, or Trp, which were the previously known precursors of NAD^+ [14]. Also, NR permitted NAD^+ synthase-depleted yeast

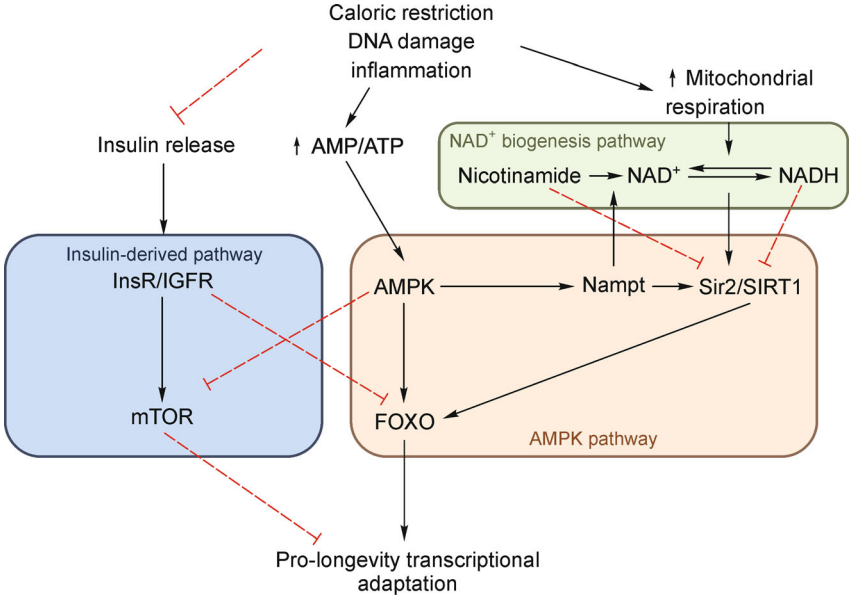

Figure 1. Integrative view of mammalian signaling pathways regulating NAD^+/NADH pool and downstream effects (modified from [3]).

cells to grow. That is because NR can use an additional salvageable pathway called Nrk pathway [12,13] to form NAD^+ and that is why NR was initially described as a growth factor, termed Factor V [15].

As downstream metabolism, NAD^+ can be transformed to Nam and multiple forms of ADP-ribose by at least three types of NAD^+-consuming enzymes (Figure 2): (i) cADP-ribose synthases cyclize the ADP-ribose moiety of NAD^+; (ii) ADP-ribosyltransferases (ARTs) post-translationally modify proteins with an ADP-ribosyl group; (iii) poly-ADP-ribosyltransferases (PARPs) form a polymer of ADP-ribose from multiple NAD^+ molecules [16]. As NAD^+-consuming enzymes, Sirtuins use the ADP-ribose moiety of NAD^+ to accept the acetyl modification of a protein lysine, forming deacetylated protein and triggering downstream regulative signals.

Since NAD^+ has various precursors, even a poor diet can meet a baseline requirement for NAD^+ synthesis [14]. The one who subsists on tryptophan-poor diets may avoid the risk of nutritional deficiency by supplementing any of other NAD^+ precursors, such as Na, Nam or NR [17]. However, there is growing evidence showing that substantially higher efficiency of NAD^+ synthesis may be beneficial to protect against neurological degeneration, *Candida glabrata* infection, and possibly to enhance reverse cholesterol transport [13]. The abundance of NAD^+ in human cells is controlled by many factors and each NAD^+ pool is sequestered in different compartments. Thus, increase or decrease of NAD^+ may act as a physiological signal in cell.

NAD^+'S FUNCTIONS IN HEALTH AND DISEASES

Deficiency of NAD^+ or its precursors may cause pellagra, which is classically described by the three Ds: dermatitis, diarrhea, and dementia [18]. Pellagra was ever thought to be an infectious disease because it was common among the rural poor in the undeveloped areas, however, it turn out to be caused by a dietary deficiency which can be cured and prevented by substituting corn-based diets with fresh milk, eggs, and meat [19]. Consistently, deep examination with pellagra animals showed a significant decrease in muscle and liver NAD^+ and $NADP^+$ [1]. It was proved that a Nam fraction from deproteinized liver could cure "black tongue" on malnourished dogs.

Aside from the most common known pellagra, NAD^+ was shown to block experimental autoimmune encephalomyelitis (EAE), a mouse model of multiple sclerosis (MS), by inducing immune homeostasis through $CD4^+$ T cells and reverses disease progression by restoring tissue integrity via remyelination and neuroregeneration. This finding demonstrated that NAD^+ might serve as a powerful therapeutic agent for the treatment of autoimmune and other diseases [20]. Additionally, NAD^+ was proved to have potential clinical use in acute or chronic disease such as acroaesthesia, chronic obstructive pulmonary, hematosepsis and immunodeficiency [13,21,22].

Recent results established protective roles for NAD^+ that might be applicable therapeutically to prevent neurodegenerative conditions. It is particularly striking that nicotinamide riboside kinase 2 (Nrk2) was identified as highly regulated proteins involved in neuron system [23] and muscle cell development [24]. The utilization of NR as a NAD^+ precursor was first demonstrated in mammalian dorsal root ganglion (DRG) neurons, in which NR induced the *NRK2* transcript when damaged by axotomy [25]. DRG neurons cannot be protected from damage-induced neuropathy by Na or Nam, which suggests that NR is a uniquely useful precursor to the

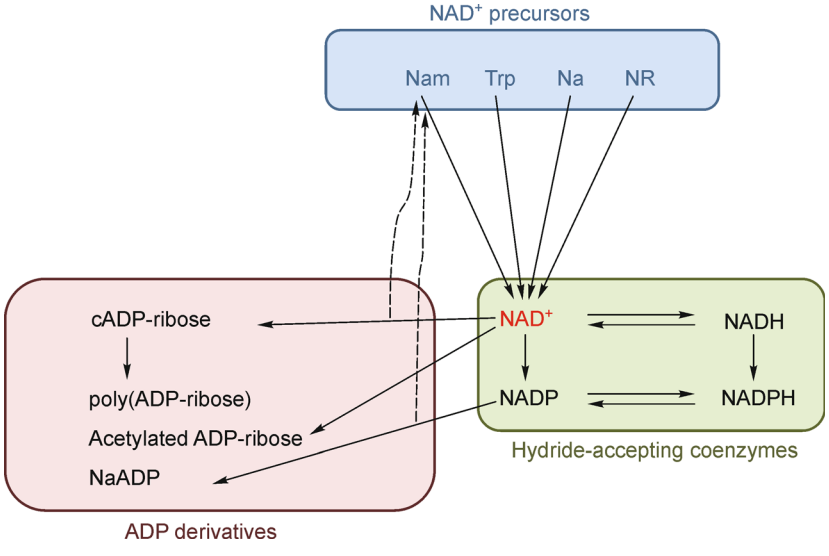

Figure 2. NAD^+ precursors and downstream NAD^+derivatives (modified from [16]).

nervous system [25] when *de novo* synthesis of NAD^+ from Trp is not sufficient. Nrk2 is present in heart, brain, and skeletal muscle, and is notably absent in kidney, liver, lung, pancreas, and placenta [24,25], which suggests utilization of NR and/or NaR [23] function in a broader range of cell types than Na or Nam. Another interesting study showed that administration of NR, even after noise exposure, prevents noise-induced hearing loss (NIHL) and spiral ganglia neurite degeneration. These effects are mediated by SIRT3, which is one member of sirtuin family as mentioned above. Under the noise exposure, NR can function in activating a NAD^+-SIRT3 pathway and further reduces neurite degeneration [26]. These findings indicate that NR can be a "star" agent in NAD^+ precursor supplementation, especially, against neurodegenerative diseases [25].

Other studies demonstrated that high dose of Na is used as an agent that elevates high-density lipoprotein cholesterol, lowers low-density lipoprotein cholesterol and lowers free fatty acids through a mechanism that is not completely understood. It was suggested that NR might possess such an activity by elevating NAD^+ in the cells responsible for reverse cholesterol transport [14]. An experiment with mice on high fat diet appears to support the potential of treatment or prevention of dyslipidemia with NR [27].

It will be important to determine how gene expression and precursor utilization changes as a function of nutrition, age, stress, and diseases. Thus, deep understanding of NAD^+ synthesis in developmental regulation and diet supplement of NAD^+ precursors remains on the forefront of NAD^+ application in clinic and medicine. Disease discussed above is summarized in Table 1.

NAD^+ AND ITS APPLICATION IN NUTRITION SUPPLEMENT

The contribution that NAD^+ metabolism makes to lifespan extension in model systems indicates that therapies to boost NAD^+ may promote effects. There is an undeniable amount of data clearly suggesting that NAD^+-Sir2/SIRT1 pathways modulate longevity, which shows a great prospect of NAD^+ and its precursor in daily nutrition supplement for aging people.

As one of the water-soluble vitamins, vitamin B3 is not readily stored and is excreted from human body, so consistent intake is important. Basically, the most fundamental use of vitamin B3 is as a kind of daily nutrition supplement contained in multivitamin tablets. The best-known agents of multivitamin supplement include Swisse, Centrum, GNC and etc.

As discussed above, NR is a natural product found in milk [12] and thus could be used as a novel supplement, potentially for people who have adverse reactions to Na or Nam, without unpleasant side effects from high-dose of Na or Nam. Considering the side effects associated with high-dose use of Nam in the prevention and treatment of diabetic disorders [29,40], NR may represent an alternative supplement [41,42]. More significantly, the specific utilization of NR may provide qualitative advantages over other NAD^+ precursors in promoting function in the central and peripheral nervous system [16].

NR has become more and more popular today as various scientific studies had proved its ability to prevent neurodegeneration, obesity and inflammation. NR has great potential as a supplement or therapeutic agent that would elevate or maintain NAD^+ in specific tissues, as the only vitamin precursor that supports neuronal NAD^+ synthesis [43,44]. One publicly trading example is that Chromadex acquired intellectual property on uses and synthesis of NR from Dartmouth College, Cornell University and Washington University and began distributing NR as NIAGEN™ in 2013.

Thanks to the development of medical and clinical technology, the average lifespan of human beings

Table 1. NAD^+ precursors and relationship to diseases.

Metabolite	Disease	Associated pathway	Refs.
Tryptophan (Trp)	Niacin deficiency	*De novo* pathway	[28]
Nicotinamide (Nam)	Diabetes, Cardiac disease, stroke, fetal ischemia, and fetal alcohol syndrome	Nam salvage pathway	[29–33]
Nicotinamide riboside (NR)	Bacterial infection (*Hemophilus influenzae*), neurodegenerative diseases, dyslipidemia	Bacterial NAD^+, Metabolism, NAD^+-SIRT pathway, Nrk pathway	[13,25,27]
Nicotinic acid (Na)	Hypercholesterolemia	Nam salvage pathway	[34–36]
NAD^+	Stroke, cardiac ischemia, acroaesthesia	NAD^+-SIRT pathway	[31,37]
Vitamin B3 (all forms of NAD^+ and its precursor)	Pellagra or B_3 deficiency; dermatitis, diarrhea, and dementia	NAD^+-SIRT pathway	[13,18,38,39]

increases to 71 year-old today. However, it is still difficult for the scientific community to precisely understand how to live a long life, because aging can be a reflection of the progressive functional decay of not just one but a complex ensemble of physiological functions. Although the final conclusions of the hypothesis that NAD^+ precursor supplements extend lifespan might still take several decades to be realized, future work will raise substantial health, safety, and efficacy concerns of utilization of NAD^+ and its precursors as nutrition supplements in animal and human systems to maintain health and to prevent disease.

ACKNOWLEDGEMENTS

We gratefully acknowledge the National Natural Science Foundation of China (No. 21502026), Shenzhen Science and Technology Innovation Committee (JCYJ20140411112047887) for their support to Yanchao Pan. We thank Dr. Zhen Xie for comments on the manuscript.

COMPLIANCE WITH ETHICS GUIDELINES

The authors Jimin Lin, Yanchao Pan and Jiangyun Wang declare that they have no conflict of interests.

This article does not contain any studies with human or animal subjects performed by any of the authors.

REFERENCES

1. Dann, W. J. (1937) Nicotinic acid and vitamin B2. Science, 86, 616–617
2. McCay, C. M., Crowell, M. F. and Maynard, L. A. (1989) The effect of retarded growth upon the length of life span and upon the ultimate body size. 1935. Nutrition, 5, 155–171, discussion 172
3. Cantó, C. and Auwerx, J. (2009) Caloric restriction, SIRT1 and longevity. Trends Endocrinol. Metab., 20, 325–331
4. Koubova, J. and Guarente, L. (2003) How does calorie restriction work? Genes Dev., 17, 313–321
5. Lane, M. A., Ingram, D. K. and Roth, G. S. (1999) Calorie restriction in nonhuman primates: effects on diabetes and cardiovascular disease risk. Toxicol. Sci., 52, 41–48
6. Schmidt, M. T., Smith, B. C., Jackson, M. D. and Denu, J. M. (2004) Coenzyme specificity of Sir2 protein deacetylases: implications for physiological regulation. J. Biol. Chem., 279, 40122–40129
7. Sinclair, D. A. and Guarente, L. (2006) Unlocking the secrets of longevity genes. Sci. Am., 294, 48–57
8. Sinclair, D. A. and Guarente, L. (1997) Extrachromosomal rDNA circles—a cause of aging in yeast. Cell, 91, 1033–1042
9. Dilova, I., Easlon, E. and Lin, S. J. (2007) Calorie restriction and the nutrient sensing signaling pathways. Cell. Mol. Life Sci., 64, 752–767
10. Kaeberlein, M. and Powers, R. W. III (2007) Sir2 and calorie restriction in yeast: a skeptical perspective. Ageing Res. Rev., 6, 128–140
11. Rongvaux, A., Andris, F., Van Gool, F. and Leo, O. (2003) Reconstructing eukaryotic NAD metabolism. BioEssays, 25, 683–690
12. Bieganowski, P. and Brenner, C. (2004) Discoveries of nicotinamide riboside as a nutrient and conserved *NRK* genes establish a Preiss-Handler independent route to NAD^+ in fungi and humans. Cell, 117, 495–502
13. Belenky, P., Racette, F. G., Bogan, K. L., McClure, J. M., Smith, J. S. and Brenner, C. (2007) Nicotinamide riboside promotes Sir2 silencing and extends lifespan via Nrk and Urh1/Pnp1/Meu1 pathways to NAD^+. Cell, 129, 473–484
14. Belenky, P., Bogan, K. L. and Brenner, C. (2007) NAD^+ metabolism in health and disease. Trends Biochem. Sci., 32, 12–19
15. Gingrich, W. and Schlenk, F. (1944) Codehydrogenase I and other pyridinium compounds as V-Factor for *hemophilus influenzae* and *H. parainfluenzae*. J. Bacteriol., 47, 535–550
16. Bogan, K. L. and Brenner, C. (2008) Nicotinic acid, nicotinamide, and nicotinamide riboside: a molecular evaluation of NAD^+ precursor vitamins in human nutrition. Annu. Rev. Nutr., 28, 115–130
17. DissertationTip (1998) Dietary Reference Intakes for Thiamin, Riboflavin, Niacin, Vitamin B6, Folate, Vitamin B12, Pantothenic Acid, Biotin, and Choline. Washington: National Academy Press
18. Hegyi, J., Schwartz, R. A. and Hegyi, V. (2004) Pellagra: dermatitis, dementia, and diarrhea. Int. J. Dermatol., 43, 1–5
19. Wilson, S.A. (1914) The Pathology of pellagra. In Proc. R. Soc. Med., 7, 31–41
20. Tullius, S. G., Biefer, H. R., Li, S., Trachtenberg, A. J., Edtinger, K., Quante, M., Krenzien, F., Uehara, H., Yang, X., Kissick, H. T., et al. (2014) NAD^+ protects against EAE by regulating $CD4^+$ T-cell differentiation. Nat. Commun., 5, 5101
21. Slegtenhorst, B. R., Dor, F. J., Rodriguez, H., Voskuil, F. J. and Tullius, S. G. (2014) Ischemia/reperfusion injury and its consequences on immunity and inflammation. Curr. Transplant. Rep., 1, 147–154
22. Bernofsky, C. (1980) Physiology aspects of pyridine nucleotide regulation in mammals. Mol. Cell. Biochem., 33, 135–143
23. Tempel, W., Rabeh, W. M., Bogan, K. L., Belenky, P., Wojcik, M., Seidle, H. F., Nedyalkova, L., Yang, T., Sauve, A. A., Park, H. W., et al. (2007) Nicotinamide riboside kinase structures reveal new pathways to NAD^+. PLoS Biol., 5, e263
24. Li, J., Mayne, R. and Wu, C. (1999) A novel muscle-specific beta 1 integrin binding protein (MIBP) that modulates myogenic differentiation. J. Cell Biol., 147, 1391–1398
25. Sasaki, Y., Araki, T. and Milbrandt, J. (2006) Stimulation of nicotinamide adenine dinucleotide biosynthetic pathways delays axonal degeneration after axotomy. J. Neurosci., 26, 8484–8491
26. Brown, K. D., Maqsood, S., Huang, J. Y., Pan, Y., Harkcom, W., Li, W., Sauve, A., Verdin, E. and Jaffrey, S. R. (2014) Activation of SIRT3 by the NAD^+ precursor nicotinamide riboside protects from noise-induced hearing loss. Cell Metab., 20, 1059–1068
27. Cantó, C., Houtkooper, R. H., Pirinen, E., Youn, D. Y., Oosterveer, M. H., Cen, Y., Fernandez-Marcos, P. J., Yamamoto, H., Andreux, P. A., Cettour-Rose, P., et al. (2012) The NAD^+ precursor nicotinamide riboside enhances oxidative metabolism and protects against high-fat diet-induced obesity. Cell Metab., 15, 838–847
28. Fukuwatari, T., Ohta, M., Kimtjra, N., Sasaki, R. and Shibata, K. (2004) Conversion ratio of tryptophan to niacin in Japanese women fed a purified diet conforming to the Japanese Dietary Reference Intakes. J. Nutr. Sci. Vitaminol., 50, 385–391
29. Knip, M., Douek, I. F., Moore, W. P., Gillmor, H. A., McLean, A. E., Bingley, P. J., Gale, E. A. and the European Nicotinamide Diabetes Intervention Trial Group. (2000) Safety of high-dose nicotinamide: a review. Diabetologia, 43, 1337–1345
30. Carson, D. (2004) European Nicotinamide Diabetes Intervention Trial

(ENDIT): a randomised controlled trial of intervention before the onset of type 1 diabetes. Lancet, 363, 925–931

31. Virág, L. and Szabó, C. (2002) The therapeutic potential of poly(ADP-ribose) polymerase inhibitors. Pharmacol. Rev., 54, 375–429
32. Ieraci, A. and Herrera, D. G. (2006) Nicotinamide protects against ethanol-induced apoptotic neurodegeneration in the developing mouse brain. PLoS Med., 3, e101
33. Feng, Y., Paul, I. A. and LeBlanc, M. H. (2006) Nicotinamide reduces hypoxic ischemic brain injury in the newborn rat. Brain Res. Bull., 69, 117–122
34. Capuzzi, D. M., Morgan, J. M., Brusco, O. A. Jr and Intenzo, C. M. (2000) Niacin dosing: relationship to benefits and adverse effects. Curr. Atheroscler. Rep., 2, 64–71
35. Carlson, L. A. (2004) Niaspan, the prolonged release preparation of nicotinic acid (niacin), the broad-spectrum lipid drug. Int. J. Clin. Pract., 58, 706–713
36. Kamanna, V. S. and Kashyap, M. L. (2000) Mechanism of action of niacin on lipoprotein metabolism. Curr. Atheroscler. Rep., 2, 36–46
37. Hassa, P. O., Haenni, S. S., Elser, M. and Hottiger, M. O. (2006) Nuclear ADP-ribosylation reactions in mammalian cells: where are we today and where are we going? Microbiol. Mol. Biol. Rev., 70, 789–829
38. Revollo, J. R., Körner, A., Mills, K. F., Satoh, A., Wang, T., Garten, A., Dasgupta, B., Sasaki, Y., Wolberger, C., Townsend, R. R., et al. (2007) Nampt/PBEF/Visfatin regulates insulin secretion in beta cells as a systemic NAD biosynthetic enzyme. Cell Metab., 6, 363–375
39. Kirkland, J. B. (2003) Niacin and carcinogenesis. Nutr. Cancer, 46, 110–118
40. Reddy, S., Bibby, N. J. and Elliott, R. B. (1990) Early nicotinamide treatment in the NOD mouse: effects on diabetes and insulitis suppression and autoantibody levels. Diabetes Res., 15, 95–102
41. Dali-Youcef, N., Lagouge, M., Froelich, S., Koehl, C., Schoonjans, K. and Auwerx, J. (2007) Sirtuins: the ‘magnificent seven’, function, metabolism and longevity. Ann. Med., 39, 335–345
42. Westphal, C. H., Dipp, M. A. and Guarente, L. (2007) A therapeutic role for sirtuins in diseases of aging? Trends Biochem. Sci., 32, 555–560
43. Zhang, R. (2013) MNADK, a novel liver-enriched mitochondrion-localized NAD kinase. Biol. Open, 2, 432–438
44. Zhang, R. (2015) MNADK, a long-awaited human mitochondrion-localized NAD kinase. J. Cell. Physiol., 230, 1697–1701

