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Role of Nicotinamide Adenine Dinucleotide and Related Precursors as Therapeutic Targets for Age-Related Degenerative Diseases: Rationale, Biochemistry, Pharmacokinetics, and Outcomes

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4 ***Role of NAD⁺ and related Precursors as Therapeutic Targets for***
5 ***Age-related Degenerative Diseases: Rationale, Biochemistry,***
6 ***Pharmacokinetics, and Outcomes***
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Abstract

Significance: Nicotinamide adenine dinucleotide (NAD⁺) is an essential pyridine nucleotide that serves as an essential cofactor and substrate for a number of critical cellular processes involved in oxidative phosphorylation and ATP production, DNA repair, epigenetically modulated gene expression, intracellular calcium signalling and immunological functions. NAD⁺ depletion may occur in response to either excessive DNA damage due to free radical or UV attack, resulting in significant PARP activation and a high turnover and subsequent depletion of NAD⁺, and/or chronic immune activation and inflammatory cytokine production resulting in accelerated CD38 activity and decline in NAD⁺ levels. Recent studies have shown that enhancing NAD⁺ levels can profoundly reduce oxidative cell damage in catabolic tissue, including the brain. Therefore, promotion of intracellular NAD⁺ anabolism represents a promising therapeutic strategy for age-associated degenerative diseases in general, and is essential to the effective realisation of multiple benefits of healthy sirtuin activity. The kynurenine pathway represents the de novo NAD⁺ synthesis pathway in mammalian cells. NAD⁺ can also be produced by the NAD⁺ salvage pathway. **Recent Advances:** In this review, we describe and discuss recent insights regarding the efficacy and benefits of the NAD⁺ precursors, nicotinamide (NAM), nicotinic acid (NA), nicotinamide riboside (NR), and nicotinamide mononucleotide (NMN), in attenuating NAD⁺ decline in degenerative disease states and physiological ageing. **Critical Issues:** Results obtained in recent years have shown that NAD⁺ precursors can play important protective roles in several diseases. However, in some cases, these precursors may vary in their ability to enhance NAD⁺ synthesis via their location in the NAD⁺ anabolic pathway. Increased synthesis of NAD⁺ promotes protective cell responses, further demonstrating that NAD⁺ is a regulatory molecule associated with several biochemical pathways. **Future Directions:** In the next few years, the refinement of personalised therapy for the use of NAD⁺ precursors, and improved detection methodologies allowing the administration of specific NAD⁺ precursors in the context of patients' NAD⁺ levels will lead to a better understanding of the therapeutic role of NAD⁺ precursors in human diseases.

Introduction

Pellagra is a syndrome caused by a diet seriously deficient in synthetic precursors for the essential pyridine nucleotide nicotinamide adenine dinucleotide (NAD⁺), namely niacin (vitamin B3), and tryptophan (75,117,255). This lethal disorder can develop within 60 days

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3 of maintaining a deficient diet due to the absence of free stores of nicotinic acid (NA) or
4 nicotinamide (NAM) (298). Pellagra is pathologically characterised by a distinct dark
5 pigmented skin rash and the three D's of dermatitis, diarrhoea, and dementia (5). Interestingly
6 the shares AIDS Dementia Complex (ADC) shares some neurological similarities with
7 pellagra in its clinical presentation (55).
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12 In the last century, pellagra was a common disease in rural areas in the poorer southern
13 United States, and was attributed to an unknown infectious pathogen (299). However, it was
14 Dr Joseph Goldberger, and his associates, of the U.S. Public Health Service, who in 1914,
15 examined the hypothesis that pellagra was due to a dietary deficiency. Subsequently, pellagra
16 was prevented using a diet rich in maize, fresh milk, eggs and cured meat in these populations
17 (1,121). Despite these advances, it was not until 1937 that Conrad Elvehjem, a biochemistry
18 Professor, first demonstrated the anti-pellagra genic effect of NAM and NA on the related
19 black tongue disease in malnourished dogs (99,100).
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27 Individuals diagnosed with pellagra-induced dementia can be successfully treated in the early
28 stages of the disease. However, untreated pellagra results in irreversible neurological damage
29 and eventually death (148). This is due to primarily to reduced NAD⁺ production and
30 availability as NAD⁺ and its phosphorylated form NADP⁺ are both essential cofactors and
31 substrates for numerous biological processes (365). A focal reduction in NAD⁺ availability
32 due to increased turnover or reduced synthesis may also be foundational to the pathology
33 seen in other conditions. It seems to fit the observation of an apparently reversible
34 dementia before frank pathology in patients with ADC. At present, pellagra is a rare
35 condition that has been reported in severe cases of alcoholism and anorexia, or
36 malnourishment in the underdeveloped world (21,332).
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45 Several biochemical studies have shown that an inefficient production of NAD⁺, where
46 catabolism exceeds anabolism, may produce cellular dysfunction simply due to dietary lack
47 of niacin (27,325). It may also be due to the rate-limiting action of co-substrate dependent
48 quinolinate phosphoribosyltransferase (QPRT) (267,304). Excess of the amino acid leucine,
49 inhibits QPRT, which prevents the formation of niacin or NA to nicotinic acid
50 mononucleotide (NAMN) (189). Reduced tryptophan availability, particularly after chronic
51 immune activation, or in the absence of a tryptophan -rich diet (i.e. soy, meat, fish, eggs and
52 peanuts), may also be associated with the development of pellagra (217). Essential
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3 differences however, may be observed between ADC and pellagra, as the latter develops as a
4 result of a global bodily deficiency of tryptophan and niacin, while ADC develops as a result
5 of increased tryptophan and NAD⁺ catabolism at specific, although possibly numerous sites
6 (Figure 1). Activation of the tryptophan catabolism may be both positive and negative in
7 ADC. Immune activated oxidative tryptophan catabolism can positively increase cell viability
8 through increased NAD⁺ metabolism in brain cells. However, chronic activation of
9 tryptophan catabolism may occur in response to increased NAD⁺ catabolism. Increased
10 astrocyte and mononuclear phagocyte activation stimulates tryptophan catabolism to maintain
11 NAD⁺ levels in the early stages of immune activation. However, prolonged immune
12 activation leads to excess macrophage recruitment and activation which reduces the
13 astrocyte-to-neuronal NAD⁺ supply, resulting in pellagra-like neuronal dysfunction which
14 may be reversible in the short term (Figure 1). The characteristic mood disorders and
15 depression of end stage HIV may be due to increased tryptophan catabolism leading to
16 reduced availability of tryptophan for catabolism via serotonergic pathways (Figure 1).
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27 It is well known that NAD⁺ concentrations increase under conditions associated with reduced
28 energy loads. These include activities such as fasting, glucose deprivation, caloric restriction,
29 and exercise (68). However, apart from pellagra, NAD⁺ levels decline in animals on high fat
30 diets, and during ageing and cellular senescence (293). Given that NAD⁺ levels are elevated
31 under conditions of increased lifespan or health span and decline under conditions of
32 accelerated ageing and/or reduced health span, suggests that reduced NAD⁺ levels may
33 represent a major contributor to the ageing process (102). Therefore, supplementation with
34 NAD⁺ and its precursors may represent a potential therapeutic strategy to mediate protection
35 against accumulation of inflammation and highly volatile reactive oxygen species during the
36 ageing process.
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45 **NAD⁺ biosynthesis pathways**

46 Several NAD⁺ precursors have been identified in our natural diet. These include the amino
47 acid tryptophan, and three forms of vitamin B3 - NA, NAM and nicotinamide riboside (NR)
48 (Figure 2). Tryptophan catabolism via the kynurenine pathway can lead to *de novo* NAD⁺
49 synthesis (128). When dietary tryptophan is limited, the efficiency of the conversion of
50 tryptophan to NAD⁺ declines below the well-established conversion ratio of 60:1 (107,164).
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55 NA and NR are precursors that are found in the basic food chain. NA is produced by plants
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3 and algae, while NR is present in milk (338). NAM is formed as a by-product of enzymatic
4 degradation of pyridine nucleotides, and is the main form of vitamin B3 that can be absorbed
5 from animal-based food. The provision of these vitamins to NAD⁺ is aided by several
6 factors, including the gut microbiome (212,213). Biosynthetic genes are also regulated by
7 circadian rhythms (243). Additionally, the expression levels of a number of enzymes
8 involved in NAD⁺ anabolism decline with age (236).
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14 ***Tryptophan catabolism via the Kynurenine Pathway***

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16 Tryptophan is the least abundant amino acid of animal and plant proteins, making up only 1-
17 1.5% of the protein amino acid content (261). Tryptophan was first isolated in 1901 by Sir
18 Frederick Gowland Hopkins and his student S. W. Cole (154), and by 1906 was reported as
19 the first amino acid necessary for growth (261). The kynurenine pathway was first described
20 as a principle route for tryptophan catabolism in 1947 (122). Two major routes for tryptophan
21 catabolism have been identified in mammals that are active independent of protein
22 anabolism. In the periphery, the kynurenine pathway accounts for up to 95% of tryptophan
23 metabolism, while only about 1% of TRYP content is converted via the indoleamine pathway
24 to form the neuroactive metabolites, serotonin and melatonin (261).
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32 ***IDO-1/2 and TDO***

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34 The kynurenine pathway proceeds with the oxidative cleavage of tryptophan by either
35 indoleamine 2,3-dioxygenase-1 (IDO-1) (EC 1.13.11.52) and its isoform indoleamine 2,3-
36 dioxygenase-2 (IDO-2), or tryptophan 2,3-dioxygenase also called tryptophan pyrolase
37 (TDO) (EC 1.13.11.11) to produce formylkynurenine (23,103,313) (Figure 2 Step a). Both
38 IDO and TDO are haem-requiring enzymes. IDO is mainly found in extrahepatic tissue
39 including the brain, placenta, spleen, lung, kidney, alimentary tract, and epididymis. It does
40 not contain activating site for tryptophan analogues and is primarily activated by
41 proinflammatory cytokines, such as interferon-gamma (IFN- γ) (109). Concomitant induction
42 of IDO and free radical production of interferon- γ (IFN- γ) may at first increase NAD⁺
43 biosynthesis to contribute to the regeneration of intracellular NAD⁺ levels in an environment
44 of increased NAD⁺ turnover and demand. This suggests a protective role for increased
45 tryptophan catabolism in activated macrophages (Figure 3). However, TDO is predominantly
46 located in the mammalian liver and can be activated by numerous factors, including fasting,
47 glucocorticoids, hydrocortisone, NA, and L-tryptophan (369).
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4 The metabolic product of IDO-1/2 and TDO activity is the unstable intermediate metabolite,
5 N-formyl kynurenine (N-f-YN) (140) which is rapidly hydrolysed by kynurenine formylase
6 (EC 3.5.1.9) to form kynurenine (Figure 2 Step b), the first appreciably stable product of the
7 kynurenine pathway. Kynurenine can cross the blood brain barrier (BBB) (240) and
8 represents a significant branch point from which three products can be synthesised with the
9 use of three different enzymes, Kynureninase (EC 3.7.1.3), Kynurenine aminotransferase
10 (KATs) (EC 2.6.1.7), and Kynurenine 3-hydroxylase (EC 1.14.13.9) (22).

17 ***Kynureninase***

18 Kynureninase is a cytosolic enzyme which produces anthranilic acid (AA) by the cleavage of
19 the alanine side chain from kynurenine (Figure 2 Step e). AA can undergo hydroxylation to
20 5- or 3- hydroxyanthranilic acid (5- or 3-HAA) via non-specific microsomal hydroxylating
21 enzymes (184,257,262). AA can also cross the BBB via passive diffusion. Kynureninase also
22 plays a role in the production of 3-HAA from 3-hydroxykynurenine (3-HK) (Figure 2 Step e).

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29 The formation of NAD⁺ from tryptophan is inhibited by inadequate levels of vitamin B6 as
30 kynureninase is dependent on pyridoxyl-5'-phosphate (PLP) (vitamin B6) as a coenzyme for
31 the conversion of kynurenine to AA, or 3-HK to 3-HAA (238). Low levels of vitamin B6 has
32 been shown to correlate with higher levels of psychological distress (173,306). The
33 mechanism of B6 involvement in depression is most likely due to the fact that B6 is a
34 cofactor for 5-hydroxytryptophan decarboxylase, the enzyme which catalyses the last step in
35 serotonin biosynthesis (126,233).

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42 However, pyridoxine is also a cofactor for several reactions in the brain neurotransmitter
43 pathway, including glutamate decarboxylase and gamma-aminobutyric acid (GABA)-
44 transaminase, the two enzymes required for the synthesis for GABA from glutamate
45 (124,125,368). In pyridoxine dependent epileptic children, inefficient B6 levels resulted in
46 markedly elevated levels of glutamate in the brain (141). Moreover, a decrease in vitamin B6
47 levels has been associated with a deficiency in both humoral and cell mediated immune
48 responses including lymphocyte differentiation and maturation (134).

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55 It has been noted that a decrease in kynureninase activity will reduce further flux through the
56 kynurenine pathway thereby decreasing production of the NMDA receptor agonist and
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excitotoxin, quinolinic acid (QUIN) (111). However, QUIN levels are increased during inflammation, suggesting that kynureninase activity may not be significantly reduced. Vitamin B6 may therefore be used preferentially by the cell for kynureninase activity (e.g. QUIN/NAD production over GABA transaminase (EC 2.6.1.19; GABA production), 5-hydroxytryptophan decarboxylase (EC 4.1.1.28; Serotonin synthesis), and glutamate decarboxylase activity (EC 4.1.1.15), indicating a cell priority for *de novo* NAD⁺ biosynthesis under these conditions. The increase in QUIN secretion by activated mononuclear phagocytes during neuroinflammation may indicate an increased demand for NAD⁺ in these cells, the production of which may be limited under certain conditions by a saturated QUIN ribosylation system (252,253).

Kynurenine aminotransferases

Kynurenine aminotransferases (KATs) produce kynurenic acid by the transmission of kynurenine (Figure 2 Step c). Kynurenic acid is a stable compound with non-specific antagonist action in the brain at the glutamate subtype, N-methyl-D-aspartate (NMDA) receptor. Both KATs and kynureninase are vitamin B6 dependent enzymes (245).

Kynurenine 3-hydroxylase

Kynurenine 3-hydroxylase is a mitochondrial enzyme that also converts kynurenine to 3-HK by the hydroxylation of the aromatic ring (Figure 2 Step d). 3-HK is an NADPH-dependent enzyme whose activity appears to be reduced with oestrogen and in conditions of hyperthyroidism (28). 3-HK can also cross the BBB, stimulate free radical production and mediate vasodilation (104).

3-hydroxyanthranilic acid oxygenase

The catabolism of 3-HAA is mediated by 3-hydroxyanthranilic acid oxygenase (3-HAAO) (EC 1.13.11.6), an enzyme that is found in both cytosol and synaptosomal fractions to produce the intermediate 2-amino-3-carboxymuconic semialdehyde (307) (Figure 2 Step f).

Picolinic acid carboxylase

The enzyme picolinic acid carboxylase (PICAC) (EC 4.1.1.45) preferentially converts 2-amino-3-carboxymuconic semialdehyde to 2-aminomuconic semialdehyde with subsequent non-enzymatic conversion to picolinic acid (PIC) (Figure 2 Step g) (188,230), a metal chelator (106,270) and NMDA-receptor antagonist or enzymatic rearrangement leading

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3 finally to acetyl CoA (30,79,230). The non-enzymatic rearrangement of 2-amino-3-
4 carboxymuconic semialdehyde occurs when PICAC is saturated with substrate to produce
5 QUIN (Figure 2 Step h). The activity of PICAC has been shown to be inversely proportional
6 to the amount of NAD⁺ synthesised from tryptophan (305).
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10 ***Quinolinic acid phosphoribosyl transferase***

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12 QUIN is converted to NAMN by the enzyme quinolinic acid phosphoribosyl transferase
13 (QPRT) (EC 2.4.2.19) (Figure 2 Step i). QPRT catalyses the reaction between 5-
14 phosphoribosyl-1-pyrophosphate (PRPP) and QUIN in the presence of Mg²⁺ to produce
15 NAMN. The maximal enzymatic rate for QPRT is apparently the lowest of all kynurenine
16 pathway enzymes, and is 80 times lower than the preceding enzyme, 3-HAAO. However, the
17 Michaelis-Menton constant (K_m) for both 3-HAO and QPRT has been calculated to be the
18 same, and this is likely due to the fact that 3-HAA provides substrate for the production of
19 PIC as well as QUIN. The relative amount of QUIN formed from 3-HAA will therefore be
20 determined by the rate of PICAC activity (168,176). The behaviour of PICAC under
21 inflammatory conditions in the human brain or elsewhere does not appear to have been
22 investigated. However, as IFN- γ appears to only induce IDO, it may cautiously be speculated
23 that PICAC activity is not increased during an inflammatory response. Thus, increased flux
24 through the kynurenine pathway will proportionately increase QUIN production.
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35 QPRT is widely distributed in several tissues including the liver and brain, and may play an
36 important role in mediating neuroprotection against QUIN-induced toxicity, associated with
37 neurodegenerative diseases, including epilepsy and Huntington's disease
38 (58,132,175,229,235,250,333,364). The physiological levels of QUIN are thought to be in the
39 low nanomolar range, and QPRT activity increases with increased levels of QUIN. However,
40 at high levels of QUIN (> 500 nM), neuronal QPRT activity is saturated (267). This leads to
41 the production of QUIN at a greater rate than the production of NAD⁺, leading to the
42 accumulation of QUIN and NMDA-mediated excitotoxicity (254).
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50 PRPP is important in the regulation of QPRT activity (Figure 4) (33,161,162,168). The rate at
51 which PRPP is synthesised and used determines its steady state concentration within the cell,
52 which then determines the metabolic progress of pathways competing for PRPP. PRPP is
53 synthesised in the cell in the reaction catalysed by 5-phosphoribose pyrophosphokinase or
54 PRPP synthetase (EC 2.7.6.1) utilising a ribose-5-phosphate and ATP. PRPP synthetase has
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3 an absolute requirement for inorganic phosphate (Pi) and is elevated in cells undergoing rapid
4 cell division. The activity of PRPP synthetase is competitively inhibited by increased levels
5 of ADP and ATP. The ribose 5-phosphate used in this reaction is generated from glucose 6-
6 phosphate metabolism via the hexose monophosphate shunt or from ribose-1-phosphate
7 (generated by the phosphorolysis of nucleotides) via a phosphoribomutase reaction (73).
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12 Disorders in PRPP-synthetase activity and NAD⁺ metabolism have been implicated in the
13 development of neurological disorders. PRPP concentrations have been positively correlated
14 with cytosolic NAD⁺ and ATP levels in whole animals, and the availability of PRPP for
15 NAD⁺ synthesis may be reduced in the presence of high turnover and *de novo* synthesis of
16 purine and pyrimidine nucleotides (96). This may occur in ADC, and neurodegenerative
17 diseases as a result of free-radical induced DNA damage and astrogliosis. The increase of
18 QUIN seen in some neuroinflammatory conditions may therefore be a combination of
19 increased flux through the kynurenine pathway coupled with decreased enzyme activity
20 possibly due to the use of PRPP for purine and pyrimidine synthesis in DNA damaged or
21 mitotic cells.
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30 ***NAD pyrophosphorylase (nicotinamide mononucleotide adenylyltransferase)***

31 Further transformations leading to the synthesis of the parent molecule of the pyridine
32 nucleotides, NAD occurs in the nucleus and possibly the mitochondria. NAMN is catalysed
33 by NAD pyrophosphorylase or nicotinamide mononucleotide adenylyltransferase (NMNAT)
34 (EC 2.7.7.1) in the presence of ATP to produce desamido NAD (193,296) (Figure 2 Step u).
35 In the presence of glutamine, desamido-NAD is amidated to the parent pyridine nucleotide,
36 NAD⁺ (Figure 2 Step m), the final product of the kynurenine pathway (367). Three isoforms
37 have been identified in humans in several different organelles, namely NMNAT-1 (nucleus),
38 NMNAT-2 (golgi complex), and NMNAT-3 (mitochondria) (31). The differential
39 localisation of these enzymes suggests an organelle-specific function for these proteins, and
40 independent nuclear, mitochondrial, and Golgi-specific NAD⁺ biosynthetic pathways. Unlike
41 NMNAT-1, which is the preferred enzyme for NAD⁺ synthesis (157), NMNAT-2 and -3 can
42 also form NADH directly from reduced nicotinamide mononucleotide (NMN) (165).
43 NMNAT activity (and predominantly NMNAT-1) is high and non-rate-limiting in catabolic
44 tissue, but not in blood (236).
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3 Apart from NAD⁺ biosynthesis, some studies have demonstrated that NMNAT isoforms can
4 protect against axonal degeneration both *in vitro* and *in vivo* (80,183,211). NMNAT has been
5 shown to serve as a stress response protein necessary for thermotolerance and attenuation of
6 oxidative-stress induced shortened lifespan (11). The same study further showed that
7 NMNAT is transcriptionally regulated by the heat shock factor (HSF) and hypoxia-inducible
8 factor 1 α (HIF1 α) *in vivo*. During conditions of heat shock, HSF can bind to the NMNAT
9 promoter, thus inducing NMNAT expression. However, under hypoxic conditions, HIF1 α
10 enhances NMNAT levels indirectly via induction of HSF (11). Additionally, NMNAT
11 isoforms may exhibit protein chaperone function, exerting neuroprotection in several
12 *Drosophila* and mouse models of neurodegeneration (54,382). Overexpression of NMNAT-1
13 has also been shown to partially maintain neuronal function and reduce the levels of
14 biochemical insoluble tau in a mouse model of chronic tauopathy with no significant effect
15 on tau phosphorylation, tau aggregation, or tau-induced inflammation and hippocampal
16 atrophy (12). Furthermore, overexpression of NMNAT-3 mediated axonal protection against
17 tumor necrosis factor-induced and intraocular pressure (IOP) elevation-induced optic nerve
18 degeneration by reducing the expression of p62 and increasing autophagic flux in a retinal
19 ganglion cell line (182). Taken together, these studies suggest the possibility for new
20 mechanisms of protection for NMNAT enzyme activity in addition to its role as an enzyme
21 for NAD⁺ biosynthesis.
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34 35 ***NAD⁺ production from the vitamin niacin***

36 In addition to its *de novo* synthesis from tryptophan, NAD⁺ can also be synthesised from
37 either the acid, amide or riboside form of the vitamin niacin (vitamin B3).
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42 ***Nicotinic acid phosphoribosyl transferase***

43 NA is converted to NAMN by the enzyme nicotinic acid phosphoribosyl transferase
44 (NAPRT) (Figure 2 Step 1) (EC 6.3.4.21) using PRPP as a co-substrate, in an ATP-dependent
45 manner. As QUIN is converted to NAMN by the enzyme QPRT, the sequence of events
46 leading to NAD⁺ production is identical after NAMN formation from either substrate (112).
47 NAPRT appears to be expressed in several catabolic tissues including the colon, heart,
48 kidney, and liver (95). The non-deamidated route of NAD⁺ synthesis displayed a higher
49 relative proportions in blood and small intestine, and higher absolute values in liver and small
50 intestines compared to the amidated (NAMPT) route, suggesting the significance of NA as a
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precursor for NAD⁺ synthesis in these tissues (236). This has been reaffirmed by several feeding studies that have shown that NA is a more favourable precursor for NAD⁺ synthesis than NAM in the liver, intestine and kidney (81). As well, NA has been shown to increase intracellular NAD⁺ levels in a kidney cell line. Additionally, overexpression of NAPRT1 has been shown to mediate protection against oxidative stress-mediated NAD⁺ depletion (142).

Although tryptophan can be converted to NAM, it cannot be used to produce NA in vertebrate cells expressing the *de novo* synthesis pathway and NAD⁺ consuming enzymes, such as poly(ADP-ribose) polymerases (PARPs). NAM can be converted to NA in the intestinal lumen by bacterial nicotinamidase (EC 3.5.1.19) (Figure 2 Step o). However, one study suggested that sufficient levels of pyrophosphate and NAMN in cells can induce NAPRT to yield NA, thus allowing for the production of NA from tryptophan (212,214). Further studies are required (and are planned) to test this hypothesis. Bacterial and fungal degradation of NAD⁺, and direct NA supplementation can also increase NA levels in the alimentary canal for distribution to the rest of the body via vascular blood flow (212).

Nicotinamide phosphoribosyl transferase

The enzyme nicotinamide phosphoribosyl transferase (NAMPT) (EC:2.4.2.12) using PRPP as a co-substrate converts NAM to NMN (Figure 2 Step p), and then to NAD⁺ by the action of NAD pyrophosphorylases in the presence of ATP (Figure 2 Step u) (351). This amidated route of NAD⁺ synthesis predominantly displayed the highest rates in liver and kidney, and lowest in blood (161). The expression of NAMPT is encoded by the pre B-cell colony enhancing factor (PBEF1) gene. NAMPT also known as PBEF or visfatin has been identified as a cytokine that promotes the maturation of B cells when other cytokines, such as IL-7, and stem cell factors are available. It also exhibits insulin mimetic effects (260,374). The intracellular domain has been shown to activate lymphocytes and function as an NAD⁺ biosynthetic enzyme (281). However, both the extracellular and intracellular domains exhibit favourable phosphoribosyl activity.

In a cisplatin-induced acute kidney injury model, pharmacological manipulation of NAMPT expression via AICAR significantly improved renal function and reduced tubular injury. This effect has been associated with increased mRNA expression of SIRT3 – a mitochondrial sirtuin – and reduced protein hyperacetylation (237). Inhibition of the NAMPT pathway can impair glucose tolerance and insulin secretion in mice, an effect which can be ameliorated by

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3 subsequent supplementation with NMN (275). Despite these findings, inhibition of NAMPT,
4 which anabolises the substrate for NMNAT in mammalian cells had no significant effect on
5 NMNAT-1 mediated axonal protection in another study (289).
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9 ***Nicotinamide N-methyltransferase***

10 The ability of a cell to salvage NAM into the generation of NAD⁺ via NAMPT versus
11 methylation of NAM by the enzyme nicotinamide N-methyltransferase (NNMT) (EC:2.1.1.1)
12 (Figure 2 Step q) to N-methylnicotinamide (MeNAM) modulates the efficiency of biological
13 processes dependent on NAD⁺ (6,263). N-methylation also regulates the biotransformation
14 and detoxification of certain drugs and other xenobiotic compounds by the liver. The
15 enzymatic activity of NNMT uses S-adenosyl methionine as the methyl donor to form
16 pyridinium ions such as S-adenosyl-L-homocysteine (287). This enzyme is predominantly
17 expressed in the liver. A lower expression has been reported in the kidney, lung, skeletal
18 muscle, placenta, heart and adipose tissue, although it was not detected in the brain or
19 pancreas (287). Increased activity of NNMT has been shown to facilitate the production of
20 toxic N-methylpyridinium compounds, which have demonstrated neurotoxic properties, and
21 which may be involved in the nigrostriatal degeneration (366).
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32 ***Nicotinamide riboside kinases***

33 NR or nicotinic acid riboside (NAR) represent newly identified precursors that can be
34 converted to NAD⁺ via the NR kinase (NRK) (EC 2.7.1.173) pathway (Figure 2 Step j), or
35 by the action of nucleoside phosphorylase and the nicotinamide salvage pathway (38). NRKs
36 are highly conserved in eukaryotic cells, and are encoded by the Nmrk genes. Two NRK
37 enzymes have been identified, NRK1 and NRK2, however, their exact physiological roles
38 remain unclear. While NRK1 is ubiquitously expressed in mammalian tissue, NRK2 is not
39 expressed in the kidney, liver, lung, pancreas and placenta (272). Using the Nmrk1-deficient
40 mouse model (NRK1KO), it has recently been shown that NRKs are rate-limiting for
41 NR/NMN-mediated NAD⁺ synthesis (272).
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50 ***Purine nucleoside phosphorylase***

51 The second NR salvage pathway is NRK-independent, whereby NR is broken into a ribosyl
52 product and NAM (Figure 2 Step k), the latter of which yields NAD⁺ by nicotinamide
53 salvage. Purine nucleoside phosphorylase (PNP) (EC 2.4.2.1) has been shown to convert
54 NAR to NA (Figure 2 Step k), which is then converted to NAMN by the catalytic action of
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3 NAPRT (331). PNP deficiency has been shown to increase the levels of deoxyGTP (dGTP)
4 levels. This in turn inhibits ribonucleotide reductase, which is required for the formation of
5 deoxynucleotides (20,285,327). The enzyme deficiency leads to the accumulation of
6 metabolites that can induce toxicity in lymphoid lineage cells (291).
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10 11 ***Cytosolic 5'-nucleotidases***

12 A recent study showed that NAR can be produced by human cells and forms a critical role in
13 intracellular NAD⁺ anabolism (190). The study showed that cytosolic 5'-nucleotidases (5'-
14 NT) can dephosphorylate NAMN, and to a lesser extent, NMN, to form NAR. The amount of
15 NAR formed appears sufficient to promote NAD⁺ synthesis in neighbouring cells that are
16 missing the machinery required to utilise non-riboside NAD⁺ precursors (190).
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22 23 **Biological roles of NAD⁺**

24 NAD⁺ is an essential pyridine nucleotide that plays major roles in a number of critical
25 biological processes, including oxidative phosphorylation and ATP production, and synthesis
26 of cholesterol, fatty acids, and steroids (224). The primary function of NAD⁺ was identified
27 by Warburg and Christian in 1936 (357). NAD⁺ serves as a hydrogen acceptor allowing the
28 transfer of electrons for oxidation-reduction (i.e. redox) reactions leading to ATP production
29 in the mitochondria. ATP represents the cellular 'energy currency', and a decline in
30 intracellular NAD⁺ levels leads to reduced levels of ATP, culminating in cell death via
31 energy restriction (373).
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38 Apart from NAD⁺, its closely related phosphate NADP (Figure 2 Step r), serves as a cofactor
39 in several anabolic processes, such as fatty acid and cholesterol synthesis (315). The reduced
40 form of NAD⁺ and NADP are NADH (Figure 2 Step t) and NADPH (Figure 2 Step s)
41 respectively. These nucleotides serve as hydride donors, in over 400 enzymatic reactions
42 throughout the body involving dehydrogenases, hydroxylases and reductases (219). These
43 reduced and phosphorylated forms can interconvert, but do not alter the levels of NAD⁺.
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49 Importantly, NADPH is an essential coenzyme required for the reduction of reactive oxygen
50 species (ROS) (29). Thioredoxin (TXN) is an antioxidant protein that is reduced by
51 thioredoxin reductase (TXNRD) in an NADPH-dependent process (61). Glutathione
52 disulphide (GSSG) is also a substrate for glutathione reductase (GSR) for reduction back to
53 glutathione (GSH) using NADPH. The generation of GSH and TXN is pivotal for the
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3 elimination of ROS such as hydrogen peroxide (H_2O_2) (123). Reduced NADPH production
4 due to **decreased** NAD⁺ anabolism (or **increased catabolism**) can lead to impairments in **the**
5 **cells redox balance leading to perturbations in mitochondrial function and genomic signalling**
6 **and stability** subsequently leading to increased vulnerability to ~~oxidative stress~~ necrotic and
7 apoptotic pathways.
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11 Apart from its roles in redox reactions, a large body of evidence has shown that NAD⁺ is
12 more than a regulator of metabolism, but rather can also participate as the required substrate
13 for several important enzymatic reactions, including DNA repair, epigenetically modulated
14 gene expression, maintenance of intracellular calcium homeostasis and immunological roles
15 (52,118,120) (Figure 5).
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20 21 ***Poly(ADP)-ribosylation and DNA repair***

22 DNA strand breaks are known to occur in response to free radicals, ultraviolet light or
23 alkylating chemicals which activate the enzyme poly(ADP-ribose) polymerase (PARP)
24 (Figure 2 Step n) (320). Neuronal and astroglial cells exposed to cytotoxic levels of glutamate
25 and QUIN show both an increase in intracellular oxidative stress and PARP activity (46).
26 PARP-1 (the dominant member of a super family of 18 PARP proteins) efficiently detects the
27 presence of DNA breaks by its N-terminal zinc-finger domain (312). The ADP-ribosylation
28 of PARP triggers the recruitment of key proteins that stimulates the repair of the DNA
29 damage in less than 15 s (85). Importantly, in order for PARP to carry out its ADP-
30 ribosylating function it uses the ADP ribose moiety of NAD⁺ for its supply. Thus, PARP
31 breaks down NAD⁺ to NAM and an ADP-ribosyl product (Figure 6A) (145). Possibly as a
32 consequence of DNA strand breaks, recent evidence suggests that the poly(ADP)ribosylation
33 of histones or transcription factors may also be involved in nuclear receptor signalling.
34 Poly(ADP-ribose) metabolism is a dynamic process in which the degradation of ADP-ribose
35 polymers occurs relatively rapidly through the action of poly(ADP-ribose) glycohydrolase
36 (295) (Figure 6A).
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49 A significant decrease in intracellular NAD⁺ has been reported in the brain and a variety of
50 other cell types as a result of DNA strand breaks and PARP activation following exposure to
51 hydrogen peroxide, nitric oxide, HIV infection, or during inflammation (7,326,330).
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53 Increased PARP activity resulting in decreased NAD⁺ has been shown to decrease ATP and
54 neurotransmitter levels in the brain as well as cause cell lysis and death (45,203) (Figure 6B).
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3 Inhibition of PARP activity, following oxidant injury has been shown to preserve NAD⁺ and
4 ATP levels preventing cell lysis (14), although damage to the DNA is probably not
5 prevented. In a pancreatic islet cell population lacking expression of PARP, NAD⁺ depletion
6 does not occur after oxidant injury despite DNA strand breaks occurring to the same degree
7 (68). This demonstrates that activation of PARP is the major cause of NAD⁺ depletion in
8 these oxidant injury cells. Elevated levels of free radicals, oxidants, and excitotoxins have
9 been reported in inflammatory mediated diseases of the brain, and in some cases, DNA
10 damage has been demonstrated (2,220,221,341,380). This suggests that NAD⁺ depletion
11 through PARP activation may play a role in CNS dysfunction and pathology under these
12 conditions (Figure 6).
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21 More recently, it has been suggested that PARP activation, rather than NAD⁺ decline may be
22 responsible for cell death following exposure to genotoxic insult. For example,
23 poly(ADP)ribosylation has been shown to directly inhibit the glycolytic enzyme hexokinase
24 leading to a significant reduction in glycolysis prior to NAD⁺ depletion, mitochondrial
25 dysfunction and neuronal cell death (17). Moreover, direct poly(ADP)ribosylation of
26 glyceraldehyde 3-phosphate dehydrogenase (GAPDH) is the primary cause of cell death in
27 kidney tubules following ischaemic injury (94). These studies suggest that the beneficial
28 effects of PARP inhibition may be due to altered metabolic effects independent of
29 maintenance of NAD⁺ levels during pathological conditions.
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37 PARP also appears to play a positive role in the up regulation of the tumor suppressor
38 protein, p53. For example, PARP-deficient cell lines derived from Chinese hamster V79 cells
39 failed to undergo poly(ADP)ribosylation and activate p53 following treatment with etoposide
40 (363). PARP can also activate DNA dependent protein kinases which regulate p53 activity
41 through phosphorylation (318). Therefore, on the contrary to reported benefits of PARP
42 inhibitors, pharmacological inhibition of PARP activity may contribute to genomic instability
43 with resulting risk of cancer formation.
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50 ***CD38/CD39/CD73/CD157 and secondary messenger signalling***

51 The immune associated ectoenzymes CD38, CD39, CD73 and CD157 represent another
52 class of NAD⁺-consuming enzymes (155) (Figure 2 Step n). These enzymes require NAD⁺ to
53 produce ADP ribose (ADPR) and hydrolyse the secondary messenger signalling molecule,
54 cyclic-ADP-ribose (cADPR) which helps mediate intracellular calcium transients (Figure 7).
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3 CD38 has also demonstrated an immunomodulatory role (135). For instance, the presence of
4 CD38 on T-lymphocytes influences the ability of antigen presenting cells to stimulate antigen
5 specific T-cells (256). Upregulation of CD38 expression also signals maturation of dendritic
6 cells during inflammatory cytokine activation and acts as a modulating adhesion and
7 signalling molecule between dendritic cells and lymphocytes (105). In cardiomyocytes,
8 exogenous stimulants may stimulate an increase in intracellular calcium, which leads to
9 activation of CD38 (147). CD38 expression has also been shown to increase with age (65),
10 and this is most likely attributed to an age-related increase in circulating inflammatory
11 cytokines, and reduced CD38 function has been associated with poor immune responses.
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19 Given that 100 molecules of NAD^+ must be hydrolysed to generate one molecular of cADPR,
20 it is highly likely that CD38 is a major regulator of intracellular NAD^+ levels (77).

21 Accordingly, we found a 5 fold increase in NAD^+ levels in CD38 knockout neuronal cells
22 compared to controls (52). Therefore, CD38 may not only represent an inefficient secondary
23 messenger enzyme, but also as a NADase which primarily regulates intracellular levels of
24 NAD^+ and its physiological processes (Figure 8).
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30 CD38 has also been shown to use $\beta\text{-NAD}^+$ as a substrate, but no $\alpha\text{-NAD}^+$ or NADH. CD38
31 can also catalyse a base exchange between NADP and NA, leading to the formation of
32 nicotinic acid adenine dinucleotide phosphate (NAADP), which is also used as a hydrolytic
33 substrate (90). It can also metabolize analogues of NAD^+ , including nicotinamide guanine
34 dinucleotide (NGD⁺) and nicotinamide hypoxanthine dinucleotide (NHD⁺), yielding cyclic
35 compounds (cGDPR and cIDPR, respectively). These compounds exhibit fluorescent
36 properties, but not calcium-releasing (383). They represent useful biochemical agents for
37 examining ADP-ribosyl cyclase activity.
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45 Prolonged activation of CD38 following cardiac stress has been shown to induce a sustained
46 Ca^{2+} release leading to cardiac hypertrophy and arrhythmias (130). Supporting evidence
47 comes from male CD38-knockout mice which reported improved cardiac function, while
48 treatment with ADPR cyclase inhibitors led to anti-arrhythmic affects in multiple in vitro
49 models and cardiac Ca^{2+} overload studies (131). Similarly, inhibition of CD73 has been
50 shown to mediate protection against renal stressors, and CD39 activity mediated protection
51 against renal ischaemic injury (268).
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3 CD38 can also regulate the activity of PARP and other NAD⁺-dependent enzymes SIRT1
4 activities by potentially reducing the accessibility of NAD⁺ to its preferred enzymatic targets
5 (348). NAM, which is generated by the catalytic activity of CD38, also represents an
6 endogenous metabolite of SIRT1 enzyme. Therefore, it has been postulated that CD38 may in
7 fact be an important regulator on intracellular NAD⁺ levels and SIRT1 activity, thus
8 influencing SIRT1 functions, including maintenance of cellular bioenergetics, obesity, and
9 senescence. Interestingly, one study reported no significant effects on NAD⁺ levels in CD38
10 knock-out mice compared to wild-type animals (377). Therefore, the amount of benefit due to
11 CD38 inhibition or ablation warrants further investigation.
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19 Novel CD38 inhibitors may also be useful for the treatment degenerative disorders where
20 optimal NAD⁺ and NADPH anabolism remains crucial to attenuate oxidative stress insult, the
21 latter of which serves as the ultimate electron donor supporting glutathione peroxidases,
22 peroxiredoxins, and glutaredoxins. However, inhibition of CD38 may also result in a
23 deleterious impact on immunological function. CD38/cADPR also signals oxytocin release,
24 which regulates many social behaviours, and inhibiting this process may induce several forms
25 of mental impairment. Moreover, niacin deficiency which has been observed in patients with
26 pellagra often progresses to a dementia similar to schizophrenia (98,264), and this could be
27 due to impaired cADPR formation.
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35 *Sirtuin activity*

36 Another important NAD⁺-dependent function is the activity of the silent information
37 regulators of gene transcription, or sirtuin family of enzymes (Figure 2 Step n). Sirtuins are a
38 family of class III NAD⁺ dependent histone deacetylases exhibit protein lysine deacetylase,
39 and partial ADP-ribose transferase activities. In the reaction mediated by sirtuins, an acetyl-
40 modified lysine is bound to a target protein and NAD⁺ in specific pockets (346).
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45 Deacetylation occurs when the modified lysine side chain is coupled to the cleavage of the
46 glycosidic bonds in NAD⁺, leading to the generation of the deacetylated lysine, acetylated
47 ADP-ribose, and NAM as byproducts (101) (Figure 9).
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51 At, present seven classes of sirtuins (SIRT1-7) have been identified in mammalian cells, each
52 of which are localised in various cellular organelles, and mediate a diverse range of important
53 biological functions (53) (Figure 10). SIRT1 and SIRT6 are nuclear proteins associated with
54 the maintenance of chromatin structure, DNA repair, and gene expression. It has been
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3 suggested that SIRT1 may play a pivotal role in promoting cellular longevity and may hold
4 the key to slowing development of the aging phenotype (290). SIRT1 has been shown to
5 influence the acetylation status of several important transcription factors, including the
6 metabolic regulator, peroxisome proliferator-activated receptor- γ (PPAR γ), tumour
7 suppressor protein (p53), and the cell growth linked FOXO forkhead family of transcription
8 factors (192). However, some evidence suggests that SIRT6 may also contribute to an age-
9 resistant phenotype (300). SIRT2 is predominantly a cytoplasmic protein where it regulates
10 gene expression by deacetylating transcription factors which shuttle from the cytoplasm to
11 the nucleus (282). SIRT3, SIRT4 and SIRT5 are found in the mitochondrion where they
12 respond to changes in mitochondrial redox status by altering the enzymatic activity of
13 specific downstream targets, including manganese superoxide dismutase (MnSOD) (249).
14 SIRT7, is localised in the nucleolus of mammalian cells and has been associated with cellular
15 growth and metabolism (347). The biological relevance of sirtuins in redox processes will be
16 discussed further in another section of this review. Importantly, the beneficial effects of
17 sirtuin activity are only achieved if NAD⁺ levels are optimal.

Principle causes of NAD⁺ decline

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29 Apart from deficiency within the NAD⁺ biosynthesis process, there are principally two
30 conditions under which NAD⁺ depletion may occur: (1) Excessive DNA damage due to free
31 radical or UV attack, leading to hyperactivation of PARP. This ultimate leads to a high
32 turnover and subsequent depletion of NAD⁺. The resulting energy crisis and reduced ATP
33 production can lead to cell death via either an apoptotic or necrotic pathway (2). A chronic
34 increase in immune activation and inflammatory cytokine production can accelerate CD38
35 activity and contributing to NAD⁺ decline. While several clinical disorders and degenerative
36 disorders can meet these criteria, chronic accumulation of oxidative stress and inflammation
37 during advanced age represents a major driver of NAD⁺ decline (49). Promotion of NAD⁺
38 anabolism using NAD⁺ precursors may represent a clinically relevant therapeutic strategy to
39 ameliorate the age-related decline in cellular energy.

Redox roles of sirtuins and transcriptional regulation

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41 Since the term of their discovery, sirtuins have been associated with lifespan extension.
42 However, while the longevity enhancing capacity of sirtuins have been established in several
43 small model systems, the modes of action of sirtuins underlying these beneficial effects
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3 remain unclear. Chronic accumulation of damage over time represents the main phenotype
4 associated with the ageing process. In particular, chronic oxidative stress can induce damage
5 to diverse macromolecules, and perturb mechanisms with which they are repaired. Recent
6 evidence suggests that the beneficial effects of sirtuins may be mediated by their ability to
7 regulate redox processes. In this section, we will investigate the association between sirtuins
8 and their redox environment, and review how sirtuin-mediated deacetylation affects target
9 enzymes and transcription factors.
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14 15 16 **SIRT1**

17 As previously mentioned, tumor suppressor p53 represents the first deacetylation substrate of
18 SIRT1. The transcription factor p53 has been shown to activate numerous pro- and
19 antioxidant genes including sestrins, manganese superoxide dismutase (MnSOD) and
20 glutathione peroxidase 1 (GSH-PX1) (284). The binding and deacetylation of p53 by SIRT1
21 at Lys382 mediates its transcriptional activity (208). SIRT1 deacetylation of p53 has been
22 shown to influence the cellular localisation of p53 in response to oxidative stress, and may
23 serve as a metabolic switch between antioxidant protection and apoptotic cell death. For
24 instance, in murine embryonic stem cells, the absence of antioxidants in cell culture media
25 induced mitochondrial translocation of p53, whilst in SIRT1 knockout cells; increased
26 oxidative stress induced nuclear translocation of p53 leading to an antioxidant response (139).
27 Similarly, up-regulation of SIRT1 in mesangial cells attenuated the induction of p53-mediated
28 apoptotic pathway following exposure to pathological concentrations of H₂O₂. However, at
29 lower concentrations of H₂O₂, the SIRT1-p53 interaction led to an induction of antioxidant
30 processes (191).
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41 While the adaptive role of SIRT1 against ROS stress has been well established *in vitro*,
42 studies using live animal have been less convincing. This is due to the high levels of
43 embryonic lethality following the production of SIRT1^{-/-} mice. However, one study using
44 heterozygous SIRT1 knockout mice reported increased vulnerability to renal oxidative stress,
45 and combined SIRT1^{+/-} p53^{+/-} showed greater susceptibility to tumor development compared
46 to p53 haploinsufficiency alone (146).
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52 SIRT1 has also been shown to deacetylate and activate FOXO3a following exposure to
53 oxidative stress (57). FOXO3a appears to be an important transcriptional activator of the
54 SOD2 gene which encodes for the production of the endogenous antioxidant protein MnSOD.
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3 The catalase enzyme, which acts directly on free radicals is predominantly localised in
4 peroxisomes, and represents another target of FOXO3a (185). As per the relationship
5 between SIRT1 and p53, low levels of H₂O₂ can mediate FOXO3a-mediated induction of
6 catalase, whilst cytotoxic levels of H₂O₂ can induce FOXO3a-mediated apoptosis (144). In
7 cardiovascular disease, increased oxidative stress can upregulate SIRT1 expression and
8 stimulation of catalase and MnSOD expression. However, higher levels of SIRT1 can lead to
9 cardiac hypertrophy and cell death via apoptotic pathways (9). Taken together, these studies
10 collectively suggest that SIRT1 serves as an ROS sensor, capable of inducing protection at
11 low-level stress, whilst inducing apoptosis at severe stress levels.
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19 Recently, it has also been shown that mechanisms responsible for the regulation of the
20 intracellular NAD⁺:NADH ratio can also affect SIRT1 function via AMP-activated kinase
21 (AMPK), an essential regulator of cellular energy homeostasis. Several studies have shown
22 that reduced glucose available in myoblasts induced activation of AMPK and upregulation of
23 NAMPT, leading to increased levels of intracellular NAD⁺ and activated SIRT1, and
24 culminating in the activation of several transcriptional mediators, including FOXO proteins
25 and PGC-1 α , thus enhancing catabolism and mitochondrial biogenesis (110). Furthermore,
26 activation of AMPK stimulated transcriptional activity downstream of SIRT1 in another
27 study (66). SIRT1 can also activate AMPK through positive feedback mechanism. For
28 instance, Liver kinase B1 (LBK1), which phosphorylates and activates AMPK under low
29 nutrient levels can be deacetylated by stimulation or overexpression of SIRT1 (either directly
30 or indirectly). This promotes translocation of LBK1 from the nucleus to the cytosol, which
31 further phosphorylates AMPK (192). As such, there seem to be multiple levels of metabolic
32 regulation occurring through the AMPK–SIRT1 axis, and many of these steps require further
33 elucidation.
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44 Apart from the AMPK-SIRT1 axis, SIRT1 can also interact and deacetylate PGC-1 α . PGC-
45 1 α is an important transcriptional coactivator that stimulates mitochondrial biogenesis and
46 indirectly also mitochondrial dynamics in a tissue-dependent manner. For instance, increased
47 hepatic SIRT1 due to fasting can deacetylation PGC-1 α leading to both inhibition of
48 glycolytic genes, and increased expression of genes associated with gluconeogenesis (276).
49 Another study showed that SIRT1 could directly interact with and deacetylate PGC-1 α in
50 adrenal PC12 cells leading to reduced PGC-1 α transcriptional activity and related
51 mitochondrial oxidative metabolism (246). However, in skeletal muscle, increased SIRT1
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3 activity and PGC-1 α deacetylation led to an increase in mitochondrial fatty-acid
4 oxidation (116). Reduced PGC-1 α activity has associated with reduced expression of the
5 mitochondrial antioxidant protein, MnSOD, providing additional support for the role of
6 SIRT1 on control of redox stressors (207).
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10 Recent studies have shown that calorie restriction, and the phytochemical resveratrol, which
11 are known another to activate SIRT1, can enhance endothelial nitric oxide synthase (NOS)
12 expression and promote mitochondrial biogenesis by upregulating transcription factors such
13 as PGC-1 α (78). Similarly, SIRT1 has been shown to deacetylated eNOS *in vivo*, leading to
14 increased eNOS activity and intracellular NO production (225). Therefore, SIRT1 represents
15 a key regulator of vascular tone dependent on eNOS.
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21 Moreover, it is well established that the transcriptional response to hypoxia is regulated
22 mainly by the HIF family of proteins, of which HIF1 α and HIF2 α are well characterised
23 (reviewed in (216)). It has been demonstrated that both HIF1 α and HIF2 α can be deacetylated
24 by SIRT1 by two separate and distinct mechanisms. Under normal physiological conditions,
25 SIRT1 can bind to, and deacetylate, HIF1 α , preventing HIF1 α from interacting with the
26 transcriptional coactivator p300, inhibiting its transcriptional activity (200). However, under
27 hypoxic conditions, the decline in the NAD⁺:NADH ratio, and available NAD⁺ for optimal
28 SIRT1 activity due to reduced oxygen levels allows HIF1 α to remain acetylated, thus
29 preventing its hypoxic transcriptional activity (200). On the contrary to its effect on HIF1 α ,
30 SIRT1 can also form a complex with SIRT1 under hypoxic conditions and is deacetylated at
31 three lysine residues (K385, K685, and K741) in the carboxy terminus, leading to increased
32 transcriptional activity of HIF2 α and related proteins and erythropoietin in particular (92).
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41 **SIRT2**

42 The expression of SIRT2 have also been shown to be upregulated at both the mRNA and
43 protein level in response to cellular stressors such as oxidative stress. Numerous studies have
44 demonstrated that Increased SIRT2 expression following oxidative insult can lead to cellular
45 apoptosis via induction of the proapoptotic protein Bim (350). Overexpression of SIRT2 has
46 also been shown to promote neurodegeneration, although the exact mechanism remains
47 unclear (322). In the absence of the SIRT2 gene, upregulation of the cytosolic chaperone 14-
48 3-3 ζ , sequesters the proapoptotic mitochondrial protein BAD in the cytosol and mediates
49 protection against anoxia–reoxygenation-induced cell death (210). SIRT2 inhibitors have
50 been shown to ameliorate α -synuclein-mediated toxicity in a cellular model of Parkinson's
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disease (210). However, under low-stress conditions, SIRT2 upregulates mitochondrial MnSOD via FOXO3a deacetylation, leading to a reduction in the levels of ROS.

SIRT3

Isocitrate dehydrogenase 2 (IDH2) represents another major target of SIRT3, a mitochondrial sirtuin. IDH2 uses NADPH to generate reduced GSH to mediate an antioxidant effect. Schlicker et al., showed SIRT3, but not SIRT5, could deacetylate IDH2 at K211 and K212 residues to promote its activity (294). It has been shown that the GSH:GSSG ratio and the level of NADPH are increased in the liver, brain, and the inner ear following CR in a SIRT3-dependent manner (311). As well, SIRT3 directly deacetylates and inhibits the activity of IDH2, and SIRT3 overexpression increased NADPH levels and reduced oxidative stress-mediated cell death (311). Taken together, these studies suggest that CR, SIRT3, and IDH2 represent important targets for the management and treatment of age-related hearing loss, and that maintenance of intracellular NAD⁺ levels modulate the cellular response to degeneration.

Like IDH2, SIRT3 has been shown to mediate SOD2 activity by regulating mitochondrial FOXO3a activity, although the exact mechanism remains unclear. One study using overexpression of SIRT3 in mouse embryonic fibroblasts shows that the levels of ROS were dramatically reduced in a SOD2-dependent manner (266). Similarly, hyperacetylation of SOD2 in SIRT3-deficient mice led to reduced SOD2 activity and upregulation of ROS production (329). However, differences in the site-specific regulation of SOD2 by SIRT3 have been reported, and this is likely due to differences in cell type, species, or stress conditions

Additional mitochondrial targets of SIRT3 and SIRT5 also been recently identified which can regulate oxidative stress. SIRT3 has been shown to deacetylate complexes I, II, III, and IV and glutamate dehydrogenase (GDH), which regulates glutamate oxidative stress, yielding NADPH, is deacetylated by SIRT3 (and antagonised by SIRT4-mediated ADP-ribosylation) (205). SIRT5 can also deacetylate cytochrome *c* (294). As well, SIRT3 and SIRT5 can both regulate mitochondrial-localized reactions of the urea cycle. To be more specific, SIRT3 can deacetylate ornithine transcarbamoylase while SIRT5 acts on carbamoyl-phosphate synthase 1, to enhance urea cycle function, and promote the clearance of oxidative stress-promoting ammonium (137).

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4 SIRT3 has been recently shown to be important for the regulation of normal cardiac function
5 and protection against cardiac pathologies. Knockout of SIRT3 has been shown to increase
6 the hyperacetylation of mitochondrial protein, leading to spontaneous cardiac hypertrophy
7 with age and greater than 50% reduction in ATP levels (319). Reduced SIRT3 expression and
8 hyperacetylation of cardiac mitochondrial enzymes have also been reported in mouse models
9 for cardiac disorders, and poor human hearts (156). As well, increased activity of acyl-CoA
10 dehydrogenase and other enzymes involved in fatty acid oxidation (FAO) have also been
11 reported in SIRT3 knockout mice (13). However, another study reported reduced rates of
12 FAO in the hearts of fasted animals (151). These differences may be attributed to variation in
13 the type of stressors which can influence the activity of protein acetylation.
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22 Renal stress has been shown to reduce the expression of SIRT3. For instance, SIRT3 mRNA
23 expression was shown to be decreased in a model of free fatty acid (FFA) associated
24 tubulointerstitial inflammation, and this occurred parallel to increased levels of ROS and
25 markers of inflammation compared to age-matched control animals (187). Interestingly,
26 retroviral overexpression of SIRT3 attenuated these changes, suggesting that optimal SIRT3
27 function is necessary for renal function (370). Similarly, high glucose levels were shown to
28 decrease the mRNA and protein expression of SIRT3, and supplementation with NAD⁺
29 ameliorated high-glucose induced mesangial hypertrophy and SIRT3 expression at both the
30 genomic and protein level (390). Taken together, these findings suggest that SIRT3 can
31 protect against renal degeneration in diabetic nephropathy.
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40 ***SIRT4***

41 Like SIRT3, SIRT4 appears to be highly expressed in catabolic tissue such as the brain, heart,
42 liver and kidney (136). SIRT4 has been shown to protect against hypoxia-induced apoptosis
43 in cardiomyoblast cells (202). However, knockout of SIRT4 protected against angiotensin-II
44 induced cardiac hypertrophy and fibrosis in mice, suggesting that SIRT4 may be directly
45 involved in the pathogenesis of cardiovascular disease (209). While both studies suggest a
46 discrepancy for the exact role of SIRT4 in cardiac function, it appears likely that these effects
47 are due to the modulatory role of SIRT4 on cellular oxidative stress levels.
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54 There also exists a strong correlation between kidney function, SIRT4 levels and the NAD⁺
55 metabolome. For instance, co-treatment with cisplatin and the phytochemical curcumin
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3 restored NAD⁺ levels and attenuated the decline in NAMPT, SIRT1, SIRT3 and SIRT4
4 expression due to cisplatin-induced nephrotoxicity (342). However, it is unlikely that these
5 effects are directly in response to SIRT4, as the levels of NAMPT, SIRT1 and SIRT3 were
6 also affected. Additional work is necessary to evaluate the role and modes of action of SIRT4
7 in degenerative disorders of the brain, heart and kidney, and other age-related conditions
8 associated with NAD⁺ depletion.
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14 ***SIRT5***

15 The exact roles of SIRT5 in maintaining normal cellular homeostasis is not well understood.
16 One study found no significant differences between the heart weight and rate, and systolic
17 blood pressure in SIRT5 knockout mice exposed to a high fat diet (379). However, another
18 study showed that protein succinylation is uniquely elevated in SIRT5 knockout mice (248).
19 These proteins include those that are involved in fatty acid metabolism, amino acid
20 catabolism, the TCA cycle, oxidative phosphorylation, ketone and pyruvate metabolism (41).
21 In mice exposed to cardiac ischemia, a larger infarct volume and elevated oxidative stress
22 were reported in SIRT5 knockout hearts compared to wild-type controls (41). These changes
23 were accompanied by increased fibrosis, and reduced shortening fraction and ejection
24 fraction compared. Increased activity of succinate dehydrogenase (SDH) was also reported in
25 SIRT5 knockout mice, and SDH inhibitors reduced infarct size to 'normal' levels (41). This
26 suggests that the protective effects of SIRT5 may be mediated by desuccinylation of SDH.
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37 Similarly, knockout of SIRT5 also resulted in hypersuccinylation of mitochondrial protein,
38 and post translational modification of malonylation and glutarylation in the kidney (198).
39 Additionally, SIRT5 has been shown to deacetylate carbamoyl-phosphate synthetase 1
40 (CPS1), leading to increased activity of CPS1 and reduced plasma urea levels (242).
41 Increased blood ammonia levels were reported in SIRT5 knockout mice compared to age-
42 matched wild-type controls. These findings provide a key role for the role of SIRT5 in the
43 regulation of ammonia.
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50 ***SIRT6***

51 While the effect of redox stressors on SIRT6 function remains nascent in current literature,
52 one study has shown that knockdown of SIRT6 can induce accelerated senescence as
53 evidenced by the development of degenerative features, shortened telomere length, and
54 reduced life span (239). Interestingly, HIF1 α has been shown to be upregulated in cells
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3 lacking SIRT6, leading to an increased glucose uptake and improved glycolysis (384). In
4 normal mice embryonic fibroblast cells, SIRT6 serves as an H3K9 histone deacetylase,
5 inhibiting HIF1 α -dependent transcription of multiple glycolytic genes, thus acting as a co-
6 repressing of HIF1 α .
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10 ***SIRT7***

11 Of the family of sirtuins, SIRT7 remains the least investigated. One study showed that
12 knockdown of SIRT7 enhances the acetylation of p53, leading to increased vulnerability to
13 genotoxic insult (241). SIRT7 has also been shown to inhibit cell proliferation following
14 exposure to high oxidative stress levels (344).
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20 ***Activation by NAD⁺ precursors***

21 A growing body of evidence suggests that upregulation of NAD⁺ anabolism can influence
22 processes regulated by sirtuins. These pathways may therefore be upregulated with NAD⁺ or
23 NAD⁺ precursors, or other means of manipulating NAD⁺ biosynthesis pathways. It has been
24 shown that the Km for SIRT3 and SIRT5 is significantly lower than the levels of
25 mitochondrial NAD⁺, suggesting that the activity of these sirtuins is rate-limited by the
26 availability of mitochondrial NAD⁺ levels (150). Current evidence suggests the importance
27 of SIRT1 and SIRT3 in regulating the beneficial effects of NAD⁺, and the effects of NAD⁺
28 supplementation on other sirtuins remains unclear. Examining whether the activity of other
29 sirtuins are affected by NAD⁺ therapy represents an emerging area of research. It is likely
30 that NAD⁺ supplementation may activate multiple members of the sirtuin family leading to
31 diverse effects on multiple biological processes, and thus improving brain, cardiac and renal
32 function under different stressors.
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43 **Distribution of the NAD⁺ metabolome**

44 It is well established that NAD⁺ (in particular the NAD⁺/NADH ratio) is a master regulator
45 of cellular bioenergetics. The total intracellular NAD⁺ content is estimated to be in the range
46 of 0.2-0.5 mM (388). This concentration is within the estimated NAD⁺ Km value of PARPs
47 (0.02-0.08 mM) (15) and SIRT1 (0.56 mM) for NAD⁺ (278). This means that the availability
48 of the essential substrate NAD⁺, is rate-limiting for PARPs and SIRT1. For instance, low
49 NAD⁺ levels due to increased PARP activity leads to reduced SIRT1 activity, whereas higher
50 NAD⁺ levels enhances PARP and SIRT1 activities. Research from our group has
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3 demonstrated that reduced levels of NAD⁺ due to chronic oxidative stress and
4 hyperactivation of PARPs are associated with significantly reduced sirtuin activity (51,223).
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8 Metabolomic profiling of the NAD⁺ metabolome in peripheral blood mononuclear cells
9 (PBMCs), plasma and urine in an overnight fasting human subject has recently been
10 published (337). The study showed that the phosphorylated NAD⁺ metabolites – NAMN,
11 NAAD, NADP⁺, NMN and ADPR – are found exclusively in blood cells, but not in plasma
12 or urine. The levels of NA, NAM and NR are considerably low in normal fasting blood (337).
13
14 Very few studies have examined the levels of these NAD⁺ metabolites due to limitations in
15 accurately measuring them. Using GC-MS, one study reported that the concentration of NAM
16 in fasting blood was about 300 nM, and the level of NA was 30 nM (72). This provides
17 evidence for the physiological importance of NAM as the preferred form of niacin to
18 extrahepatic tissue. The blood levels of both NA and NAM can be significantly increased
19 following supplementation with vitamin B3. These pharmacological doses range between 1-3
20 grams of NA or NAM. In comparison, a niacin-rich meal contains about 10 grams of vitamin
21 B3 composed of a mixture of NA and NAM, the concentrations of which vary with their
22 content in plant and animal foods (231,232).
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32 It has been previously shown that small amounts of NA can be converted to NAD⁺ in the
33 intestine and liver, and NA may not be detected in systemic blood. Moreover, the catalytic
34 activity of NAD⁺ glycohydrolases or ADP-ribosylation in the small intestine or liver can
35 induce the release of NAM into the blood stream (274). NAM from the diet may also be used
36 to form NAD⁺ in the small intestine and liver, and may also be released into the blood
37 stream. Expression of hepatic NNMT leads to the formation of MeNAM from NAM, thus
38 maintaining SIRT1 activity in the liver (152).
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45 It remains unclear whether NAM can accumulate in the blood stream following a NAM-rich
46 meal, or stored in several tissue for generation of NAD⁺ as required, and later released into
47 the blood stream to maintain threshold levels in the blood stream. However, one study
48 previously showed that up to 60% of the total NAD⁺ levels are depleted in red blood cells in
49 a rat model of niacin deficiency. The remaining 40% appeared resistant to depletion (274).
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51 On the contrary, the levels of NAD⁺ in the liver were considerably higher and depleted at a
52 slower rate during deficiency (274). Short- or long-term storage of NAD⁺ may take place in
53 the liver and red blood cells, where it regulates blood NAM levels during periods of niacin
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3 deficiency eg. during fasting. Under normal physiological conditions, high affinity
4 transporters are required to facilitate the transfer of NA and NAM into extrahepatic tissues,
5 which are present in the blood stream at low to mid nanomolar concentrations. Understanding
6 the interactions between these precursors can help us to elucidate appropriate
7 pharmacological doses of NA and NAM.
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12 Recently NR has been identified as an NAD⁺ precursor vitamin that is uniquely and orally
13 bioavailable in mice and humans (337). Blood NAD⁺ levels have been shown to increase by
14 2.7 fold following a single daily dose of NR (1000 mg) for 7 days, with a concurrent increase
15 in NAAD by up to 45 fold in PBMCs. While it is unclear how an oral dose of NR can raise
16 NAAD levels, it has been suggested that NR may be partially converted to NAM via the
17 NAD⁺ salvage pathway (337). Such conversion may stimulate bacterial hydrolysis of NAM
18 to NA, culminating in the production of NAD⁺ using an NAAD intermediate. Another study
19 showed that NMN is metabolised extracellularly to yield NR, which is then converted to
20 NAD⁺ intracellularly (272). Therefore, NR and NMN represent convergent supplementation
21 strategies to enhance NAD⁺ anabolism.
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30 **Subcellular compartmentalisation of NAD⁺**

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32 Traditionally, it was thought that NAD⁺ was distributed in the nucleus, as only one form of
33 NMNAT was identified as nuclear in origin (296). Nuclear NAD⁺ was therefore available to
34 catalyse poly(ADP-ribose) formation, but could also equilibrate in the cytosol via nuclear
35 pores (32). Until recently, the significance of mitochondrial NAD⁺ was unclear, and it was
36 thought that NAD⁺ could be transported in its intact form into the mitochondria (138).
37 However, it is now understood that there are three intracellular NAD⁺ compartments – the
38 nucleus, cytosol and mitochondria (31). Subcellular compartmentalisation of NAD⁺ is
39 thought to play a critical role following niacin depletion. As total intracellular NAD⁺ levels
40 decline, distinct subcellular stores of NAD⁺ may influence the outcome of competition
41 between biochemical processes dependent on NAD⁺ consumption, leading to significant
42 alterations in metabolic pathways that are involved in tissue pathologies.
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52 Recently, three distinct NMNAT enzymes have been discovered, localised to the nucleus
53 (NMNAT-1), mitochondria (NMNAT-2), and the Golgi apparatus (NMNAT-3) (31). While
54 the levels of NMN required for the catalytic activity of NMNAT-1 and NMNAT-2 are very
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3 close, a higher amount of NMN is required for NMNAT-2 activity (121). The differential
4 expression of NMNAT enzymes in different intracellular compartments suggests multiple
5 roles for promoting optimal metabolic function in a variety of cells, or an additional
6 mechanism for adaptive response to stress. For example, one study showed that niacin
7 deficiency with normoxia reduced lung NAD⁺ levels in Fisher-344 rats by 40 % (273).
8 Interestingly, exposure to chronic hypoxic conditions induced poly(ADP-ribose) formation in
9 lung tissue, but did not reduce lung NAD⁺ content, rather NAD⁺ levels remained at near
10 control non-treated levels in niacin-deficient lung tissue.
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17 NMNAT-1 plays an important role in mediating NAD⁺ synthesis to close proximity of the
18 main enzyme responsible for ADP-ribosylation, PARP-1, but also including PARP-2 and 3,
19 tankrases, and sirtuins. While it is likely that nuclear NAD⁺ may enter the cytosol via
20 specific nuclear pores, there are also additional benefits for the formation of NAD⁺ in the
21 nucleus. Overexpression of NMNAT-1 has been shown to rescue neurons from axonal
22 degeneration, known as Wallerian degeneration (381). Similarly, inactive mutant forms of
23 NMNAT-1 also demonstrated beneficial effects against neural loss, possibly due to a
24 chaperone effect (54,382). NMNAT-1 can direct NAD⁺ synthesis towards the active site of
25 automodified PARP-1 via non-covalent interactions between NMNAT-1 and poly(ADP-
26 ribose) (32).
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35 Moreover, the mitochondrion represents the main site for important redox reactions,
36 including the TCA cycle and oxidative phosphorylation for ATP production. As well, it is
37 also home to mitochondrial poly(ADP-ribose) metabolism and SIRT3-5 activities (247).
38 These fundamental processes need to be maintained if possible even in the presence of NAD⁺
39 decline due to increased cellular ADP-ribosylation and niacin deficiency. NAD⁺ can be
40 released from the mitochondria and into the cytosol and nucleus through specific
41 permeability transition pores during conditions of apoptosis or necrosis (89,149). Therefore,
42 high starting mitochondrial levels of NAD⁺, which are an order of a magnitude greater than
43 cytosolic levels, are necessary to maintain optimal redox function.
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51 On the other hand, the Golgi apparatus is involved in packaging and transfer of
52 macronutrients to other organelles, and for clearance from the cell. It is likely that the Golgi
53 apparatus may regulate NAD⁺ levels in other organelles, although this remains uncertain.
54 NAD⁺ may be excreted from the Golgi apparatus and into the cytosol, or it may be released
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3 in the extracellular space to act as a substrate for important ecto-mono(ADP-
4 ribosyl)transferases and/or ADP-ribosylcyclases, which do not normally have access to
5 significant amounts of NAD⁺ (31).
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9 The effect of NAD⁺ precursors in the subcellular distribution remain uncertain and several
10 questions remain unanswered. Will nuclear NAD⁺ be made more available following
11 treatment with high levels of vitamin B3, since it has the greatest capacity to modulate
12 poly(ADP)ribosylation and repair of DNA damage? Will there also be an increase in
13 cytosolic NAD⁺, given that brain cyclic ADP-ribose levels can increase? What are the effects
14 of high levels of vitamin B3 on the mitochondrial NAD⁺ pool? Interestingly, NAPRT, the
15 enzyme responsible for the conversion of NA to NAD⁺ is found in the cytoplasm (142).
16 Therefore, supplementation with high levels of NA may alter the subcellular contents of
17 NAD⁺.
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26 **Modulation of NAD⁺ metabolism by caloric restriction**

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28 It is well established that caloric restriction (CR) represents the most efficacious intervention
29 to promote longevity in several short-lived species including mice and rats, and maintain a
30 healthy and average lifespan in primates. CR is defined as a 20% reduction in calorie intake
31 compared to ad libitum feeding without incurring malnutrition or reduction in important
32 vitamins and nutrients (222). Although the molecular basis of CR remains unclear, it is
33 thought that CR regulates fat and carbohydrate metabolism, ameliorates oxidative stress and
34 inflammation, activates a stress-induced hormetic response that down-regulates insulin and
35 insulin-like signalling (ILS), amino signalling target of rapamycin (TOR)-S6 kinase pathway,
36 and the glucose signalling Ras-protein kinase A (PKA) pathway (36). It is believed that
37 regulation of macromolecule consumption is a direct response to reduced diet, while
38 hormesis and down-regulation of TOR and PKA is most likely the molecular aspect of CR.
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48 Several studies have examined the effect of CR in a variety of model organisms. In yeast,
49 exposure to sublethal stress conditions increases expression of nicotinamidases, thus altering
50 NAD⁺ metabolism and enhancing the activity of Sir2, a yeast homolog of mammalian SIRT2
51 (16). This is evidenced by repression of age-associated extrachromosomal ribosomal DNA
52 circles (309). Down-regulation of TOR and PKA also mediates the beneficial effects of CR
53 on lifespan as reported in cell survival studies (361). On the contrary, longevity in worms is
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3 mediated by inactivation of ILS or Forkhead FoxO transcription factor daf-16 (25). While
4 additional mechanisms may be attributed to CR in mammals, alterations in the NAD⁺
5 metabolome and increased sirtuin activity may play a prominent role in mediating health
6 benefits reported in the brain and liver following a CR diet.
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11 In rodents, brain total NAD levels were reportedly increased in CR-treated mice, while NAM
12 levels decreased concurrently (265). These observations occurred in parallel to increased
13 neuronal SIRT1 activity which lowered Alzheimer's associated-neuropathology. In another
14 study, hepatic total NAD levels increased in fasted mice, and these changes were
15 accompanied by increased SIRT1 activation, PGC1 α deacetylation, and increased
16 mitochondrial biogenesis (277) (Figure 11). Three mechanisms have been developed to
17 explain these changes in the NAD⁺ metabolome following CR: (1) Increased systemic
18 mobilisation of NAD⁺ precursors, NAM and NR, since increased L-tryptophan and NA
19 availability is dependent on dietary availability; (2) Reduced NAD⁺ catabolism if major
20 NAD-consuming enzymes such as PARPs and CD38 are negatively modulated by CR; and
21 (3) CR-mediated negative regulation of the NR and/or NAR pathways may increase brain and
22 hepatic NAD⁺ levels.
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32 Information obtained from experimental small model organisms has provided insight into the
33 molecular basis of CR and ageing. However, there are still discrepancies in studies using
34 larger animals. CR has been investigated in rhesus monkeys, which are the closest
35 experimental model organism to human in a controlled environment (226). One study
36 conducted by the National Institute of Aging (NIA) reported no significant improvement in
37 lifespan. However, a positive trend to slow-down the onset of age-related degenerative
38 diseases was observed (227). In contrast, another study by the Wisconsin National Primate
39 Research Center (WNPRC) showed significant improvements in both lifespan and health
40 span (82,83). These discrepancies have been attributed to differences in dietary composition
41 and heterogenic genetic backgrounds of the subjects (Figure 11).
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50 Nevertheless, the beneficial effects of CR have been documented in the Comprehensive
51 Assessment of Long-term Effects of Reducing Intake of Energy (CALERIE) study conducted
52 by the National Institute of Health (NIH). The study showed that a two-year 25% CR regimen
53 provided significant health benefits in non-obese humans, including reduced inflammatory
54 markers and cardiometabolic risk factors (280). However, given the results observed in
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3 rhesus monkeys, longer studies with a larger sample size need to be conducted to validate the
4 potential effects of CR on human lifespan and normal physiological function.
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8 **Beneficial effects of NAD⁺ precursors**

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10 NAD⁺ anabolism in mammalian cells is known to occur through two major pathways; the *de*
11 *novo* and the salvage pathways. To determine nutritional and therapeutic benefits due to
12 maintenance of NAD⁺ levels in tissues, organs and cells, supplementation with either NAD⁺
13 and its reduced form NADH, or its precursors represents a potential therapeutic strategy to
14 slow down the ageing process and/or improve the management of age-related degenerative
15 disease. Oral supplementation with NAD⁺ and NADH have not shown any significant
16 elevation in plasma or tissue levels of NAD⁺, potentially due to inefficient metabolism of
17 NAD⁺ through the gut, thus leading to poor bioavailability (177). Additionally, oral NADH
18 may not be oxidised to NAD⁺ in the body, may not be efficiently absorbed by the
19 gastrointestinal system, or may be converted to a product prior to absorption that cannot yield
20 NAM (34,35). At present, intravenous infusion of NAD⁺ is the only recognised effective
21 means of clinically increasing systemic NAD⁺ levels. However, it is anticipated that some of
22 the alternative NAD⁺ precursors, including NA, NAM, NMN, NR and NAR (Figure 12) are
23 likely to provide some benefits.
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34 ***Nicotinic acid***

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36 NA represents the acid form of niacin. It is commonly prescribed clinically for the treatment
37 of hyperlipidemia. It has been reported that daily intake of 1-3 grams reduces blood
38 triglyceride levels and low-density lipoproteins (LDL), whilst increasing the level of high-
39 density lipoprotein (HDL), thus favourably regulating the LDL:HDL ratio (133,343). Our
40 research group was the first to show that exogenous NA efficiently increased intracellular
41 NAD⁺ levels in brain cells (127). However, NA therapy induces significant skin flushing in a
42 majority of individuals, thus limiting its clinical uses. A mild skin flush has been reported in
43 patients exposed to 50 mg oral NA, and the upper tolerable limit for NA has been set to 35
44 mg per day for adults in USA and Canada (314). The lipid lowering effects of NA are thought
45 to be mediated by binding of NA to the cell surface of a G-protein coupled receptor known as
46 HM74A or GPR109A (314). This association in adipocytes suppresses triglyceride lipolysis,
47 culminating in the reduction of circulating fatty acids, and reduced liver VLDL formation and
48 circulating LDL-cholesterol (314) (Figure 13).
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4 The uncomfortable side effect occurs because of an NA mediated stimulation of HM74A in
5 some skin immune cells which results in the conversion of the omega-6 metabolite
6 arachidonic acid (AA) into prostaglandin E2, stimulating vasodilation of skin capillaries,
7 causing skin flush (314) (Figure 14). RUP25, a receptor that differs from HM74A by only
8 one amino acid, has been identified. RUP25 has been shown to exhibit greater affinity to NA
9 than HM74A, and has been associated with extreme skin flush reactions in some people
10 (314). This often dramatic and unwelcome side effect has therefore restricted NA
11 applications to essentially a treatment-resistant lipid lowering therapy (170).
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19 In addition, a ketone body, beta-hydroxybutyrate is the natural ligand for HM74A, which is
20 produced during fasting (314). While NA demonstrated a greater affinity to HM74A (100 nM
21 required for half maximal) compared to beta-hydroxybutyrate (700 nM required for half
22 maximal), endogenous NA levels do not reach the concentrations required to activate this
23 receptor, whilst ketone bodies circulate at the required levels (314). However, other
24 mechanisms have been prescribed to account for the effects of NA on dyslipidemia. These
25 include but are not limited to inhibition of liver diacylglycerol acetyltransferase, inhibition of
26 pathways associated with the clearance of HDL in the liver (74,345), and activation of PPAR-
27 mediated cholesterol transport from extrahepatic tissue (84,169,171). More recently, SIRT1
28 has been shown to be a positive regulator of the liver X receptor (LXR). SIRT1-mediated
29 deacetylation of LXR at conserved lysine residues can lead to activation of LXR, which
30 regulates cholesterol levels, HDL biogenesis, and lipid homeostasis (199).
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40 Likewise, elevated levels of NA have been shown to improve genomic integrity by reducing
41 micronucleus frequency, and NA deficiency results in chromosomal instability (178-181).
42 Treatment with NA has been reported to delay carcinogenesis, enhance repair efficiency
43 following γ - and X-irradiation in mouse melanoma cells and human PBMCs, and improved
44 neuronal function following hypoxic insult (244,362). NA has also been shown to enhance
45 endothelial protection by increasing endothelial levels of NADP⁺ and glutathione (GSH)
46 (114). However, these studies were performed using concentrations ranging between 250-
47 1000 μ M in the culture medium, which is beyond the physiological concentrations in
48 humans.
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Nicotinamide

NAM, the amide form of vitamin B3 is also generated as a by-product of SIRT-mediated deacetylation, PARP-mediated ADP-ribosylation and CD38 NADase and ADP-ribosylation activities which can be converted back to NAD⁺ via the salvage pathway. High levels of NAM have been used to enhance radiotherapy or chemosensitise solid tumours by promoting microvascular flow inside the tumour (3,4). The clinical regimen describes oral doses (3-6 g) aimed at increasing systemic blood levels to 700 μ M or higher, combined with inhalation of 95 % oxygen/5 % carbon dioxide (358,359). This leads to improved tumour blood flow and oxygen generation, thus enhancing the effect of radiation by inhibiting myosin light chain kinase (MLCK). Decreased phosphorylation of MLCK disrupts vascular smooth muscle contraction, promoting vasodilation (283). However, the concentrations used *in vitro* are an order of magnitude higher than clinical systemic levels, and the macrovascular effects appear to be independent of its effects on NAD⁺ production.

NAM has also been shown to prevent or slow down the progression of several types of diabetes in animal models, although this effect was not reproducible using gram amounts of NAM in a randomised control trial (10,113,215,323,371,386,387). NAM has also been used as a potential therapeutic strategy to limit vascular injury and ischemia to the brain and other tissue in response hypoxic and/or chemotoxic insult in several animal models with some success (91,301,302,334,352).

Topical NAM formulations have also been successfully used for the treatment of inflammatory skin conditions, including rosacea, autoimmune bullous dermatoses, and acne (251). NAM has also been previously used for the maintenance of skin integrity, lowering sebum levels, and reducing hyperpigmentation spots and redness (328). NAM has also been shown to reduce acute and chronic effects of UV-induced skin damage by preventing the expression the inflammatory mediators IL-6 and TNF α , and the DNA damage markers cyclobutane pyrimidine dimers and 8-oxo-7,8-dihydro-2-deoxyguanosine (234). As well, NAM has been shown to improve UV-induced immunosuppression and photocarcinogenesis in rodent models and human studies (115). Similarly, human clinical studies have shown that oral NAM can significantly reduce actinic keratosis compared to a placebo, and may likely to also be useful for the prevention of non-melanoma skin cancer (321).

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4 However, it is well established that as a by-product of NAD⁺ catabolism, NAM also serves
5 as a natural feedback inhibitor for NAD-dependent enzymes (Figure 15). For example,
6 PARP, sirtuin and CD38 activities are proportionately inhibited as NAM concentrations
7 increase, and this has been postulated as the mechanism for the anti-diabetic effects of NAM
8 in humans. While NAD⁺ levels are still elevated, the important NAD-dependent functions
9 (e.g. SIRT1 activity) are inhibited. Moreover, NAM supplementation worsened liver fat
10 accumulation in a choline deficient rat model, and this effect was attributed to the
11 accumulation of poly(ADP-ribose), and a reduction in epigenetic methylation due to the use
12 of methyl groups in NAM excretion (19,172). Therefore, though exogenous NAM can be
13 converted to NAD⁺ it is again not considered an ideal supplement, particularly in the medium
14 to longer term due to its enzyme inhibiting and methyl depleting potential.
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24 ***Nicotinamide mononucleotide***

25 NMN is an important precursor for NAD⁺ synthesis from NAM. Supplementation with NMN
26 has been shown to have a positive effect on insulin levels most likely through action on
27 pancreatic β cells (316). NMN supplementation has also been shown to reduce obesity and
28 vascular damage in several *in vitro* and *in vivo* models (87,355). NMN has also been reported
29 to improve CNS function by increasing brain mitochondrial respiratory deficits, protecting
30 against amyloid-beta (A β) oligomer-induced toxicity and cognitive impairment, and
31 ameliorate reactive glial-induced motor neuron loss, and maintenance of neural stem and
32 progenitor cells (356). It has also been shown that NMN can protect against cerebral
33 ischemia-induced apoptosis and enhances neurogenesis following cerebral injury (360).
34 NMN treatment also up-regulated Nrf2 and HP-1 protein expression and promoted Nrf2
35 nuclear translocation for its transactivation following post-ischemic neuroinflammation, to
36 attenuate secondary neurological injury (360). Similarly, overexpression of NAMPT, the
37 enzyme required for NMN anabolism, appears neuroprotective in stroke (353,354).
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48 A recent study showed that NMN treatment significantly improved major pathological
49 hallmarks of Alzheimer's disease (AD) in APP^{swe}/PS1^{dE9} AD transgenic (tg) mice,
50 including cognitive impairment, neuroinflammation, A β deposition, and synaptic loss (372).
51 Another study also found that NMN treatment inhibited JNK activation and APP-mediated
52 amyloidogenic processing by APP-cleavage secretase in AD-tg mice (349,356). Accordingly,
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3 it is likely that NMN may represent a new therapeutic target for the treatment and
4 management of AD and other age-related degenerative diseases.
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8 In NDUSF4KO mice – a mouse model for cardiac-specific complex I deficiency – mice
9 exhibited a reduced NAD⁺/NADH ratio, hyperacetylation of mitochondrial protein including
10 the mitochondrial permeability transition pore (mPTP), impaired oxidative phosphorylation
11 and increased vulnerability to cardiac stress (174). Treatment with NMN was able to partially
12 restore the intracellular levels of NAD⁺ and attenuated the hyperphosphorylation of mPTP
13 and improved heart failure following exposure to chronic stress. Moreover, these mice also
14 exhibited increased activation of SIRT3, and its deacetylation of key protein including mPTP
15 due to enhanced activity following increased NAD⁺ levels following NMN treatment may
16 also explain the beneficial effects of NAD⁺ in NDUSF4KO (174).
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20 Despite these reported benefits of NMN treatment, evidence also suggests that as NMN is
21 effectively contained within the cells membranes and is not subject to high diffusion
22 gradients. This has raised the question of whether NMN is able to effectively traffic across
23 most cells. Interestingly, extracellular NMN may be actively produced from the direct
24 metabolism of exogenous NAD⁺ (388). However, further work is required to establish the
25 range of conditions for which NMN may prove beneficial in humans.
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33 ***Nicotinamide riboside***

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35 NR is a naturally occurring precursor of NAD⁺ originally isolated from fresh milk (338).
36 Exogenous treatment with NR has been shown to increase intracellular NAD⁺ levels in a
37 variety of cell lines. Supplementation with NR protected murine dorsal root ganglion neurons
38 from axonopathy via a mechanism involving the transcriptional induction of NRK2 gene
39 (288). As this effect is not reproducible by NA or NAM, NR represents a major precursor in
40 the CNS when the *de novo* synthesis of NAD⁺ by the kynurenine pathway is impaired. NR
41 has been shown to efficiently increase NAD⁺ levels without causing any adverse skin
42 flushing in contrast to NA, or liver damage in contrast NAM (337). NR has also been shown
43 to serve as a cholesterol-lowering agent in obese mice (67). Recent studies have shown that
44 NR is the mitochondrially favoured NAD⁺ precursor, and the beneficial *in vivo* effects of
45 NR have been attributed to modulation of mitochondrial sirtuin activities, as well as
46 nucleocytosolic targets including PARPs, sirtuins, CD38, NAD-dependent oxidoreductases,
47 and NADPH-dependent ROS detoxification enzymes. Supplementation with NR has also
48 been shown to reduce the acetylation state of several protein targets of SIRT3, including
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3 SOD2 and NADH ubiquinone oxidoreductase subunit A9, suggesting that NR may be used to
4 pharmacologically activate SIRT3 (67). Moreover, administration of NR slowed down
5 neurite degeneration after noise exposure by the NAD⁺-SIRT3 pathway (56).
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9 As yet, the effect on metabolic health of NR as an exclusive source of niacin remains unclear.
10 Supplementation of dietary NR in mice overexpressing the putative human oncogene,
11 unconventional prefoldin RBP5 interactor (URI) reduced dysplastic lesions and prevented
12 tumour development, thus providing evidence for NAD⁺ supplementation as a novel
13 approach for the treatment and management of hepatocellular carcinoma (340).
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16 Overexpression of human URI drives the development of dysplasia in hepatocytes via
17 mechanisms involving the aryl hydrocarbon receptor (AhR) and oestrogen receptor (OR), and
18 impaired kynurenine pathway metabolism (340). End-stage tumours in hURI-overexpressing
19 mice regressed with increased apoptosis in mice supplemented with NR (320,340).
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22 Despite the beneficial effects of NR supplementation, the doses often used to produce
23 beneficial effects are remarkably high (400 mg NR/kg body weight/day) compared to current
24 commercially available supplements (6-500 mg/ kg body weight/day). One study showed that
25 NR (300 mg/kg body weight/day) reduced exercise performance in rats, and previously
26 reported ergogenic effects of NR could not be confirmed (186). Two hypotheses have been
27 postulated to explain this observation: (1) Based on similar effects of NA and NAM, it is
28 likely that NR may also reduce fatty acid oxidation during exercise, leading to earlier fatigue;
29 and/or (2) NR may also alter the redox properties of NAD⁺ and NADP⁺, leading to a non-
30 optimal reductive state (186). Additional studies are warranted to examine the effects of NR
31 on exercise performance.
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42 Recently, the effect of a wide range of dietary NR concentrations on metabolic flexibility and
43 gene expression in epididymal white adipose tissue was examined in mice exposed to a
44 mildly obesogenic (40 % fat) diet (303). The study showed that 30 mg NR per kg diet was
45 most beneficial for improving metabolic health, with regards to metabolic flexibility and
46 increased expression of PPAR- γ , a master regulator of adipogenesis, and SOD2 and PRDX3,
47 two antioxidant genes (303). The study concluded that 30 mg NR/kg diet represented the
48 optimal concentration to potentiate metabolic health.
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54 *Nicotinic acid riboside*

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3 The least examined of the NAD⁺ precursors, NAR has been shown to be produced in human
4 cells through NMN and NAMN dephosphorylation by cytosolic 5'-NTs (38,190). It is
5 anticipated that this metabolite will represent an important precursor for NAD⁺ generation.
6 Low micromolar concentrations of NAR have already been demonstrated to produce
7 sufficient amounts of NAD⁺ to maintain cell viability. One study showed that NAR can be
8 produced and delivered by cells at physiologically sufficient levels (190). It is likely that
9 other cell types may use NAR and transport it between each other.
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16 **Pharmacokinetics of NAD⁺ precursors**

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18 Boosting the NAD⁺ pool by utilising precursor molecules may have multiple health benefits
19 and a diverse range of therapeutic implications. NA, NAM, NMN and NR have been
20 publicised as potent NAD⁺ boosters. NMN and NR may also be used as a general
21 supplement in patients who have adverse responses to NA and NAM. The pharmacokinetics
22 of NA and NAM has been extensively investigated, while the pharmacokinetic properties of
23 NR have only recently been determined in mice and a middle-aged human subject (337).
24 However, pharmacokinetics effects of NMN and more so, NAR have not yet been fully
25 investigated in either the human or murine models.
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32 *Nicotinic acid*

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34 The pharmacokinetics of NA have been previously examined using pharmacological doses of
35 NA and several extended release formulations. An open-label, dose-rate escalation, crossover
36 study administered 12 human subjects with 2000 mg NA in solution form in slow (25 mg
37 niacin aqueous solution administered every 10 minutes for 80 doses), intermediate (50 mg
38 niacin aqueous solution administered every 10 minutes for 40 doses), or fast (100 mg niacin
39 aqueous solution administered every 10 minutes for 20 doses) (232). Peak NA levels varied
40 between 10 μ M (slow release) to 240 μ M (fast release). Interestingly, the area under the curve
41 (AUC) for the slow release formulation was 25-fold lower than the fast release counterpart.
42 NA in the slow release preparation is taken up by the intestine and liver, forming NAD⁺, and
43 the released into the circulation as NAM (232). Importantly, a concentration of 10 μ M is
44 estimated to be about 30-fold higher than physiological levels of NAM in the blood. Fast
45 release formulation of NA not only yields higher peak levels and AUC, it also elevates peak
46 NAM levels to 16 μ M. NA is preferentially removed from circulation at high levels with a
47 half-life of 1 h, compared to a half-life of 4 h for NAM (231,232). Therefore, high doses of
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3 NA also elevated the levels of NAM to supra-physiological levels. Therefore, the effects of
4 NA on lipids may also be due to the protective effects of NAM and increased NAD⁺
5 anabolism.
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10 In a recently study comparing the efficiency of NAD⁺ precursors to generate NAD⁺ in mice
11 following oral gavage, NA produced the lowest levels of NAD⁺ (337). However, the kinetics
12 of hepatic NAD⁺ accumulation was 4-6 h faster than either NAM or NR. Oral administration
13 of NA doubled hepatic NAD⁺ (from 1 to 2 mM) by increasing the level of NAAD (an
14 intermediate), and enhanced NAD⁺ catabolism as reported by increased levels of MeNAM
15 (337). The liver promoted NAD⁺ anabolism as long as enough NA is available, while
16 increasing the activity of NAD-dependent processes, some of which generate NAM as a by-
17 product (337). Increased NNMT expression due to increased levels of MeNAM stabilises
18 hepatic SIRT1 protein and regulates lipid levels in mice and humans (152,194,336).
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26 Another study reported significant changes in the levels of NAD⁺ following oral
27 supplementation over a 2-3 week feeding interval (26). It was previously thought that NAD⁺
28 levels will continue to increase in response to time before reaching a plateau. In rats
29 supplemented with NA (30 and 4000 mg/kg), bone marrow NAD⁺ significantly increased in
30 animal fed with 4000 mg/kg (26). However, NAD⁺ levels were down-regulated as
31 consumption became chronic, and it was unclear whether this effect was due to either NA
32 uptake alone, associated with the conversion of NA to NAM, or altered NAD⁺ catabolism in
33 bone marrow. Therefore, it has been postulated that pharmacological responses to long-term
34 supplementation with NAD⁺ precursors may change over time (26). This also raises the
35 important question of whether higher NAD⁺ level have the potential to induce a deleterious
36 impact on cellular function, thus stimulating an adaptive response.
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45 *Nicotinamide*

46 The pharmacokinetics of 3-6 g of oral NAM in humans has been previously investigated (93).
47 Higher doses are prone to produce adverse reactions including nausea and vomiting. The
48 peak blood levels of NAM were between 1-2 mM. This is estimated to be more than 3000-
49 fold higher than circulating levels (93). This figure is also well above the minimum
50 concentration associated with radiation sensitivity. The half-life of NAM is 4-5 h, which
51 provides sufficient time to facilitate carbogen breathing and radiation therapy. It has been
52 suggested that if MLCK represents the likely target of NAM, then it is required in order to
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3 accumulate inhibitory concentrations inside a cell. While intracellular transporters for NAM
4 have been previously identified, mechanisms for cellular responses to high concentrations of
5 NAM remain unclear. One study showed that radiation sensitivity remained after a decline in
6 circulating levels of NAM, suggesting that the beneficial effects of NAM may be related to
7 additional downstream effects, including increased NAD⁺ generation (279), and/or inhibition
8 of poly(ADP-ribose) and DNA damage-induced apoptosis (153).
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14 While high doses of NA have been shown to increase NAM levels, the effect of increased
15 NAM on NA levels remains unclear. At present, enzymatic conversion of NAM to NA has
16 not been identified. However, the deamidation of NAM via oral and intestinal microflora is
17 possible (212). One study showed that significant amounts of salivary NAM were converted
18 to NA, although circulating levels of NA following large oral dosing of NAM remained
19 undetected (317). It is likely that the quantification methods used in this study were not
20 sensitive enough to clearly delineate changes in the NAD⁺ metabolome. Skin flushing, a
21 common adverse effect following NA treatment, has also been reported with NAM,
22 suggesting that NA may be increased following administration of high doses of NAM (167).
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30 A recent study comparing three NAD⁺ precursor vitamins provided in bolus at equivalent
31 oral doses also demonstrated increased hepatic NMN, NAAD, NAD⁺ and NADP⁺ levels,
32 and NAD⁺ catabolic activity as evidenced by elevations in MeNAM and ADP-ribose (337).
33 However, while the AUC of increased NAD⁺ due to NAM showed a 50% benefit compared
34 to NA, the study demonstrated a 50% deficit in NAM-mediated accumulation of ADP-ribose
35 compared to NA (337). These studies also suggest that NAAD may represent a biomarker for
36 increased NAD⁺ synthesis, and is independent of traditional NAAD precursors such as L-
37 tryptophan and NA (337). Unlike NA, NAM is not a potent cholesterol-lowering agent, and
38 high levels of NAM may inhibit PARP and sirtuin activities.
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46 Evidence for potential adaptive responses following high doses of NAM has been previously
47 reported. Rats supplemented with high doses of NAM (4g/kg) exhibited elevated levels of
48 NAD⁺ and ADP-ribose in the brain. Interestingly, these changes were accompanied by
49 impaired cognition as reported by impaired performance in a hippocampal-dependent spatial
50 learning test (197). Increased NAD⁺ can ultimately lead to increased activity of a diverse
51 range of enzymes including PARPs, sirtuins and CD38/CD157. This in turn may have
52 dynamic effects in cellular function.
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Nicotinamide mononucleotide

The detection of NMN in blood remains challenging. While the concentration of NMN has been reported to be around 50 μM in plasma (275), NMN levels were undetectable in another study (272). These differences can be attributed to different detection techniques for NMN. For instance, using an HPLC-based method, intracellular concentrations of NMN and NAD^+ were reported to increase up to 500 pmol/mg and 50 pmol/mg of white adipose and pancreatic tissue 15 min after intraperitoneal injection of 500 mg/kg of NMN (376). However, hepatic NMN and NAD^+ levels were reported to reach 10 and 4000 pmol/mg respectively, 6 h after oral gavage of 185 mg/kg (337). Similarly, the level of NMN has been reported to be around 1.5 pmol/mg tissue in tumours, and 80 nM in ascite fluid (310).

NMN appears to be stable in plasma and cell media supplemented with 10 % foetal bovine serum (FBS), and no increases in NAM levels were reported after 1 h incubation (272). However, NMN injections led to significant increases in NAM levels in plasma, which suggests that NMN may be partially converted to NAM following intraperitoneal injection. The presence of NAM in mice plasma following NMN injection suggests that NMN may be initially converted to NR (272).

A recent study demonstrated that dephosphorylation of NMN into NR, which is required to produce NAD^+ in yeast, represents a major step as an exogenous NAD^+ precursor in mammalian cells (272). It is thought that the extracellular receptor CD73 may act as an NR-releasing enzyme. CD73 has both pyrophosphatase and 5'NT activity which facilitates the conversion of extracellular NAD^+ and NMN to NR, which in turn can be used to stimulate further NAD^+ synthesis. This is supported by another study which showed that gene silencing of CD73 inhibits the use of NMN as a potential NAD^+ precursor (310). Additionally, NRK1 has recently been identified as an important rate-limiting enzyme for the conversion of NMN to NR for NAD^+ synthesis (272), and provides a reliable explanation to account for overlapping effects reported for NMN and NR.

Nicotinamide riboside

Evaluating the pharmacokinetics of NR in mammalian tissue has been limited by poor sensitivity and detection of NR in biological samples. As well, results of a clinical trial aimed at investigating the pharmacokinetics of NR in healthy human subjects are not yet available.

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3 However, NR was degraded rapidly after incubation in murine plasma (~10% NR was
4 degraded after 10 min, and 66% was degraded after 1 h), leading to comparable increases in
5 NAM. These results hint at the presence of plasma factors that can degrade NR to NAM
6 (272). NR degradation followed by detection of NAM has also been observed in cell media
7 containing 10 % FBS (272). Importantly, NR is stable in protein fractions in milk with a
8 potential lifetime of one week (338). NR may also be circulated in a cell-bound form for
9 several hours.
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16 One study showed that NR is safe and orally bioavailable in mice and humans with no
17 adverse effects reported (337). However, a future study will need to incorporate a validated
18 flushing symptom questionnaire to assess whether NR may be associated with any flushing
19 episodes. Higher levels of NMN, NAMN, NAM, NAAD, NAD⁺ and NADP⁺ were produced
20 following oral gavage of NR compared to oral NAM (337). In addition, ADP-ribose levels
21 were more significantly elevated compared to NA and NAM, suggesting that NR can enhance
22 the activity of NAD⁺ consuming enzymes more than mole-equivalent doses of NAM and NA
23 (337). More recently, a randomised double-blinded placebo-controlled study showed that NR
24 in combination with pterostilbene, a naturally occurring phytochemical found in blueberries,
25 can increase NAD⁺ in a dose-dependent manner in whole blood lysates throughout the entire
26 8 week trial (88). Taken together, this suggests that NR is a more potent precursor of NAD⁺
27 synthesis and NAD-dependent activities than amidated and acidic forms of niacin.
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37 *Nicotinic acid riboside*

38 To our knowledge, the pharmacokinetic properties of NAR have not been reported in the
39 literature. Understanding the pharmacology of NAR has remained difficult due to its low
40 physiological sub-micromolar concentration. The ¹H-NMR method has shown some success
41 in detecting NAR in cell culture medium because of the observed chemical shifts (190). Also,
42 low sensitivity of detection methodologies requires acquisition of spectra over extensive
43 periods of time. Overexpression of NAPRT in HEK293 cells led to the detection of NAR in
44 the cell culture medium, and this affect is due to increased NA catabolism (190). In HeLa
45 cells, NAR but not NR, is released in amounts that are sufficient to maintain NAD⁺
46 biosynthesis and cell survival (190).
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54 *Nicotinic acid adenine dinucleotide – a biomarker of elevated NAD⁺ metabolism*

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3 As mentioned above, NA, NAM and NR have been shown to increase the levels of the
4 intermediate, NAAD. However, the increase in the levels of NAAD was lowest following
5 ingestion of NA (337). This finding suggests that increased NAD⁺ anabolism by
6 supplementation with NAD⁺ precursors not only increases the accumulation of by-products
7 of NAD⁺ catabolism (such as ADP-ribose and MeNAM), but also stimulates retrograde
8 synthesis of NAAD and NAMN. As the rate of NAD⁺ anabolism increases, NAD⁺ is
9 deamidated to form NAAD by a yet unknown mechanism. Similarly, it is also possible that
10 deamidation of NMN can lead to increased levels of NAMN and NAAD. While the
11 deamidation reaction is yet to be verified, it is possible that NAAD may also be formed from
12 NAADP, although the mechanism responsible for this elusive biochemical reaction is yet to
13 be identified.
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22 **Effects of NAD⁺ precursors on NAD-dependent processes**

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24 Apart from the beneficial effects of NAD⁺ precursors on normal cellular function, including
25 NAD⁺ and NADP-dependent reactions and ADP-ribosylation, these substrates for NAD⁺
26 anabolism share a common effect of increasing intracellular NAD⁺ levels in multiple cellular
27 compartments. It has been considered that normal metabolic processes may be fulfilled
28 following recommended daily intake of vitamin B3, and that higher doses may induce
29 alternate mechanisms. However, it is now clear that physiological roles of NAD⁺ precursors
30 may be different than the doses present naturally in the diet.
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37 *Nicotinamide and PARPs*

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39 Whether NAM can inhibit PARP-1 activity remains controversial. The K_i value for NAM-
40 mediated inhibition of PARP-1 ranges between 30-200 μM in a cell-free system. The K_i
41 value in cultured cells was 3-fold greater than in a cell-free system (271). This suggests that
42 the uptake and/or conversion of NAM to NAD⁺ may be limited in cell cultures. In mammals,
43 oral NAM is metabolised by the small intestine and liver before it enters the blood stream.
44 NAM is taken up by extrahepatic tissue in small amounts where it is immediately converted
45 to NAD⁺. Moreover, blood volumes are significantly lower than tissue volumes. Therefore, it
46 is less likely that tissue NAM levels will reach the concentrations needed to inhibit PARP-1
47 activity.
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3 On the other hand, the conversion of NAM to NAD⁺ due to increased substrate has been
4 shown to promote poly(ADP-ribose) levels. For instance, supplementation with NAM (1
5 g/kg) increased hepatic NAD⁺ levels by 50%, while basal poly(ADP-ribose) levels increased
6 by 2 fold (163). This suggests that NAM was more effective at enhancing substrate pools
7 than mediating PARP-1 inhibition. However, poly(ADP-ribose) content was the same when
8 the same animals were exposed to a hepato-carcinogen to enhance PARP-1 activity. NA also
9 promoted higher levels of poly(ADP-ribose) formation (163). Taken together, it is likely that
10 basal PARP-1 activity may be regulated differently than DNA-damage induced PARP-1
11 activity, and NAM may be more effective at inhibiting the latter form of PARP-1 activity.
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19 In another study, increased PARP-1 activity was reported in extrahepatic tissue in response to
20 oral dosing of NAM (4 g/kg). In that study, bone marrow NAD⁺ levels increased by 2.5 fold,
21 basal poly(ADP-ribose) levels increased by 5-fold, while DNA-damage-induced poly(ADP-
22 ribose) increased by 2-fold (42,43). Similarly, studies in radiation sensitisation models
23 showed that radiation sensitivity due to NAM was due to mechanism(s) independent of
24 inhibition of PARP activity and DNA repair processes (279).
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30 *Nicotinamide and sirtuins*

31 Mammalian sirtuins have developed low NAD⁺ binding affinities which ensured that their
32 deacetylase activities can be efficiently regulated by minor changes in the intracellular
33 concentrations of NAD⁺, thus serving as potent NAD⁺ sensors. Reduced intracellular levels
34 of NAD⁺ during ageing can downregulate sirtuin activity and SIRT1-mediated deacetylation
35 of p53 (51). On the other hand, increased intracellular NAD⁺ levels, either due to CR or
36 NAD⁺ supplementation, can upregulate sirtuin activity. While resveratrol, a plant-derived
37 stilbene putatively allosterically activates SIRT1 only, NAD⁺ supplementation can activate
38 almost all seven forms of mammalian sirtuins. For example, regulation of SIRT3 by
39 intracellular NAD⁺ levels has been demonstrated to be the major determinant of cellular
40 resilience against apoptosis (143).
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50 If the increase in NAM in biological systems is capable of inhibiting PARP-1 activity, then it
51 may also inhibit other NAD⁺ dependent processes such as sirtuins. High levels of NAM may
52 inhibit NAM cleavage reactions or mediate competitive inhibition at NAD⁺-binding sites
53 leading to altered function or sirtuin enzymes, to ultimately enhance the levels of NAD⁺.
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3 Physiological levels of NAM are within the same range as the IC₅₀ of several sirtuins (159),
4 therefore suggesting that sirtuins may act as NAM sensors as well as NAD⁺ sensors .
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8 NAM has been shown to bind to a specific conserved region in the catalytic site of sirtuins,
9 inducing a reverse base-exchange reaction with an intermediate, rather than deacetylation,
10 thus inhibiting sirtuin deacetylase activity (129). The base-exchange equilibrium constant has
11 been estimated to be about 20 for SIRT1(37). This means that the maximum possible
12 activation of SIRT1 by full inhibition of the base exchange reaction at any NAM
13 concentration is greater than for Sir2 in yeast. Recently, isonicotinamide (isoNAM), a
14 synthetic analogue of NAM has been shown to compete with NAM for binding at the
15 catalytic site (228). However, unlike NAM, isoNAM does not substantially react with the
16 intermediate, leading to increased Sir2 activity.
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24 NAM represents a physiological inhibitor of sirtuins. The IC₅₀ values for inhibition of
25 bacterial Sir2, yeast Sir2, mouse Sir2, SIRT1, SIRT2, SIRT3, and SIRT5 were measured to
26 be 26, 120, 160, 50, 100, 36.7 μM, and 1.6 mM respectively (129). The concentration of
27 NAM in yeast nuclei has been estimated to be 10–150 μM, which suggests that NAM is a
28 regulator of Sir2 activity *in vivo* (292). Yeast and bacterial sirtuins have lower Km's and Kd's
29 for NAD⁺ compared to mammalian sirtuins, and therefore may be less sensitive to changes in
30 intracellular NAD⁺ concentrations than their mammalian counterpart (68). Therefore,
31 increased NAD⁺ levels are more likely to result in activation of mammalian sirtuins. In
32 mammalian cells, low levels of NAM have been reported in several rat tissues (142). This is
33 likely due to rapid catabolism of NAM for the production of NAD⁺ and related pyridine
34 nucleotides. However, high concentrations of NAM (up to 300 μM) have been reported in the
35 brain of Tg2576 mice (265), providing additional evidence for NAM as a regulator of sirtuin
36 activity in mammalian tissue. Moreover, the ratio of NAD⁺ to NAM in subcellular sub-
37 compartments can decline with age, therefore lowering sirtuin activities (223). This effect
38 may be due to increased utilisation of NAD⁺ by PARPs and reduced NAD⁺ anabolism from
39 NAM by NAMPT-mediated salvage pathway (160).
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51 Depending on their physiological roles, several other mechanisms have been attributed to
52 account for the regulation of sirtuins by NAM. For instance, NAM can only partially inhibit
53 Sir2AF2 (SIRT2 homolog from Archaeon *Archaeoglobus fulgidus*), whereas it is a full
54 inhibitor of SIRT1 (129). As well, NAM is a competitive inhibitor of SIRT3, in contrast to
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3 non-competitive inhibition reported for other sirtuins (129). Mammalian SIRT3 is a
4 mitochondrial sirtuin that has demonstrated tumour suppressive effects and regulates
5 glycolytic metabolism (18). Inhibition of SIRT3 by NAM may increase glycolytic
6 metabolism and inhibit aerobic glycolysis and thus reducing cancer cell biomass, and
7 improving chronic tissue damage.
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12 Apart from base exchange, which is known to increase the rate of forward reaction for sirtuin
13 activity, direct competition has been postulated as another mechanism to explain the greater
14 degree of competition between NAM and NAD^+ in the inhibition kinetics of SIRT3
15 compared to that of SIRT1 (129). This involves NAD^+ binding to the catalytic site in the
16 presence of NAM (129). Elucidating the mechanisms of action of NAM against SIRT3 and
17 other sirtuins can help to develop more efficient inhibitors and activators of sirtuins that can
18 be translated to the clinic.
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24 ***CD38-mediated processes***

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26 It has been proposed by our group and others that CD38 can regulate SIRT1 activity by
27 modulating the availability of the essential substrate NAD^+ , and NAM to the SIRT1 enzyme
28 (52). This can have a profound effect on modulating obesity, metabolic disorders, cellular
29 energy homeostasis and cellular senescence, and ageing. By promoting intracellular NAD^+
30 anabolism whilst reducing NAM levels, inhibition of CD38 can increase SIRT1 activity.
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37 Several CD38 inhibitors have been identified. These include NAM and NA, NAD^+ analogs
38 such as arabiono-NAD, and reducing agents including dithiothreitol (76). CD38, due to its
39 effect on calcium generation, also serves as an important mediator of smooth muscle
40 contraction, cell death, and apoptosis, neural and hormonal signalling, and egg fertilisation
41 (76). Therefore, CD38 inhibition may be useful under pathological conditions where calcium
42 homeostasis is impaired, including hypertension, cardiac ischemia, asthma and dysfunctional
43 labour.
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50 However, CD38 is also involved in the release of hormones such as oxytocin and ACTH
51 which regulate maternal and social behaviour (166). Inhibition of CD38 in these conditions
52 may have significant negative effects on psychological function. In addition, CD38 plays an
53 important role in the immune system, and knock-out of CD38 has been shown to increase
54 susceptibility to bacterial infection (201). Therefore, the effect of NAD^+ precursors and
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changes to the NAD⁺ metabolome may have previously unknown effects of CD38 activity and NAD-dependent processes, and may serve as important therapeutic strategies for the treatment of metabolic and inflammatory conditions if appropriate dosage regimens are devised and adapted to meet individual patient requirements.

Redox reactions

In eukaryotic cells, the generation of ATP is achieved predominantly by mitochondrial oxidative phosphorylation. In this process, free energy released following the break-down of carbon substrates is captured by exchanges between electron donors and electron acceptors via the electron transport chain (ETC) leading to ATP production (308). NAD⁺ serves as an electron acceptor, and its reduction leads to the generation of NADH, which can be subsequently oxidised by complex I of the ETC to produce NAD⁺. The NAD⁺/NADH ratio serves as an important indicator of several oxidoreductase enzymes. Elevated levels of NADH can inhibit NAD-dependent processes. A metabolic imbalance in oxidative phosphorylation has been associated with several cardiac, neurological and renal pathologies (206). Alterations to the ETC can lead to a significant decline in ATP production, increased intracellular Ca²⁺ influx and free radical production, and lowered NAD⁺/NADH ratio. A switch between oxidative to anaerobic metabolism in response to several cardiac stressors has been shown to reduce oxidative damage and maintain ATP levels. However, this compensatory mechanism impairs oxidative phosphorylation whilst limiting the mitochondrial NAD⁺ pool (97).

Similarly, the NAD⁺/NADH ratio appears to play a crucial role in the heart and kidney and supplementation with NAD⁺ precursors has been shown to protect against impairments in oxidative phosphorylation due to cardiac stressors and AKI-induced renal damage (150). In PGC1 α -deficient mice, treatment with NAM increased fatty acid oxidation, ATP generation and the NAD⁺/NADH ratio to protect against AKI toxicity (339). Therefore, under degenerative conditions associated with impaired oxidative phosphorylation, or other abnormality leading to a decline in NAD⁺, upregulation of NAD⁺ anabolism through NAD⁺ precursors may improve redox function.

While human *in vivo* studies regarding the effect of NAD⁺/NADH ratio remain nascent. However, using two-photon microscopy for the quantification of NADH and NADPH in epidermal skin layers, one study reported a significant increase in NADH fluorescence

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3 following arterial occlusion, suggesting that there is a reduction in oxidative phosphorylation
4 due to a decline in the need for electron donation for the oxidation of NADH to NAD⁺ (24).

5 Similarly, reduced NADPH fluorescence emission has been reported in the facial skin of
6 older aged females compared to younger subjects (286). Taken together, these studies provide
7 supportive evidence for the role of NAD⁺ in regulating cellular bioenergetics.
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12 Impaired poly(ADP)ribosylation has been associated with increased sensitivity to DNA
13 damage underlying skin lesions reported in the human disease of niacin deficiency better
14 known as pellagra (274). Additionally, impairment in the formation of cyclic ADP-ribose,
15 which regulates intracellular calcium levels, may contribute to neuronal loss observed in
16 pellagrous dementia (378). However, redox reactions corresponding to the ratio of
17 NAD⁺/NADH are less prone to be affected by altered NAD⁺ levels as ADP-ribosylation
18 reactions. NAD⁺/NADP⁺ serve as soluble cofactors in a multitude of oxidation/reduction
19 reactions. The catalytic enzymes utilise riboflavin-based nucleotides as a source of prosthetic
20 groups. Others contain iron to facilitate electron transfer. Unlike poly- and mono(ADP-
21 ribosylation) reactions, iron and riboflavin deficiencies are not known to induce sun
22 sensitivity of the skin, and dementia, which are two main characteristics of pellagra .
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32 One study investigated the effect of NADH, the reduced form of NAD⁺, on proliferation,
33 cytokine release, and cell redox status of lymphocytes collected from healthy aged subjects
34 (40). Cells exposed to NADH (500 μM/L) showed increased levels of glutathione (GSH), and
35 catalase activities, while malondialdehyde and carbonyl proteins are markedly decreased
36 (40), suggesting a decline in oxidative stress. Recently, it has been shown that treatment with
37 1 mM NADH increased the expression of nuclear Nrf2, catalase activity, and total GSH by
38 increasing SIRT2 function (69).
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45 As well, the effect of niacin deficiency on endogenous antioxidant defence mechanisms,
46 NADPH:NADP⁺ and GSH:GSSG redox couples remains unclear. Two studies showed that
47 niacin deficiency increased markers of oxidative stress, but did not induce either NADPH or
48 GSH decline (27,325). This suggests that niacin deficiency impaired poly(ADP-ribose)
49 accumulation but did not stimulate further tissue damage, whilst maintaining GSH defences.
50 Several mechanisms have been postulated to account for the maintenance of redox reactions
51 during periods of niacin deficiency. These include variations in substrate affinity for NAD⁺,
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3 subcellular localisation of enzymes and cofactors, and direct modulation of enzyme
4 activity/expression levels.
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8 **Does NAD⁺ and related precursors display hormesis**

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10 There is a growing body of evidence which suggests that NAD⁺ decline is a major
11 contributor to the ageing process and may be involved in the pathogenesis of several age-
12 related degenerative diseases affecting the heart, brain, liver, kidney and skin. These results
13 collectively highlight the potential for NAD⁺ supplementation, whether using NAD⁺ alone
14 or NAD⁺ precursors to protect against ageing and associated pathologies. While such
15 prospects are of major clinical significance, the role of NAD⁺ and its modulation in human
16 ageing remains only partially understood. In particular, little is understood regarding the
17 impact of having 'very high' NAD⁺ levels. We suggest that modulation of NAD⁺ levels may
18 induce a hormetic dose response that may confound numerous clinical outcomes.
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26 The term hormesis was first incorporated into the biomedical context by Southam and Ehrlich
27 in 1943 to account for the effects of Red cedar tree extracts on wood-rotting fungi (62). The
28 study showed that various species of fungi exhibited low-dose stimulation and a high dose
29 inhibitory effect on cellular metabolism. By the 21st century, hormesis is now used to define
30 the biphasic dose response that occurs following exposure to a chemical or physical agent, or
31 as an over-compensatory response to cytotoxic insult (63). Resveratrol, an activator of
32 sirtuins has recently been shown to induce a hormetic dose response in a variety of biological
33 models including breast, prostate, colon, lung, uterine and leukemia tumor cell lines (64). In
34 these studies, lower concentrations of resveratrol enhanced tumor cell proliferation. However,
35 at higher concentrations, resveratrol induced an inhibitory effect. For instance, resveratrol
36 increased the activity of the vitamin D receptor and promoted proliferation of T47D breast
37 cancer cells up to 4 μ M, above which led to reduced proliferation of the tumor cell line (64).
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47 Other studies have shown that resveratrol can protect cultured hippocampal neurons against
48 oxidative stress at concentrations between 5-25 μ M (108). Resveratrol could also ameliorate
49 inflammation and oxidative stress in cultured tumor cells by inhibition of COX-2. (391)
50 However, when these cells are under conditions of reduced oxygen and glucose availability,
51 resveratrol can induce apoptotic cell death (64).
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3 In light of these findings, it is likely that upregulation of NAD⁺ anabolism may also conform
4 to a hormesis biphasic dose response. For example, where neuronal cells are exposed to
5 cellular stress, as may occur due to ischemic insult, or cytotoxins such as glutamate, and A β
6 aggregates, increasing NAD⁺ levels may provide both beneficial and deleterious effects
7 which may be dependent on the dosage and duration of administration relative to the
8 cytotoxic stimulant. For instance, *in vitro* incubation of naive T cells with NAD⁺ induced
9 apoptosis, while activated T cells incubated with NAD⁺ showed no signs of apoptosis (204).
10 It was suggested that ecto-NAD, as substrate of ADP ribosylation, acts on naive, but not on
11 activated T cells (297). This indicates that many effects of NAD⁺ are dependent on
12 environmental factors that would seem to produce a favourable response.
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21 Competition between NAD⁺-consuming enzymes also displays hormesis. For example, as
22 previously mentioned, PARP1 activity increases with age due to accumulation of oxidative
23 DNA damage, and in response to high energy intake. Since the K_m for NAD⁺ for PARP1
24 and SIRT1 is relatively similar, the decline in NAD⁺ levels following PARP1 activation can
25 also induce a decline in SIRT1 activity. Therefore, while low levels of PARP1 activity can
26 repair DNA damage following exposure to mild oxidative stress levels, increased PARP1
27 activity can lead to cell death via reduced SIRT1 activity and energy restriction, therefore
28 exacerbating disease progression (269). Therefore, rigorous double blind and placebo
29 controlled clinical trials are needed to assess the nature of the dose-response effect of NAD⁺
30 in humans. Further work will be required to gain further understanding of the role of NAD⁺
31 anabolism against ageing and age-related diseases.
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41 **Limitation of using *in vitro* and *in vivo* studies**

42 Despite the importance of NAD⁺ metabolism to human health and diseases, determining the
43 levels of NAD⁺ remains a challenge. As well, while there is clinical significance for
44 supplementation with NAD⁺ precursor in the clinic, evidence showing increased NAD⁺
45 levels upon such supplementation is limited. While cell culture and animal models are
46 commonly used in research studies, they are not a true representation of human physiology.
47 Moreover, biochemical assays or analytical methods that are currently used to analyse tissue
48 samples or cell homogenates are vulnerable to changes in pH, temperature, light, and
49 chemical agent or buffer solution. Therefore, more accurate and reliable quantification and
50 extrapolation to *in vivo* conditions are warranted.
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Cell-culture systems

Accumulating evidence suggests the involvement of oxidative stress, inflammation and increased L-tryptophan catabolism in several degenerative disorders. This has paved the way for investigation of basic mechanisms of free-radical damage and modulation of the NAD⁺ metabolome as a therapeutic strategy to protect against it. For example, primary murine and human brain cell cultures, and immortalised cell lines remain highly useful as models for examining the effect of oxidative damage and adaptive cellular responses. The most common approach to modelling CNS oxidative stress and altered kynurenine pathway metabolism is through exposure of primary glial and neuronal cells to deleterious conditions, and addition of exogenous prooxidants and neuroprotective agents (44,46,48,50,71,195,333). Cell culture models have also been used to examine the effects of niacin deficiency, and inhibition of NAD-dependent processes (such as PARP, sirtuin and CD38 inhibition) on cellular function in several *in vitro* disease models (46,47,52,119,267). However, most cell culture components, which are fundamental to these studies, are aimed at maximising cell growth and survival in culture, and do not fully, recapitulate natural *in vivo* biological processes. There is also strong evidence that the beneficial effects of NAD⁺ precursors in culture systems may be incurred via non-physiological mechanisms.

Vitamin B3 is present in cell culture in both its amide and acidic form. However, NAM is present at highest concentrations, and this reflects the significance of NAM as the main form of niacin in the blood stream. Commonly used cell culture media (e.g. MEM, Williams, RPMI, BME, L-15, Dulbecco's) contain between 1-4 mg/L of NAM, although more specialised media (MCDB 131, BGjB) may contain between 6-20 mg/L. The equivalent molar concentration for 4 mg/L is approximately 33 μ M. This amount is about 300-fold greater than the average levels of NAM in plasma. It is likely that these concentrations may have a profound effect on intracellular NAD⁺ storage, cyclic-ADP- and monoribosylation, and inhibitor studies. Other cell culture media contain equal contents of NAM and NA at concentrations of up to 4 μ M. Similarly, the concentration is well above the physiological concentration of NA in systemic circulation. Moreover, these levels are significantly greater than the amount required to activate HM74A receptors if these are expressed in cells in culture.

In vivo models

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3 The human life-span is much longer than smaller mammalian species, making it difficult to
4 fully characterise the influence of NAD⁺ metabolism during normal human aging. Therefore,
5 traditional *in vivo* studies have been performed using animals with phenotypically accelerated
6 ageing or prolonged longevity, transgenic, mutant, and knockout models that focus on a
7 single gene's role, to generate reproducible results. Due to their short lifespan, inbred
8 laboratory rodents, particularly rats and mice (e.g. senescence accelerated mice – SAM), are
9 used as models to investigate the effects of intrinsic and extrinsic factors on lifespan (324).
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11 However, this is quite limited since these inbred models do not provide significant genetic
12 diversity to be comparable to humans and correlate poorly with human conditions. To date,
13 more than 150 clinical trial candidates to attenuate inflammation in critically ill patients have
14 failed due to over-reliance on inadequate animal models.
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23 We have addressed the conceptual translation of biochemical data collected from aging
24 female Wistar rats to further enlighten our understanding of the role of NAD⁺ metabolism
25 and other molecular changes occurring as part of 'normal' human ageing (51-53). Our
26 physiologically aged Wistar rats were an outbred model which displays significant genetic
27 diversity within a small number of individuals. This diversity-outcrossed rat model is more
28 representative of a natural population and is therefore a powerful tool in identifying the
29 genetic basis for assessing the efficacy of these pharmacological strategies, and identify
30 adverse effects in first-line therapeutic tests which are otherwise nascent in previously inbred
31 animals. Additional effects of aging previously demonstrated in this animal model include a
32 marked decrease in the astrocyte-neuronal ratio; altered pericyte-endothelial relations
33 affecting 'vessel stability'; marked inflammatory cascade, CNS neovascularization and
34 breakdown of the blood-retinal barrier, and a decline in defence-related Fos expression
35 (158,218).
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46 ***Methods of Detection***

47 NAD⁺ and its related metabolites have been previously measured using a variety of methods.
48 For instance, enzymatic and colorimetric assay which provide indirect measurements, have
49 inherent difficulties that can affect reliability and are susceptible to significant variation in
50 metabolite levels due to minor differences in temperature and pH, and cannot detect low
51 picomolar levels. Moreover, reverse phase HPLC, which relies on mobile phases containing
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3 buffer salts and ion pairing agents have been used to increase sensitivity, but are still limited
4 to low micromolar detection levels (70).
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7 In contrast, tandem LC-MS/MS allows more robust quantification of trace levels of NAD⁺
8 metabolites in different biological samples with high specificity and sensitivity. It represents
9 the gold standard in NAD⁺ metabolomics. However, unlike Nuclear Magnetic Resonance
10 (NMR), complex sampling processes are required (335). The diversity of NAD⁺ metabolites
11 (i.e. free bases, mono and di-nucleotides) makes their simultaneous differential analysis a
12 major challenge (335). We recently developed an improved method to quantify the NAD⁺
13 metabolome and adenosine phosphates across biological samples, including brain and
14 reproductive cells. Its principle features are enhanced resolution and simultaneous
15 quantification of 17 analytes on an amino phase column, avoiding the need for two separate
16 (i.e alkaline and acidic chromatographic gradients) (60).
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24 Development of non-destructive detection and quantification of the NAD⁺ metabolome is
25 desirable to elucidate intracellular NAD⁺ levels and redox state in the intact human and
26 animal body. Recently, a novel magnetic resonance imaging (MRI)-based has been
27 developed to determine the endogenous ³¹P MR signals of the NAD⁺ molecules in live
28 animal brains (389). This technique can resolve the MRI signal of NADH from that of
29 NAD⁺ by utilising specific spectroscopic characteristics at a given magnetic field strength.
30 This approach requires ultrahigh fields of 9.4 and 16.4 T. This non-invasive technique has
31 been further used to measure intracellular NAD⁺ and NADH contents and NAD⁺/NADH
32 redox state in healthy human brains using a 7-T human MR scanner (389). We were the first
33 to show that intracellular NAD⁺ levels, which is the essential substrate for sirtuin activity,
34 decline with age in humans and physiologically aged rats (51,52,223). MRI was used to
35 reaffirm these age-dependent increase of intracellular NADH and age-dependent reductions
36 in NAD⁺, total NAD contents, and NAD⁺/NADH redox potential of the healthy human
37 brains.
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48 It is anticipated that improvement in methods to quantify the NAD⁺ metabolome will be
49 developed to help standardise NAD⁺ research across different laboratories, and to overcome
50 challenges associated with translation of preclinical studies towards clinical practice.
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54 **Prospects of using NAD⁺ precursors in the clinic**

55 Pellagra, a syndrome caused by a diet deficient in either NA or L-tryptophan can lead to
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3 psychotic symptoms leading to pre-senile dementia likely due to upregulation of IDO, which
4 can deplete neurons of the essential amino acid, L-tryptophan causing neurodegeneration.
5 Administration of the NAD⁺ precursors, NA or NAM previously improved the neurological
6 state of dementia patients in the 1930's. Pharmacological doses of either NA or NAM have
7 also provided dramatic therapeutic benefits for other diseases, including, lipid
8 dyshomeostasis, rheumatoid arthritis, type I diabetes, colitis, multiple sclerosis (MS), and
9 schizophrenia in both animal models and in the clinical setting. Among these precursors, NA
10 appears to specifically activate the G-protein coupled receptor, GPR109, leading to the
11 release of the prostaglandins, PGE₂ and PGD₂ (314). These prostaglandins exert potent anti-
12 inflammatory effects through endogenous signalling mechanisms. While NAM can prevent
13 MS in animal models, it is also an inhibitor of sirtuins, and may therefore prove detrimental
14 on long-term cell survival and longevity (258,259).
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24 There is growing evidence suggesting that NAD⁺ administration may also reduce cellular
25 injury in multiple oxidative stress induced degenerative diseases. NAD⁺ treatment has been
26 shown to reduce PARP1-induced astrocyte death (7). PARP1 has been implicated in the
27 pathogenesis of several diseases including diabetes, Alzheimer's disease (AD) and
28 Parkinson's disease (PD) (196,221). Since supplementation with NAD⁺ can protect against
29 PARP1 mediated cell death, NAD⁺ administration may improve cell viability in these
30 diseases by at least partially ameliorating PARP1 toxicity. *In vitro* studies have shown that
31 NAD⁺ remains protective even when administered at 3-4 hours following PARP1 activation,
32 suggesting that NAD⁺ administration has a long window period for reducing cellular injury
33 (8). In addition, NAD⁺ may also improve cell viability by enhancing sirtuin activities and/or
34 improving energy metabolism.
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43 While the potential involvement of NAD⁺ metabolic pathways in energy metabolism and
44 mitochondrial function have been known for quite some time, suggestions of the involvement
45 of NAD⁺ in DNA repair and longevity have grown at a rapid rate in the last decade.
46 Characterisation of the NAD⁺ synthetic pathways has not only made these advancements
47 possible, but also contributed extensively to the understanding of the diverse roles of pyridine
48 nucleotides in cellular biology. Despite this, information regarding the fundamental roles of
49 NAD⁺ in neurodegeneration and ageing remains limited. Further investigations are necessary
50 in this increasingly relevant field.
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3 While the current review herein focussed on PARP1 in cellular degeneration, the role of other
4 PARPs such as tankyrases in cellular function remains largely unknown. Since NAD-
5 dependent tankyrases are primary mediators of telomerase activity it is highly likely that
6 NAD⁺ may also affect the aging process through regulation of tankyrase activity (385). It
7 would therefore be intriguing to study the effects of NAD⁺ precursors on tankyrases and
8 telomerases on certain biological functions, including neurogenesis, which might be relevant
9 in the aging brain.
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16 Additionally, NAD⁺ regulates diverse pathways which may control lifespan. The importance of
17 NAD⁺ is further underscored by recent work providing genetic evidence for the existence of
18 several pathways necessary for NAD⁺ synthesis. For example, the newly identified NAD⁺
19 precursor, NR has been shown to contribute to NAD⁺ synthesis by at least two unique pathways
20 in the yeast *Saccharomyces cerevisiae*, and can upregulate intracellular NAD⁺ levels in mice and
21 humans (338). Both pathways require the NAM ring for entry into the previously established
22 pathways for NAD⁺ synthesis. Future studies are required to address the importance of NR in
23 human health and disease, and whether it can be effectively used to replenish lowered NAD⁺
24 levels in age-related diseases, such as AD. Given the adverse effects associated with high-dose
25 use of NA and NAM, NR may represent an alternative precursor to enhance NAD⁺ levels.
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33 As well, changes in the NADH level, NAD⁺ redox potential and NAD⁺ levels are likely to
34 be present in other pathological conditions and may be associated with disease progression.
35 In particular, increased oxidative stress and immune activation in AD, PD, ADC and
36 Amyotrophic lateral sclerosis (ALS) may influence the available concentration of these
37 molecules. Future investigation into the metabolism and biological function of NAD⁺ in
38 these and other degenerative diseases may expose fundamental properties that may involve
39 the use of NAD⁺ precursors as adjunct therapy for treatment in these diseases, and perhaps
40 may help in slowing down the age-related disease process.
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48 Resveratrol is a polyphenol with major health benefits that is thought to operate through
49 direct activation of the 'anti-aging' enzyme SIRT1. However, recent reports have challenged
50 this 'direct-activation' hypothesis, suggesting that the mechanism by which resveratrol
51 increases SIRT1 function is still unknown (39,86). Previous work from our group has shown
52 for the first time that resveratrol induces a dose dependent increase in NMNAT-1 activity. As
53 SIRT1 requires NAD⁺ as a substrate to perform its gene silencing function, higher NAD⁺
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3 levels will enhance SIRT1 activity. This finding suggests that resveratrol may promote
4 SIRT1 function by enhancing NAD⁺ synthesis in whole cell systems without requiring direct
5 activation. Our observation that resveratrol increases NAD⁺ levels in primary human brain
6 cells by acting on NMNAT, together with the neuroprotective effects of green tea
7 polyphenols against QUIN-mediated excitotoxicity (47), supports the view that polyphenols
8 have considerable therapeutic potential, particularly for the treatment of neurodegenerative
9 diseases. As NMNAT can accelerate NAD⁺ synthesis from all six substrates, QUIN, NR,
10 NMN, NA, NAR and NAM, NMNAT activation by resveratrol may represent an ideal natural
11 therapeutic to replenish NAD⁺ levels. Maintenance of higher cellular NAD⁺ will enhance
12 SIRT1 activity and other NAD⁺-dependent pathways, impacting positively on cell viability
13 and longevity.
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22 Finally, increased NAMPT has been reported in a mice model of collagen-induced arthritis
23 both in the serum and in the arthritic paw (59). NAMPT inhibition reduced arthritic severity
24 comparable to etanercept, and significantly lowered the levels of cytokine release in affected
25 joints, and reduced intracellular NAD⁺ concentrations in inflammatory cells (59). Therefore,
26 NAMPT may play an important role during inflammatory diseases associated by cytokine
27 secretion from leukocytes. Therefore, increasing NAD⁺ levels may be deleterious in
28 inflammatory conditions and may exacerbate the disease due to increased NAMPT activity
29 and NAD⁺ use in immune cells. This represents an additional potential negative to 'one-size-
30 fits-all' use of NAD⁺.
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38 While most of the evidence reviewed in this paper strongly supports the current enthusiasm to
39 investigate and develop strategies for increasing NAD⁺ levels in conditions where NAD⁺
40 turnover is high and/or concentrations are reduced, the use of NAD⁺ enhancing therapeutics
41 in circumstances where cellular NAD⁺ levels are already adequate may be unwise. Given the
42 complexity of the biochemical systems affected by NAD⁺ and its associated metabolites a
43 simplistic, one size fits all, approach to NAD⁺ therapeutics will likely limit the true potential
44 of NAD⁺ treatment and may in fact cause harm under some circumstances. As well, while
45 several NAD⁺ precursors have been recently identified and examined in several models, a
46 side-by-side comparison of these precursors is nascent in current literature. It is anticipated
47 that these precursors may exhibit important differences in their effect in various pathological
48 disorders (375).
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3 To circumvent this, in addition to the many studies focussed on identifying efficient ways of
4 increasing NAD⁺, additional effort must be applied to the development of cost-effective
5 methods of measuring and correlating NAD⁺ levels in both tissue and extracellular fluids to
6 cellular and organ health in an effort to establish a clear understanding of what a 'healthy'
7 NAD⁺ level actually is. Armed with this knowledge the clinician may confidently apply
8 NAD⁺ therapy after an appropriate assessment of NAD⁺ levels to determine whether the
9 treatment is likely to be effective in each client's case.
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15 **Concluding remarks**

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17 NAD⁺ research has generated multiple discoveries in the last two decades. Identification of
18 the important role of NAD⁺ as a cofactor in cellular respiration and energy production was
19 followed by discoveries of numerous NAD⁺ biosynthesis pathways. In recent years, NAD⁺
20 has been shown to play a unique role in DNA repair and epigenetic control through protein
21 deacetylation. Elucidation of the pivotal roles played by NAD⁺ in linking the key
22 biochemical and cellular processes of oxidative stress and immune activation, energy
23 metabolism, epigenetic control and cell viability in degenerative disorders and ageing will
24 likely prove seminal to the advancement of effective therapeutics in degenerative diseases.
25 NAD⁺ remains the central molecule in the metabolism and functions of NAD⁺, NADH,
26 NADP⁺ and NADPH. Of these four molecules, only NAD⁺ can be synthesised *de novo* via
27 the kynurenine pathway, while the generation of NADH, NADP⁺ and NADPH requires
28 NAD⁺ as the original precursor. Maintenance of intracellular NAD⁺ levels is pivotal for the
29 regulation of DNA repair, stress resistance, and cell death, suggesting that NAD⁺ synthesis
30 through the kynurenine pathway and/or salvage pathway is an attractive target for therapeutic
31 intervention in age associated degenerative disorders. Agents such as NR, and to a lesser
32 degree, NA and NAM, have been shown to protect severed axons from degeneration in
33 animal models for Wallerian degeneration, and extend lifespan in small organisms. However,
34 further studies are necessary to clarify the conditions under which specific NAD⁺ precursors
35 should be used to efficiently promote intracellular NAD⁺ anabolism. This involves
36 evaluating the pharmacokinetics, safety, and efficacy in healthy and disease models to
37 develop targeted therapies that ameliorate degenerative processes and help maintain and
38 improve health span and longevity.
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While it will almost certainly be proved true that NAD⁺ therapy alone is not the mythical 'elixir of life', its foundational role in cellular energetics, nuclear signalling and viability suggest it just may be a key ingredient.

References

1. Public Health Reports, June 26, 1914. The etiology of pellagra. The significance of certain epidemiological observations with respect thereto. *Public Health Rep* 90: 373-5, 1975.
2. Abeti R, Duchon MR. Activation of PARP by oxidative stress induced by beta-amyloid: implications for Alzheimer's disease. *Neurochem Res* 37: 2589-96, 2012.
3. Agote M, Viaggi M, Kreimann E, Krawiec L, Dargosa, Juvenal GJ, Pisarev. Influence of nicotinamide on the radiosensitivity of normal and goitrous thyroid in the rat. *Thyroid* 11: 1003-7, 2001.
4. Agote Robertson M, Finochietto P, Gamba CA, Dargosa MA, Viaggi ME, Franco MC, Poderoso JJ, Juvenal GJ, Pisarev MA. Nicotinamide increases thyroid radiosensitivity by stimulating nitric oxide synthase expression and the generation of organic peroxides. *Horm Metab Res* 38: 12-5, 2006.
5. Aikawa H, Suzuki K. Lesions in the skin, intestine, and central nervous system induced by an antimetabolite of niacin. *Am J Pathol* 122: 335-42, 1986.
6. Aksoy S, Brandriff BF, Ward A, Little PF, Weinshilboum RM. Human nicotinamide N-methyltransferase gene: molecular cloning, structural characterization and chromosomal localization. *Genomics* 29: 555-61, 1995.
7. Alano CC, Garnier P, Ying W, Higashi Y, Kauppinen TM, Swanson RA. NAD⁺ depletion is necessary and sufficient for poly(ADP-ribose) polymerase-1-mediated neuronal death. *J Neurosci* 30: 2967-78, 2010.
8. Alano CC, Ying W, Swanson RA. Poly(ADP-ribose) polymerase-1-mediated cell death in astrocytes requires NAD⁺ depletion and mitochondrial permeability transition. *J Biol Chem* 279: 18895-902, 2004.
9. Alcendor RR, Gao S, Zhai P, Zablocki D, Holle E, Yu X, Tian B, Wagner T, Vatner SF, Sadoshima J. Sirt1 regulates aging and resistance to oxidative stress in the heart. *Circ Res* 100: 1512-21, 2007.
10. Alenzi FQ. Effect of nicotinamide on experimental induced diabetes. *Iran J Allergy Asthma Immunol* 8: 11-8, 2009.
11. Ali YO, McCormack R, Darr A, Zhai RG. Nicotinamide mononucleotide adenyltransferase is a stress response protein regulated by the heat shock factor/hypoxia-inducible factor 1alpha pathway. *J Biol Chem* 286: 19089-99, 2011.
12. Ali YO, Ruan K, Zhai RG. NMNAT suppresses tau-induced neurodegeneration by promoting clearance of hyperphosphorylated tau oligomers in a Drosophila model of tauopathy. *Hum Mol Genet* 21: 237-50, 2012.
13. Alrob OA, Sankaralingam S, Ma C, Wagg CS, Fillmore N, Jaswal JS, Sack MN, Lehner R, Gupta MP, Michelakis ED, Padwal RS, Johnstone DE, Sharma AM, Lopaschuk GD. Obesity-induced lysine acetylation increases cardiac fatty acid oxidation and impairs insulin signalling. *Cardiovasc Res* 103: 485-97, 2014.
14. Altmeyer M, Hottiger MO. Poly(ADP-ribose) polymerase 1 at the crossroad of metabolic stress and inflammation in aging. *Aging-Us* 1: 458-469, 2009.
15. Altmeyer M, Messner S, Hassa PO, Fey M, Hottiger MO. Molecular mechanism of poly(ADP-ribosylation) by PARP1 and identification of lysine residues as ADP-ribose acceptor sites. *Nucleic Acids Res* 37: 3723-38, 2009.

16. Anderson RM, Bitterman KJ, Wood JG, Medvedik O, Sinclair DA. Nicotinamide and PNC1 govern lifespan extension by calorie restriction in *Saccharomyces cerevisiae*. *Nature* 423: 181-5, 2003.
17. Andrabi SA, Umanah GKE, Chang C, Stevens DA, Karuppagounder SS, Gagne JP, Poirier GG, Dawson VL, Dawson TM. Poly(ADP-ribose) polymerase-dependent energy depletion occurs through inhibition of glycolysis. *Proceedings of the National Academy of Sciences of the United States of America* 111: 10209-10214, 2014.
18. Ansari A, Rahman MS, Saha SK, Saikot FK, Deep A, Kim KH. Function of the SIRT3 mitochondrial deacetylase in cellular physiology, cancer, and neurodegenerative disease. *Aging Cell* 16: 4-16, 2017.
19. ApSimon MM, Rawling JM, Kirkland JB. Nicotinamide megadosing increases hepatic poly(ADP-ribose) levels in choline-deficient rats. *J Nutr* 125: 1826-32, 1995.
20. Arpaia E, Benveniste P, Di Cristofano A, Gu Y, Dalal I, Kelly S, Hershfield M, Pandolfi PP, Roifman CM, Cohen A. Mitochondrial basis for immune deficiency. Evidence from purine nucleoside phosphorylase-deficient mice. *J Exp Med* 191: 2197-208, 2000.
21. Badawy AA. Pellagra and alcoholism: a biochemical perspective. *Alcohol Alcohol* 49: 238-50, 2014.
22. Badawy AA. Kynurenine Pathway of Tryptophan Metabolism: Regulatory and Functional Aspects. *Int J Tryptophan Res* 10: 1178646917691938, 2017.
23. Ball HJ, Yuasa HJ, Austin CJ, Weiser S, Hunt NH. Indoleamine 2,3-dioxygenase-2; a new enzyme in the kynurenine pathway. *Int J Biochem Cell Biol* 41: 467-71, 2009.
24. Balu M, Mazhar A, Hayakawa CK, Mittal R, Krasieva TB, Konig K, Venugopalan V, Tromberg BJ. In vivo multiphoton NADH fluorescence reveals depth-dependent keratinocyte metabolism in human skin. *Biophys J* 104: 258-67, 2013.
25. Bansal A, Kwon ES, Conte D, Jr., Liu H, Gilchrist MJ, MacNeil LT, Tissenbaum HA. Transcriptional regulation of *Caenorhabditis elegans* FOXO/DAF-16 modulates lifespan. *Longev Healthspan* 3: 5, 2014.
26. Bartleman AP, Jacobs R, Kirkland JB. Niacin supplementation decreases the incidence of alkylation-induced nonlymphocytic leukemia in Long-Evans rats. *Nutr Cancer* 60: 251-8, 2008.
27. Benavente CA, Jacobson EL. Niacin restriction upregulates NADPH oxidase and reactive oxygen species (ROS) in human keratinocytes. *Free Radic Biol Med* 44: 527-37, 2008.
28. Bender DA, McCreanor GM. Kynurenine hydroxylase: a potential rate-limiting enzyme in tryptophan metabolism. *Biochem Soc Trans* 13: 441-3, 1985.
29. Benfeitas R, Uhlen M, Nielsen J, Mardinoglu A. New Challenges to Study Heterogeneity in Cancer Redox Metabolism. *Front Cell Dev Biol* 5: 65, 2017.
30. Beninger RJ, Colton AM, Ingles JL, Jhamandas K, Boegman RJ. Picolinic acid blocks the neurotoxic but not the neuroexcitant properties of quinolinic acid in the rat brain: evidence from turning behaviour and tyrosine hydroxylase immunohistochemistry. *Neuroscience* 61: 603-12, 1994.
31. Berger F, Lau C, Dahlmann M, Ziegler M. Subcellular compartmentation and differential catalytic properties of the three human nicotinamide mononucleotide adenylyltransferase isoforms. *J Biol Chem* 280: 36334-41, 2005.
32. Berger F, Lau C, Ziegler M. Regulation of poly(ADP-ribose) polymerase 1 activity by the phosphorylation state of the nuclear NAD biosynthetic enzyme NMN adenylyl transferase 1. *Proc Natl Acad Sci U S A* 104: 3765-70, 2007.
33. Bhatia R, Calvo KC. The sequencing expression, purification, and steady-state kinetic analysis of quinolinate phosphoribosyl transferase from *Escherichia coli*. *Arch Biochem Biophys* 325: 270-8, 1996.

- 1
 - 2
 - 3
 - 4
 - 5
 - 6
 - 7
 - 8
 - 9
 - 10
 - 11
 - 12
 - 13
 - 14
 - 15
 - 16
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 - 46
 - 47
 - 48
 - 49
 - 50
 - 51
 - 52
 - 53
 - 54
 - 55
 - 56
 - 57
 - 58
 - 59
 - 60
34. Birkmayer JG, Nadlinger K. Safety of stabilized, orally absorbable, reduced nicotinamide adenine dinucleotide (NADH): a 26-week oral tablet administration of ENADA/NADH for chronic toxicity study in rats. *Drugs Exp Clin Res* 28: 185-92, 2002.
35. Birkmayer JG, Vrecko C, Volc D, Birkmayer W. Nicotinamide adenine dinucleotide (NADH)--a new therapeutic approach to Parkinson's disease. Comparison of oral and parenteral application. *Acta Neurol Scand Suppl* 146: 32-5, 1993.
36. Blagosklonny MV. Hormesis does not make sense except in the light of TOR-driven aging. *Aging (Albany NY)* 3: 1051-62, 2011.
37. Blum CA, Ellis JL, Loh C, Ng PY, Perni RB, Stein RL. SIRT1 modulation as a novel approach to the treatment of diseases of aging. *J Med Chem* 54: 417-32, 2011.
38. Bogan KL, Brenner C. Nicotinic acid, nicotinamide, and nicotinamide riboside: a molecular evaluation of NAD+ precursor vitamins in human nutrition. *Annu Rev Nutr* 28: 115-30, 2008.
39. Borra MT, Smith BC, Denu JM. Mechanism of human SIRT1 activation by resveratrol. *J Biol Chem* 280: 17187-95, 2005.
40. Bouamama S, Merzouk H, Medjdoub A, Merzouk-Saidi A, Merzouk SA. Effects of exogenous vitamins A, C, and E and NADH supplementation on proliferation, cytokines release, and cell redox status of lymphocytes from healthy aged subjects. *Appl Physiol Nutr Metab* 42: 579-587, 2017.
41. Boylston JA, Sun J, Chen Y, Gucek M, Sack MN, Murphy E. Characterization of the cardiac succinylome and its role in ischemia-reperfusion injury. *J Mol Cell Cardiol* 88: 73-81, 2015.
42. Boyonoski AC, Spronck JC, Gallacher LM, Jacobs RM, Shah GM, Poirier GG, Kirkland JB. Niacin deficiency decreases bone marrow poly(ADP-ribose) and the latency of ethylnitrosourea-induced carcinogenesis in rats. *J Nutr* 132: 108-14, 2002.
43. Boyonoski AC, Spronck JC, Jacobs RM, Shah GM, Poirier GG, Kirkland JB. Pharmacological intakes of niacin increase bone marrow poly(ADP-ribose) and the latency of ethylnitrosourea-induced carcinogenesis in rats. *J Nutr* 132: 115-20, 2002.
44. Braidy N, Brew BJ, Inestrosa NC, Chung R, Sachdev P, Guillemin GJ. Changes in Cathepsin D and Beclin-1 mRNA and protein expression by the excitotoxin quinolinic acid in human astrocytes and neurons. *Metab Brain Dis* 29: 873-83, 2014.
45. Braidy N, Grant R. Kynurenine pathway metabolism and neuroinflammatory disease. *Neural Regen Res* 12: 39-42, 2017.
46. Braidy N, Grant R, Adams S, Brew BJ, Guillemin GJ. Mechanism for quinolinic acid cytotoxicity in human astrocytes and neurons. *Neurotox Res* 16: 77-86, 2009.
47. Braidy N, Grant R, Adams S, Guillemin GJ. Neuroprotective effects of naturally occurring polyphenols on quinolinic acid-induced excitotoxicity in human neurons. *FEBS J* 277: 368-82, 2010.
48. Braidy N, Grant R, Brew BJ, Adams S, Jayasena T, Guillemin GJ. Effects of Kynurenine Pathway Metabolites on Intracellular NAD Synthesis and Cell Death in Human Primary Astrocytes and Neurons. *Int J Tryptophan Res* 2: 61-9, 2009.
49. Braidy N, Guillemin G, Grant R. Promotion of cellular NAD(+) anabolism: therapeutic potential for oxidative stress in ageing and Alzheimer's disease. *Neurotox Res* 13: 173-84, 2008.
50. Braidy N, Guillemin GJ, Grant R. Effects of Kynurenine Pathway Inhibition on NAD Metabolism and Cell Viability in Human Primary Astrocytes and Neurons. *Int J Tryptophan Res* 4: 29-37, 2011.
51. Braidy N, Guillemin GJ, Mansour H, Chan-Ling T, Poljak A, Grant R. Age related changes in NAD+ metabolism oxidative stress and Sirt1 activity in wistar rats. *PLoS One* 6: e19194, 2011.
52. Braidy N, Poljak A, Grant R, Jayasena T, Mansour H, Chan-Ling T, Guillemin GJ, Smythe G, Sachdev P. Mapping NAD(+) metabolism in the brain of ageing Wistar rats: potential targets for influencing brain senescence. *Biogerontology* 15: 177-98, 2014.

- 1
- 2
- 3 53. Braidy N, Poljak A, Grant R, Jayasena T, Mansour H, Chan-Ling T, Smythe G, Sachdev P,
4 Guillemin GJ. Differential expression of sirtuins in the aging rat brain. *Front Cell Neurosci* 9:
5 167, 2015.
- 6 54. Brazill JM, Li C, Zhu Y, Zhai RG. NMNAT: It's an NAD⁺ synthase... It's a chaperone... It's a
7 neuroprotector. *Curr Opin Genet Dev* 44: 156-162, 2017.
- 8 55. Brousseau KM, Filley CM, Kaye K, Kiser JJ, Adler LE, Connick E. Dementia with features of
9 Alzheimer's disease and HIV-associated dementia in an elderly man with AIDS. *AIDS* 23:
10 1029-31, 2009.
- 11 56. Brown KD, Maqsood S, Huang JY, Pan Y, Harkcom W, Li W, Sauve A, Verdin E, Jaffrey SR.
12 Activation of SIRT3 by the NAD(+) precursor nicotinamide riboside protects from noise-
13 induced hearing loss. *Cell Metab* 20: 1059-68, 2014.
- 14 57. Brunet A, Sweeney LB, Sturgill JF, Chua KF, Greer PL, Lin Y, Tran H, Ross SE, Mostoslavsky R,
15 Cohen HY, Hu LS, Cheng HL, Jedrychowski MP, Gygi SP, Sinclair DA, Alt FW, Greenberg ME.
16 Stress-dependent regulation of FOXO transcription factors by the SIRT1 deacetylase. *Science*
17 303: 2011-5, 2004.
- 18 58. Busse M, Hettler V, Fischer V, Mawrin C, Hartig R, Dobrowolny H, Bogerts B, Frodl T, Busse S.
19 Increased quinolinic acid in peripheral mononuclear cells in Alzheimer's dementia. *Eur Arch*
20 *Psychiatry Clin Neurosci*, 2017.
- 21 59. Busso N, Karababa M, Nobile M, Rolaz A, Van Gool F, Galli M, Leo O, So A, De Smedt T.
22 Pharmacological inhibition of nicotinamide phosphoribosyltransferase/Visfatin enzymatic
23 activity identifies a new inflammatory pathway linked to NAD. *PLoS One* 3: e2267, 2008.
- 24 60. Bustamante S, Jayasena T, Richani D, Gilchrist RB, Wu LE, Sinclair DA, Sachdev P, Braidy N.
25 Quantifying the cellular NAD⁺ metabolome using a tandem liquid chromatography mass
26 spectrometry approach. 14, 2018.
- 27 61. Cadenas C, Franckenstein D, Schmidt M, Gehrman M, Hermes M, Geppert B, Schormann W,
28 Maccoux LJ, Schug M, Schumann A, Wilhelm C, Freis E, Ickstadt K, Rahnenfuhrer J, Baumbach
29 JI, Sickmann A, Hengstler JG. Role of thioredoxin reductase 1 and thioredoxin interacting
30 protein in prognosis of breast cancer. *Breast Cancer Res* 12: R44, 2010.
- 31 62. Calabrese EJ. Hormesis: a fundamental concept in biology. *Microb Cell* 1: 145-149, 2014.
- 32 63. Calabrese EJ, Mattson MP. How does hormesis impact biology, toxicology, and medicine?
33 *NPJ Aging Mech Dis* 3: 13, 2017.
- 34 64. Calabrese EJ, Mattson MP, Calabrese V. Resveratrol commonly displays hormesis:
35 occurrence and biomedical significance. *Hum Exp Toxicol* 29: 980-1015, 2010.
- 36 65. Camacho-Pereira J, Tarrago MG, Chini CCS, Nin V, Escande C, Warner GM, Puranik AS,
37 Schoon RA, Reid JM, Galina A, Chini EN. CD38 Dictates Age-Related NAD Decline and
38 Mitochondrial Dysfunction through an SIRT3-Dependent Mechanism. *Cell Metabolism* 23:
39 1127-1139, 2016.
- 40 66. Canto C, Gerhart-Hines Z, Feige JN, Lagouge M, Noriega L, Milne JC, Elliott PJ, Puigserver P,
41 Auwerx J. AMPK regulates energy expenditure by modulating NAD⁺ metabolism and SIRT1
42 activity. *Nature* 458: 1056-60, 2009.
- 43 67. Canto C, Houtkooper RH, Pirinen E, Youn DY, Oosterveer MH, Cen Y, Fernandez-Marcos PJ,
44 Yamamoto H, Andreux PA, Cettour-Rose P, Gademann K, Rinsch C, Schoonjans K, Sauve AA,
45 Auwerx J. The NAD(+) precursor nicotinamide riboside enhances oxidative metabolism and
46 protects against high-fat diet-induced obesity. *Cell Metab* 15: 838-47, 2012.
- 47 68. Canto C, Menzies KJ, Auwerx J. NAD(+) Metabolism and the Control of Energy Homeostasis:
48 A Balancing Act between Mitochondria and the Nucleus. *Cell Metab* 22: 31-53, 2015.
- 49 69. Cao W, Hong Y, Chen H, Wu F, Wei X, Ying W. SIRT2 mediates NADH-induced increases in
50 Nrf2, GCL, and glutathione by modulating Akt phosphorylation in PC12 cells. *FEBS Lett* 590:
51 2241-55, 2016.
- 52 70. Casabona G, Sturiale L, L'Episcopo MR, Raciti G, Fazio A, Sarpietro MG, Genazzani AA,
53 Cambria A, Nicoletti F. HPLC analysis of cyclic adenosine diphosphate ribose and adenosine
54
55
56
57
58
59

- diphosphate ribose: determination of NAD⁺ metabolites in hippocampal membranes. *Ital J Biochem* 44: 258-68, 1995.
71. Castellano-Gonzalez G, Pichaud N, Ballard JW, Bessede A, Marcal H, Guillemin GJ. Epigallocatechin-3-gallate induces oxidative phosphorylation by activating cytochrome c oxidase in human cultured neurons and astrocytes. *Oncotarget* 7: 7426-40, 2016.
72. Catz P, Shinn W, Kapetanovic IM, Kim H, Kim M, Jacobson EL, Jacobson MK, Green CE. Simultaneous determination of myristyl nicotinate, nicotinic acid, and nicotinamide in rabbit plasma by liquid chromatography-tandem mass spectrometry using methyl ethyl ketone as a deproteinization solvent. *J Chromatogr B Analyt Technol Biomed Life Sci* 829: 123-35, 2005.
73. Chambers DA, Martin DW, Jr., Weinstein Y. The effect of cyclic nucleotides on purine biosynthesis and the induction of PRPP synthetase during lymphocyte activation. *Cell* 3: 375-80, 1974.
74. Chapman MJ, Le Goff W, Guerin M, Kontush A. Cholesteryl ester transfer protein: at the heart of the action of lipid-modulating therapy with statins, fibrates, niacin, and cholesteryl ester transfer protein inhibitors. *Eur Heart J* 31: 149-64, 2010.
75. Chaudhuri RN, Chakravarti H. An outbreak of pellegra syndrome in a rural area of Bengal. *Ind Med Gaz* 82: 657-60, 1947.
76. Chini EN. CD38 as a regulator of cellular NAD: a novel potential pharmacological target for metabolic conditions. *Curr Pharm Des* 15: 57-63, 2009.
77. Chini EN. CD38 as a Regulator of Cellular NAD: A Novel Potential Pharmacological Target for Metabolic Conditions. *Current Pharmaceutical Design* 15: 57-63, 2009.
78. Clementi E, Nisoli E. Nitric oxide and mitochondrial biogenesis: a key to long-term regulation of cellular metabolism. *Comp Biochem Physiol A Mol Integr Physiol* 142: 102-10, 2005.
79. Cockhill J, Jhamandas K, Boegman RJ, Beninger RJ. Action of picolinic acid and structurally related pyridine carboxylic acids on quinolinic acid-induced cortical cholinergic damage. *Brain Res* 599: 57-63, 1992.
80. Coleman MP, Freeman MR. Wallerian degeneration, wld(s), and nmnat. *Annu Rev Neurosci* 33: 245-67, 2010.
81. Collins PB, Chaykin S. The management of nicotinamide and nicotinic acid in the mouse. *J Biol Chem* 247: 778-83, 1972.
82. Colman RJ, Anderson RM, Johnson SC, Kastman EK, Kosmatka KJ, Beasley TM, Allison DB, Cruzen C, Simmons HA, Kemnitz JW, Weindruch R. Caloric restriction delays disease onset and mortality in rhesus monkeys. *Science* 325: 201-4, 2009.
83. Colman RJ, Beasley TM, Kemnitz JW, Johnson SC, Weindruch R, Anderson RM. Caloric restriction reduces age-related and all-cause mortality in rhesus monkeys. *Nat Commun* 5: 3557, 2014.
84. Cooper DL, Murrell DE, Roane DS, Harirforoosh S. Effects of formulation design on niacin therapeutics: mechanism of action, metabolism, and drug delivery. *Int J Pharm* 490: 55-64, 2015.
85. Dantzer F, Ame JC, Schreiber V, Nakamura J, Menissier-de Murcia J, de Murcia G. Poly(ADP-ribose) polymerase-1 activation during DNA damage and repair. *Methods Enzymol* 409: 493-510, 2006.
86. Dasgupta B, Milbrandt J. Resveratrol stimulates AMP kinase activity in neurons. *Proc Natl Acad Sci U S A* 104: 7217-22, 2007.
87. de Picciotto NE, Gano LB, Johnson LC, Martens CR, Sindler AL, Mills KF, Imai S, Seals DR. Nicotinamide mononucleotide supplementation reverses vascular dysfunction and oxidative stress with aging in mice. *Aging Cell* 15: 522-30, 2016.
88. Dellinger RW, Santos SR, Morris M, Evans M, Alminana D, Guarente L, Marcotulli E. Repeat dose NRPT (nicotinamide riboside and pterostilbene) increases NAD(+) levels in humans safely and sustainably: a randomized, double-blind, placebo-controlled study. *NPJ Aging Mech Dis* 3: 17, 2017.

- 1
2
3 89. Di Lisa F, Ziegler M. Pathophysiological relevance of mitochondria in NAD(+) metabolism. *FEBS Lett* 492: 4-8, 2001.
- 4 90. Di Stefano M, Conforti L. Diversification of NAD biological role : the importance of location. *Febs Journal* 280: 4711-4728, 2013.
- 5
6 91. Diamond MP, Fletcher NM, Neubauer BR, Saed MG, H MA, Saed GM. Hypoxia-Induced
7 Genotype Switch in Nicotinamide Adenine Dinucleotide Phosphate (NADPH) Oxidase
8 Through the Up-Regulation of Cytidine Deaminase Regulates Postoperative Adhesion
9 Development. *J Minim Invasive Gynecol* 22: S159, 2015.
- 10 92. Dioum EM, Chen R, Alexander MS, Zhang Q, Hogg RT, Gerard RD, Garcia JA. Regulation of
11 hypoxia-inducible factor 2alpha signaling by the stress-responsive deacetylase sirtuin 1.
12 *Science* 324: 1289-93, 2009.
- 13 93. Dragovic J, Kim SH, Brown SL, Kim JH. Nicotinamide pharmacokinetics in patients. *Radiother
14 Oncol* 36: 225-8, 1995.
- 15 94. Du X, Matsumura T, Edelstein D, Rossetti L, Zsengeller Z, Szabo C, Brownlee M. Inhibition of
16 GAPDH activity by poly(ADP-ribose) polymerase activates three major pathways of
17 hyperglycemic damage in endothelial cells. *J Clin Invest* 112: 1049-57, 2003.
- 18 95. Duarte-Pereira S, Pereira-Castro I, Silva SS, Correia MG, Neto C, da Costa LT, Amorim A, Silva
19 RM. Extensive regulation of nicotinate phosphoribosyltransferase (NAPRT) expression in
20 human tissues and tumors. *Oncotarget* 7: 1973-83, 2016.
- 21 96. Duley JA, Christodoulou J, de Brouwer AP. The PRPP synthetase spectrum: what does it
22 demonstrate about nucleotide syndromes? *Nucleosides Nucleotides Nucleic Acids* 30: 1129-
23 39, 2011.
- 24 97. Easlson E, Tsang F, Skinner C, Wang C, Lin SJ. The malate-aspartate NADH shuttle components
25 are novel metabolic longevity regulators required for calorie restriction-mediated life span
26 extension in yeast. *Genes Dev* 22: 931-44, 2008.
- 27 98. Easton T, Richardson AJ, Hall AD, Kidane L, Das I, Puri BK. The niacin flush test as an index of
28 fatty acid deficiency in schizophrenia. *Schizophrenia Research* 36: 307-308, 1999.
- 29 99. Elvehjem CA, Madden RJ, Strong FM, Wolley DW. The isolation and identification of the anti-
30 black tongue factor. 1937. *J Biol Chem* 277: e22, 2002.
- 31 100. Elvehjem CA, Madden RJ, Strong FM, Woolley DW. The isolation and identification of the
32 anti-black tongue factor. *Nutr Rev* 32: 48-50, 1974.
- 33 101. Fan J, Krautkramer KA, Feldman JL, Denu JM. Metabolic regulation of histone post-
34 translational modifications. *ACS Chem Biol* 10: 95-108, 2015.
- 35 102. Fang EF, Lautrup S, Hou Y, Demarest TG, Croteau DL, Mattson MP, Bohr VA. NAD(+) in Aging:
36 Molecular Mechanisms and Translational Implications. *Trends Mol Med* 23: 899-916, 2017.
- 37 103. Fatokun AA, Hunt NH, Ball HJ. Indoleamine 2,3-dioxygenase 2 (IDO2) and the kynurenine
38 pathway: characteristics and potential roles in health and disease. *Amino Acids* 45: 1319-29,
39 2013.
- 40 104. Fazio F, Carrizzo A, Lionetto L, Damato A, Capocci L, Ambrosio M, Battaglia G, Bruno V,
41 Madonna M, Simmaco M, Nicoletti F, Vecchione C. Vasorelaxing Action of the Kynurenine
42 Metabolite, Xanthurenic Acid: The Missing Link in Endotoxin-Induced Hypotension? *Front
43 Pharmacol* 8: 214, 2017.
- 44 105. Fedele G, Frasca L, Palazzo R, Ferrero E, Malavasi F, Ausiello CM. CD38 is expressed on
45 human mature monocyte-derived dendritic cells and is functionally involved in CD83
46 expression and IL-12 induction. *Eur J Immunol* 34: 1342-50, 2004.
- 47 106. Fernandez-Pol JA. Transition metal ions induce cell growth in NRK cells synchronized in G1 by
48 picolinic acid. *Biochem Biophys Res Commun* 76: 413-9, 1976.
- 49 107. Fu CS, Swendseid ME, Jacob RA, McKee RW. Biochemical markers for assessment of niacin
50 status in young men: levels of erythrocyte niacin coenzymes and plasma tryptophan. *J Nutr*
51 119: 1949-55, 1989.
- 52
53
54
55
56
57
58
59
60

- 1
2
3 108. Fukui M, Choi HJ, Zhu BT. Mechanism for the protective effect of resveratrol against
4 oxidative stress-induced neuronal death. *Free Radic Biol Med* 49: 800-13, 2010.
- 5 109. Fukunaga M, Yamamoto Y, Kawasoe M, Arioka Y, Murakami Y, Hoshi M, Saito K. Studies on
6 tissue and cellular distribution of indoleamine 2,3-dioxygenase 2: the absence of IDO1
7 upregulates IDO2 expression in the epididymis. *J Histochem Cytochem* 60: 854-60, 2012.
- 8 110. Fulco M, Cen Y, Zhao P, Hoffman EP, McBurney MW, Sauve AA, Sartorelli V. Glucose
9 restriction inhibits skeletal myoblast differentiation by activating SIRT1 through AMPK-
10 mediated regulation of Nampt. *Developmental Cell* 14: 661-673, 2008.
- 11 111. Gaertner FH, Shetty AS. Kynureninase-type enzymes and the evolution of the aerobic
12 tryptophan-to-nicotinamide adenine dinucleotide pathway. *Biochim Biophys Acta* 482: 453-
13 60, 1977.
- 14 112. Galassi L, Di Stefano M, Brunetti L, Orsomando G, Amici A, Ruggieri S, Magni G.
15 Characterization of human nicotinate phosphoribosyltransferase: Kinetic studies, structure
16 prediction and functional analysis by site-directed mutagenesis. *Biochimie* 94: 300-9, 2012.
- 17 113. Gale EA, Bingley PJ, Emmett CL, Collier T, European Nicotinamide Diabetes Intervention Trial
18 G. European Nicotinamide Diabetes Intervention Trial (ENDIT): a randomised controlled trial
19 of intervention before the onset of type 1 diabetes. *Lancet* 363: 925-31, 2004.
- 20 114. Ganji SH, Qin S, Zhang L, Kamanna VS, Kashyap ML. Niacin inhibits vascular oxidative stress,
21 redox-sensitive genes, and monocyte adhesion to human aortic endothelial cells.
22 *Atherosclerosis* 202: 68-75, 2009.
- 23 115. Gensler HL. Prevention of photoimmunosuppression and photocarcinogenesis by topical
24 nicotinamide. *Nutr Cancer* 29: 157-62, 1997.
- 25 116. Gerhart-Hines Z, Rodgers JT, Bare O, Lerin C, Kim SH, Mostoslavsky R, Alt FW, Wu Z,
26 Puigserver P. Metabolic control of muscle mitochondrial function and fatty acid oxidation
27 through SIRT1/PGC-1alpha. *EMBO J* 26: 1913-23, 2007.
- 28 117. Gillman J, Gillman T. Malnutrition and pellegra in South Africa. *Nutr Rev* 5: 353-5, 1947.
- 29 118. Godoy JA, Rios JA, Zolezzi JM, Braidy N, Inestrosa NC. Signaling pathway cross talk in
30 Alzheimer's disease. *Cell Commun Signal* 12: 23, 2014.
- 31 119. Godoy JA, Zolezzi JM, Braidy N, Inestrosa NC. Role of Sirt1 during the ageing process:
32 relevance to protection of synapses in the brain. *Mol Neurobiol* 50: 744-56, 2014.
- 33 120. Godoy JA, Zolezzi JM, Braidy N, Inestrosa NC. Role of Sirt1 During the Ageing Process:
34 Relevance to Protection of Synapses in the Brain. *Mol Neurobiol*, 2014.
- 35 121. Goldberger J. The etiology of pellagra. 1914. *Public Health Rep* 121 Suppl 1: 77-9; discussion
36 76, 2006.
- 37 122. Gonzalez Esquivel D, Ramirez-Ortega D, Pineda B, Castro N, Rios C, Perez de la Cruz V.
38 Kynurenine pathway metabolites and enzymes involved in redox reactions.
39 *Neuropharmacology* 112: 331-345, 2017.
- 40 123. Gorrini C, Harris IS, Mak TW. Modulation of oxidative stress as an anticancer strategy.
41 *Nature Reviews Drug Discovery* 12: 931-947, 2013.
- 42 124. Gospe SM, Jr., Olin KL, Keen CL. Reduced GABA synthesis in pyridoxine-dependent seizures.
43 *Lancet* 343: 1133-4, 1994.
- 44 125. Goto T, Matsuo N, Takahashi T. CSF glutamate/GABA concentrations in pyridoxine-
45 dependent seizures: etiology of pyridoxine-dependent seizures and the mechanisms of
46 pyridoxine action in seizure control. *Brain Dev* 23: 24-9, 2001.
- 47 126. Gougeon L, Payette H, Morais JA, Gaudreau P, Shatenstein B, Gray-Donald K. Intakes of
48 folate, vitamin B6 and B12 and risk of depression in community-dwelling older adults: the
49 Quebec Longitudinal Study on Nutrition and Aging. *Eur J Clin Nutr* 70: 380-5, 2016.
- 50 127. Grant RS, Kapoor V. Murine glial cells regenerate NAD, after peroxide-induced depletion,
51 using either nicotinic acid, nicotinamide, or quinolinic acid as substrates. *J Neurochem* 70:
52 1759-63, 1998.
- 53
54
55
56
57
58
59
60

- 1
2
3 128. Grant RS, Passey R, Matanovic G, Smythe G, Kapoor V. Evidence for increased de novo
4 synthesis of NAD in immune-activated RAW264.7 macrophages: a self-protective
5 mechanism? *Arch Biochem Biophys* 372: 1-7, 1999.
- 6 129. Guan X, Lin P, Knoll E, Chakrabarti R. Mechanism of inhibition of the human sirtuin enzyme
7 SIRT3 by nicotinamide: computational and experimental studies. *PLoS One* 9: e107729, 2014.
- 8 130. Guan XH, Hong X, Zhao N, Liu XH, Xiao YF, Chen TT, Deng LB, Wang XL, Wang JB, Ji GJ, Fu M,
9 Deng KY, Xin HB. CD38 promotes angiotensin II-induced cardiac hypertrophy. *J Cell Mol Med*
10 21: 1492-1502, 2017.
- 11 131. Guan XH, Liu XH, Hong X, Zhao N, Xiao YF, Wang LF, Tang L, Jiang K, Qian YS, Deng KY, Ji GJ,
12 Fu MG, Xin HB. CD38 Deficiency Protects the Heart from Ischemia/Reperfusion Injury
13 through Activating SIRT1/FOXOs-Mediated Antioxidative Stress Pathway. *Oxidative Medicine*
14 *and Cellular Longevity*, 2016.
- 15 132. Guillemin GJ, Brew BJ. Implications of the kynurenine pathway and quinolinic acid in
16 Alzheimer's disease. *Redox Rep* 7: 199-206, 2002.
- 17 133. Guyton JR. Niacin in cardiovascular prevention: mechanisms, efficacy, and safety. *Curr Opin*
18 *Lipidol* 18: 415-20, 2007.
- 19 134. Ha C, Miller LT, Kerkvliet NI. The effect of vitamin B6 deficiency on cytotoxic immune
20 responses of T cells, antibodies, and natural killer cells, and phagocytosis by macrophages.
21 *Cell Immunol* 85: 318-29, 1984.
- 22 135. Haag F, Adriouch S, Brass A, Jung C, Moller S, Scheuplein F, Bannas P, Seman M, Koch-Nolte
23 F. Extracellular NAD and ATP: Partners in immune cell modulation. *Purinergic Signal* 3: 71-81,
24 2007.
- 25 136. Haigis MC, Mostoslavsky R, Haigis KM, Fahie K, Christodoulou DC, Murphy AJ, Valenzuela
26 DM, Yancopoulos GD, Karow M, Blander G, Wolberger C, Prolla TA, Weindruch R, Alt FW,
27 Guarente L. SIRT4 inhibits glutamate dehydrogenase and opposes the effects of calorie
28 restriction in pancreatic beta cells. *Cell* 126: 941-54, 2006.
- 29 137. Hallows WC, Yu W, Smith BC, Devries MK, Ellinger JJ, Someya S, Shortreed MR, Prolla T,
30 Markley JL, Smith LM, Zhao S, Guan KL, Denu JM. Sirt3 promotes the urea cycle and fatty
31 acid oxidation during dietary restriction. *Mol Cell* 41: 139-49, 2011.
- 32 138. Hammen PK, Allali-Hassani A, Hallenga K, Hurley TD, Weiner H. Multiple conformations of
33 NAD and NADH when bound to human cytosolic and mitochondrial aldehyde
34 dehydrogenase. *Biochemistry* 41: 7156-68, 2002.
- 35 139. Han MK, Song EK, Guo Y, Ou X, Mantel C, Broxmeyer HE. SIRT1 regulates apoptosis and
36 Nanog expression in mouse embryonic stem cells by controlling p53 subcellular localization.
37 *Cell Stem Cell* 2: 241-51, 2008.
- 38 140. Han Q, Robinson H, Li J. Biochemical identification and crystal structure of kynurenine
39 formamidase from *Drosophila melanogaster*. *Biochem J* 446: 253-60, 2012.
- 40 141. Hansson O. Tryptophan load test and pyridoxine treatment in epileptic children. *Acta Neurol*
41 *Scand* 43: Suppl 31:65, 1967.
- 42 142. Hara N, Yamada K, Shibata T, Osago H, Hashimoto T, Tsuchiya M. Elevation of cellular NAD
43 levels by nicotinic acid and involvement of nicotinic acid phosphoribosyltransferase in
44 human cells. *J Biol Chem* 282: 24574-82, 2007.
- 45 143. Harkcom WT, Ghosh AK, Sung MS, Matov A, Brown KD, Giannakakou P, Jaffrey SR. NAD⁺ and
46 SIRT3 control microtubule dynamics and reduce susceptibility to antimicrotubule agents.
47 *Proc Natl Acad Sci U S A* 111: E2443-52, 2014.
- 48 144. Hasegawa K, Wakino S, Yoshioka K, Tatematsu S, Hara Y, Minakuchi H, Washida N, Tokuyama
49 H, Hayashi K, Itoh H. Sirt1 protects against oxidative stress-induced renal tubular cell
50 apoptosis by the bidirectional regulation of catalase expression. *Biochem Biophys Res*
51 *Commun* 372: 51-6, 2008.
- 52
53
54
55
56
57
58
59
60

- 1
2
3 145. Hassa PO, Haenni SS, Elser M, Hottiger MO. Nuclear ADP-ribosylation reactions in
4 mammalian cells: where are we today and where are we going? *Microbiol Mol Biol Rev* 70:
5 789-829, 2006.
- 6 146. He W, Wang Y, Zhang MZ, You L, Davis LS, Fan H, Yang HC, Fogo AB, Zent R, Harris RC, Breyer
7 MD, Hao CM. Sirt1 activation protects the mouse renal medulla from oxidative injury. *J Clin*
8 *Invest* 120: 1056-68, 2010.
- 9 147. Hegab Z, Mohamed TMA, Stafford N, Mamas M, Cartwright EJ, Oceandy D. Advanced
10 glycation end products reduce the calcium transient in cardiomyocytes by increasing
11 production of reactive oxygen species and nitric oxide. *FEBS Open Bio* 7: 1672-1685, 2017.
- 12 148. Hegyi J, Schwartz RA, Hegyi V. Pellagra: dermatitis, dementia, and diarrhea. *Int J Dermatol*
13 43: 1-5, 2004.
- 14 149. Herrero-Yraola A, Bakhit SM, Franke P, Weise C, Schweiger M, Jorcke D, Ziegler M.
15 Regulation of glutamate dehydrogenase by reversible ADP-ribosylation in mitochondria.
16 *EMBO J* 20: 2404-12, 2001.
- 17 150. Hershberger KA, Martin AS, Hirschey MD. Role of NAD(+) and mitochondrial sirtuins in
18 cardiac and renal diseases. *Nat Rev Nephrol* 13: 213-225, 2017.
- 19 151. Hirschey MD, Shimazu T, Goetzman E, Jing E, Schwer B, Lombard DB, Grueter CA, Harris C,
20 Biddinger S, Ilkayeva OR, Stevens RD, Li Y, Saha AK, Ruderman NB, Bain JR, Newgard CB,
21 Farese RV, Jr., Alt FW, Kahn CR, Verdin E. SIRT3 regulates mitochondrial fatty-acid oxidation
22 by reversible enzyme deacetylation. *Nature* 464: 121-5, 2010.
- 23 152. Hong S, Moreno-Navarrete JM, Wei X, Kikukawa Y, Tzamelis I, Prasad D, Lee Y, Asara JM,
24 Fernandez-Real JM, Maratos-Flier E, Pissios P. Nicotinamide N-methyltransferase regulates
25 hepatic nutrient metabolism through Sirt1 protein stabilization. *Nat Med* 21: 887-94, 2015.
- 26 153. Hong SJ, Dawson TM, Dawson VL. Nuclear and mitochondrial conversations in cell death:
27 PARP-1 and AIF signaling. *Trends Pharmacol Sci* 25: 259-64, 2004.
- 28 154. Hopkins FG, Cole SW. A contribution to the chemistry of proteids: Part I. A preliminary study
29 of a hitherto undescribed product of tryptic digestion. *J Physiol* 27: 418-28, 1901.
- 30 155. Horenstein AL, Sizzano F, Lusso R, Besso FG, Ferrero E, Deaglio S, Corno F, Malavasi F. CD38
31 and CD157 ectoenzymes mark cell subsets in the human corneal limbus. *Mol Med* 15: 76-84,
32 2009.
- 33 156. Horton JL, Martin OJ, Lai L, Riley NM, Richards AL, Vega RB, Leone TC, Pagliarini DJ, Muoio
34 DM, Bedi KC, Jr., Margulies KB, Coon JJ, Kelly DP. Mitochondrial protein hyperacetylation in
35 the failing heart. *JCI Insight* 2, 2016.
- 36 157. Houtkooper RH, Canto C, Wanders RJ, Auwerx J. The secret life of NAD+: an old metabolite
37 controlling new metabolic signaling pathways. *Endocr Rev* 31: 194-223, 2010.
- 38 158. Hunt GE, Van Nieuwenhuijzen PS, Chan-Ling T, McGregor IS. 'When an old rat smells a cat': A
39 decline in defense-related, but not accessory olfactory, Fos expression in aged rats.
40 *Neurobiol Aging* 32: 737-49, 2011.
- 41 159. Hwang ES, Song SB. Nicotinamide is an inhibitor of SIRT1 in vitro, but can be a stimulator in
42 cells. *Cell Mol Life Sci*, 2017.
- 43 160. Imai S, Yoshino J. The importance of NAMPT/NAD/SIRT1 in the systemic regulation of
44 metabolism and ageing. *Diabetes Obes Metab* 15 Suppl 3: 26-33, 2013.
- 45 161. Ishidoh K, Kamemura N, Imagawa T, Oda M, Sakurai J, Katunuma N. Quinolate
46 phosphoribosyl transferase, a key enzyme in de novo NAD(+) synthesis, suppresses
47 spontaneous cell death by inhibiting overproduction of active-caspase-3. *Biochim Biophys*
48 *Acta* 1803: 527-33, 2010.
- 49 162. Iwai K, Taguchi H. Distribution of quinolate phosphoribosyl-transferase in animals, plants
50 and microorganisms. *J Nutr Sci Vitaminol (Tokyo)* 19: 491-9, 1973.
- 51 163. Jackson TM, Rawling JM, Roebuck BD, Kirkland JB. Large supplements of nicotinic acid and
52 nicotinamide increase tissue NAD+ and poly(ADP-ribose) levels but do not affect
53
54
55
56
57
58
59
60

- 1
2
3 diethylnitrosamine-induced altered hepatic foci in Fischer-344 rats. *J Nutr* 125: 1455-61,
4 1995.
- 5 164. Jacob RA, Swendseid ME, McKee RW, Fu CS, Clemens RA. Biochemical markers for
6 assessment of niacin status in young men: urinary and blood levels of niacin metabolites. *J*
7 *Nutr* 119: 591-8, 1989.
- 8 165. Jayaram HN, Kusumanchi P, Yalowitz JA. NMNAT expression and its relation to NAD
9 metabolism. *Curr Med Chem* 18: 1962-72, 2011.
- 10 166. Jin D, Liu HX, Hirai H, Torashima T, Nagai T, Lopatina O, Shnayder NA, Yamada K, Noda M,
11 Seike T, Fujita K, Takasawa S, Yokoyama S, Koizumi K, Shiraishi Y, Tanaka S, Hashii M,
12 Yoshihara T, Higashida K, Islam MS, Yamada N, Hayashi K, Noguchi N, Kato I, Okamoto H,
13 Matsushima A, Salmina A, Munesue T, Shimizu N, Mochida S, Asano M, Higashida H. CD38 is
14 critical for social behaviour by regulating oxytocin secretion. *Nature* 446: 41-5, 2007.
- 15 167. Kaanders JH, Stratford MR, Liefers J, Dennis MF, van der Kogel AJ, van Daal WA, Rojas A.
16 Administration of nicotinamide during a five- to seven-week course of radiotherapy:
17 pharmacokinetics, tolerance, and compliance. *Radiother Oncol* 43: 67-73, 1997.
- 18 168. Kalikin L, Calvo KC. Inhibition of quinolinate phosphoribosyl transferase by pyridine analogs
19 of quinolinic acid. *Biochem Biophys Res Commun* 152: 559-64, 1988.
- 20 169. Kamanna VS, Kashyap ML. Mechanism of action of niacin on lipoprotein metabolism. *Curr*
21 *Atheroscler Rep* 2: 36-46, 2000.
- 22 170. Kamanna VS, Kashyap ML. Nicotinic acid (niacin) receptor agonists: will they be useful
23 therapeutic agents? *Am J Cardiol* 100: S53-61, 2007.
- 24 171. Kamanna VS, Kashyap ML. Mechanism of action of niacin. *Am J Cardiol* 101: 20B-26B, 2008.
- 25 172. Kang-Lee YA, McKee RW, Wright SM, Swendseid ME, Jenden DJ, Jope RS. Metabolic effects
26 of nicotinamide administration in rats. *J Nutr* 113: 215-21, 1983.
- 27 173. Kang L, Da Vanzo JP. Is the tryptophan load test a valid index for a chemically-induced
28 vitamin B6 deficiency? *Proc Soc Exp Biol Med* 123: 340-3, 1966.
- 29 174. Karamanlidis G, Lee CF, Garcia-Menendez L, Kolwicz SC, Jr., Suthammarak W, Gong G,
30 Sedensky MM, Morgan PG, Wang W, Tian R. Mitochondrial complex I deficiency increases
31 protein acetylation and accelerates heart failure. *Cell Metab* 18: 239-50, 2013.
- 32 175. Khan NA, Khan I, Abid SMA, Zaib S, Ibrar A, Andleeb H, Hameed S, Iqbal J. Quinolinic
33 carboxylic acid derivatives as potential multi-target compounds for neurodegeneration:
34 monoamine oxidase and cholinesterase inhibition. *Med Chem*, 2017.
- 35 176. Khan S, Pandey SS, Jyotshna, Shanker K, Khan F, Rahman LU. Cloning and functional
36 characterization of quinolinic acid phosphoribosyl transferase (QPT) gene of *Nicotiana*
37 *tabacum*. *Physiol Plant*, 2017.
- 38 177. Kimura N, Fukuwatari T, Sasaki R, Shibata K. Comparison of metabolic fates of nicotinamide,
39 NAD⁺ and NADH administered orally and intraperitoneally; characterization of oral NADH. *J*
40 *Nutr Sci Vitaminol (Tokyo)* 52: 142-8, 2006.
- 41 178. Kirkland JB. Niacin status and treatment-related leukemogenesis. *Mol Cancer Ther* 8: 725-32,
42 2009.
- 43 179. Kirkland JB. Niacin status impacts chromatin structure. *J Nutr* 139: 2397-401, 2009.
- 44 180. Kirkland JB. Niacin requirements for genomic stability. *Mutat Res* 733: 14-20, 2012.
- 45 181. Kirkland JB. Niacin status and genomic instability in bone marrow cells; mechanisms favoring
46 the progression of leukemogenesis. *Subcell Biochem* 56: 21-36, 2012.
- 47 182. Kitaoka Y, Munemasa Y, Kojima K, Hirano A, Ueno S, Takagi H. Axonal protection by Nmnat3
48 overexpression with involvement of autophagy in optic nerve degeneration. *Cell Death Dis* 4:
49 e860, 2013.
- 50 183. Kitay BM, McCormack R, Wang Y, Tsoulfas P, Zhai RG. Mislocalization of neuronal
51 mitochondria reveals regulation of Wallerian degeneration and NMNAT/WLD(S)-mediated
52 axon protection independent of axonal mitochondria. *Hum Mol Genet* 22: 1601-14, 2013.
- 53
54
55
56
57
58
59
60

184. Komrower GM, Wilson V, Clamp JR, Westall RG. Hydroxykynureninuria: A Case of Abnormal Tryptophan Metabolism Probably Due to a Deficiency of Kynureninase. *Arch Dis Child* 39: 250-6, 1964.
185. Kops GJ, Dansen TB, Polderman PE, Saarloos I, Wirtz KW, Coffey PJ, Huang TT, Bos JL, Medema RH, Burgering BM. Forkhead transcription factor FOXO3a protects quiescent cells from oxidative stress. *Nature* 419: 316-21, 2002.
186. Kourtzidis IA, Stoupas AT, Gioris IS, Veskokouk AS, Margaritelis NV, Tsantarliotou M, Taitzoglou I, Vrabas IS, Paschalis V, Kyparos A, Nikolaidis MG. The NAD(+) precursor nicotinamide riboside decreases exercise performance in rats. *J Int Soc Sports Nutr* 13: 32, 2016.
187. Koyama T, Kume S, Koya D, Araki S, Isshiki K, Chin-Kanasaki M, Sugimoto T, Haneda M, Sugaya T, Kashiwagi A, Maegawa H, Uzu T. SIRT3 attenuates palmitate-induced ROS production and inflammation in proximal tubular cells. *Free Radic Biol Med* 51: 1258-67, 2011.
188. Krieger I, Statter M. Tryptophan deficiency and picolinic acid: effect on zinc metabolism and clinical manifestations of pellagra. *Am J Clin Nutr* 46: 511-7, 1987.
189. Krishnaswamy K, Bapurao S. Effect of leucine at different levels of pyridoxine on hepatic quinolinate phosphoribosyl transferase (EC 2.4.2.19) and leucine aminotransferase (EC 2.6.1.6) in rats. *Br J Nutr* 39: 61-4, 1978.
190. Kulikova V, Shabalin K, Nerinovski K, Dolle C, Niere M, Yakimov A, Redpath P, Khodorkovskiy M, Migaud ME, Ziegler M, Nikiforov A. Generation, Release, and Uptake of the NAD Precursor Nicotinic Acid Riboside by Human Cells. *J Biol Chem* 290: 27124-37, 2015.
191. Kume S, Haneda M, Kanasaki K, Sugimoto T, Araki S, Isono M, Isshiki K, Uzu T, Kashiwagi A, Koya D. Silent information regulator 2 (SIRT1) attenuates oxidative stress-induced mesangial cell apoptosis via p53 deacetylation. *Free Radic Biol Med* 40: 2175-82, 2006.
192. Lan F, Cacicedo JM, Ruderman N, Ido Y. SIRT1 modulation of the acetylation status, cytosolic localization, and activity of LKB1. Possible role in AMP-activated protein kinase activation. *J Biol Chem* 283: 27628-35, 2008.
193. Lau C, Niere M, Ziegler M. The NMN/NaMN adenylyltransferase (NMNAT) protein family. *Front Biosci (Landmark Ed)* 14: 410-31, 2009.
194. Lee HJ, Hong YS, Jun W, Yang SJ. Nicotinamide Riboside Ameliorates Hepatic Metaflammation by Modulating NLRP3 Inflammasome in a Rodent Model of Type 2 Diabetes. *J Med Food* 18: 1207-13, 2015.
195. Lee MC, Ting KK, Adams S, Brew BJ, Chung R, Guillemin GJ. Characterisation of the expression of NMDA receptors in human astrocytes. *PLoS One* 5: e14123, 2010.
196. Lehmann S, Costa AC, Celardo I, Loh SH, Martins LM. Parp mutations protect against mitochondrial dysfunction and neurodegeneration in a PARKIN model of Parkinson's disease. *Cell Death Dis* 7: e2166, 2016.
197. Li D, Tian YJ, Guo J, Sun WP, Lun YZ, Guo M, Luo N, Cao Y, Cao JM, Gong XJ, Zhou SS. Nicotinamide supplementation induces detrimental metabolic and epigenetic changes in developing rats. *Br J Nutr* 110: 2156-64, 2013.
198. Li F, He X, Ye D, Lin Y, Yu H, Yao C, Huang L, Zhang J, Wang F, Xu S, Wu X, Liu L, Yang C, Shi J, He X, Liu J, Qu Y, Guo F, Zhao J, Xu W, Zhao S. NADP(+)-IDH Mutations Promote Hypersuccinylation that Impairs Mitochondria Respiration and Induces Apoptosis Resistance. *Mol Cell* 60: 661-75, 2015.
199. Li X, Zhang S, Blander G, Tse JG, Krieger M, Guarente L. SIRT1 deacetylates and positively regulates the nuclear receptor LXR. *Mol Cell* 28: 91-106, 2007.
200. Lim JH, Lee YM, Chun YS, Chen J, Kim JE, Park JW. Sirtuin 1 modulates cellular responses to hypoxia by deacetylating hypoxia-inducible factor 1alpha. *Mol Cell* 38: 864-78, 2010.

- 1
2
3 201. Lischke T, Heesch K, Schumacher V, Schneider M, Haag F, Koch-Nolte F, Mittrucker HW. CD38 controls the innate immune response against *Listeria monocytogenes*. *Infect Immun* 81: 4091-9, 2013.
- 4
5
6 202. Liu B, Che W, Xue J, Zheng C, Tang K, Zhang J, Wen J, Xu Y. SIRT4 prevents hypoxia-induced apoptosis in H9c2 cardiomyoblast cells. *Cell Physiol Biochem* 32: 655-62, 2013.
- 7
8 203. Liu D, Gharavi R, Pitta M, Gleichmann M, Mattson MP. Nicotinamide prevents NAD⁺ depletion and protects neurons against excitotoxicity and cerebral ischemia: NAD⁺ consumption by SIRT1 may endanger energetically compromised neurons. *Neuromolecular Med* 11: 28-42, 2009.
- 9
10
11
12 204. Liu ZX, Azhipa O, Okamoto S, Govindarajan S, Dennert G. Extracellular nicotinamide adenine dinucleotide induces t cell apoptosis in vivo and in vitro. *J Immunol* 167: 4942-7, 2001.
- 13
14 205. Lombard DB, Alt FW, Cheng HL, Bunkenborg J, Streeper RS, Mostoslavsky R, Kim J, Yancopoulos G, Valenzuela D, Murphy A, Yang Y, Chen Y, Hirschey MD, Bronson RT, Haigis M, Guarente LP, Farese RV, Jr., Weissman S, Verdin E, Schwer B. Mammalian Sir2 homolog SIRT3 regulates global mitochondrial lysine acetylation. *Mol Cell Biol* 27: 8807-14, 2007.
- 15
16
17 206. Lopez-Otin C, Blasco MA, Partridge L, Serrano M, Kroemer G. The hallmarks of aging. *Cell* 153: 1194-217, 2013.
- 18
19
20 207. Lu Z, Xu X, Hu X, Fassett J, Zhu G, Tao Y, Li J, Huang Y, Zhang P, Zhao B, Chen Y. PGC-1 alpha regulates expression of myocardial mitochondrial antioxidants and myocardial oxidative stress after chronic systolic overload. *Antioxid Redox Signal* 13: 1011-22, 2010.
- 21
22 208. Luo J, Nikolaev AY, Imai S, Chen D, Su F, Shiloh A, Guarente L, Gu W. Negative control of p53 by Sir2alpha promotes cell survival under stress. *Cell* 107: 137-48, 2001.
- 23
24 209. Luo YX, Tang X, An XZ, Xie XM, Chen XF, Zhao X, Hao DL, Chen HZ, Liu DP. SIRT4 accelerates Ang II-induced pathological cardiac hypertrophy by inhibiting manganese superoxide dismutase activity. *Eur Heart J* 38: 1389-1398, 2017.
- 25
26
27 210. Lynn EG, McLeod CJ, Gordon JP, Bao JJ, Sack MN. SIRT2 is a negative regulator of anoxia-reoxygenation tolerance via regulation of 14-3-3 zeta and BAD in H9c2 cells. *Febs Letters* 582: 2857-2862, 2008.
- 28
29
30 211. Mack TG, Reiner M, Beirowski B, Mi W, Emanuelli M, Wagner D, Thomson D, Gillingwater T, Court F, Conforti L, Fernando FS, Tarlton A, Andressen C, Addicks K, Magni G, Ribchester RR, Perry VH, Coleman MP. Wallerian degeneration of injured axons and synapses is delayed by a Ube4b/Nmnat chimeric gene. *Nat Neurosci* 4: 1199-206, 2001.
- 31
32
33 212. Magni G, Amici A, Emanuelli M, Orsomando G, Raffaelli N, Ruggieri S. Enzymology of NAD⁺ homeostasis in man. *Cell Mol Life Sci* 61: 19-34, 2004.
- 34
35 213. Magni G, Amici A, Emanuelli M, Raffaelli N, Ruggieri S. Enzymology of NAD⁺ synthesis. *Adv Enzymol Relat Areas Mol Biol* 73: 135-82, xi, 1999.
- 36
37
38 214. Magni G, Amici A, Orsomando G. The enzymology of cytosolic pyrimidine 5'-nucleotidases: functional analysis and physiopathological implications. *Curr Med Chem* 20: 4304-16, 2013.
- 39
40
41 215. Maier KG. Nicotinamide adenine dinucleotide phosphate oxidase and diabetes: vascular implications. *Vasc Endovascular Surg* 42: 305-13, 2008.
- 42
43
44 216. Majmundar AJ, Wong WJ, Simon MC. Hypoxia-inducible factors and the response to hypoxic stress. *Mol Cell* 40: 294-309, 2010.
- 45
46
47 217. Maltos AL, Portari GV, Moraes GV, Monteiro MC, Vannucchi H, da Cunha DF. Niacin metabolism and indoleamine 2,3-dioxygenase activation in malnourished patients with flaky paint dermatosis. *Nutrition* 31: 890-2, 2015.
- 48
49
50 218. Mansour H, McColm JR, Cole L, Weible M, 2nd, Korlimbinis A, Chan-Ling T. Connexin 30 expression and frequency of connexin heterogeneity in astrocyte gap junction plaques increase with age in the rat retina. *PLoS One* 8: e57038, 2013.
- 51
52
53 219. Marohnic CC, Bewley MC, Barber MJ. Engineering and characterization of a NADPH-utilizing cytochrome b5 reductase. *Biochemistry* 42: 11170-82, 2003.
- 54
55
56
57
58
59
60

- 1
2
3 220. Martire S, Fusco A, Mosca L, Forte E, Correani V, Fontana M, Scarpa S, Maras B, d'Erme M. Bioenergetic Impairment in Animal and Cellular Models of Alzheimer's Disease: PARP-1 Inhibition Rescues Metabolic Dysfunctions. *J Alzheimers Dis* 54: 307-24, 2016.
- 4
5 221. Martire S, Mosca L, d'Erme M. PARP-1 involvement in neurodegeneration: A focus on Alzheimer's and Parkinson's diseases. *Mech Ageing Dev* 146-148: 53-64, 2015.
- 6
7 222. Marzetti E, Wohlgemuth SE, Aulisa AG, Bernabei R, Pahor M, Leeuwenburgh C. Calorie restriction for optimal cardiovascular aging: the weight of evidence. *Curr Cardiovasc Risk Rep* 4: 340-346, 2010.
- 8
9
10
11 223. Massudi H, Grant R, Braidy N, Guest J, Farnsworth B, Guillemin GJ. Age-associated changes in oxidative stress and NAD⁺ metabolism in human tissue. *PLoS One* 7: e42357, 2012.
- 12
13 224. Massudi H, Grant R, Guillemin GJ, Braidy N. NAD⁺ metabolism and oxidative stress: the golden nucleotide on a crown of thorns. *Redox Rep* 17: 28-46, 2012.
- 14
15 225. Mattagajasingh I, Kim CS, Naqvi A, Yamamori T, Hoffman TA, Jung SB, DeRicco J, Kasuno K, Irani K. SIRT1 promotes endothelium-dependent vascular relaxation by activating endothelial nitric oxide synthase. *Proc Natl Acad Sci U S A* 104: 14855-60, 2007.
- 16
17 226. Mattison JA, Colman RJ, Beasley TM, Allison DB, Kemnitz JW, Roth GS, Ingram DK, Weindruch R, de Cabo R, Anderson RM. Caloric restriction improves health and survival of rhesus monkeys. *Nat Commun* 8: 14063, 2017.
- 18
19 227. Mattison JA, Roth GS, Beasley TM, Tilmont EM, Handy AM, Herbert RL, Longo DL, Allison DB, Young JE, Bryant M, Barnard D, Ward WF, Qi W, Ingram DK, de Cabo R. Impact of caloric restriction on health and survival in rhesus monkeys from the NIA study. *Nature* 489: 318-21, 2012.
- 20
21
22 228. McClure JM, Wierman MB, Maqani N, Smith JS. Isonicotinamide enhances Sir2 protein-mediated silencing and longevity in yeast by raising intracellular NAD⁺ concentration. *J Biol Chem* 287: 20957-66, 2012.
- 23
24 229. McLin JP, Thompson LM, Steward O. Differential susceptibility to striatal neurodegeneration induced by quinolinic acid and kainate in inbred, outbred and hybrid mouse strains. *Eur J Neurosci* 24: 3134-40, 2006.
- 25
26 230. Mehler AH. Formation of picolinic and quinolinic acids following enzymatic oxidation of 3-hydroxyanthranilic acid. *J Biol Chem* 218: 241-54, 1956.
- 27
28 231. Menon RM, Adams MH, Gonzalez MA, Tolbert DS, Leu JH, Cefali EA. Plasma and urine pharmacokinetics of niacin and its metabolites from an extended-release niacin formulation. *Int J Clin Pharmacol Ther* 45: 448-54, 2007.
- 29
30 232. Menon RM, Gonzalez MA, Adams MH, Tolbert DS, Leu JH, Cefali EA. Effect of the rate of niacin administration on the plasma and urine pharmacokinetics of niacin and its metabolites. *J Clin Pharmacol* 47: 681-8, 2007.
- 31
32 233. Mesripour A, Hajhashemi V, Kuchak A. Effect of concomitant administration of three different antidepressants with vitamin B6 on depression and obsessive compulsive disorder in mice models. *Res Pharm Sci* 12: 46-52, 2017.
- 33
34 234. Monfrecola G, Gaudiello F, Cirillo T, Fabbrocini G, Balato A, Lembo S. Nicotinamide downregulates gene expression of interleukin-6, interleukin-10, monocyte chemoattractant protein-1, and tumour necrosis factor-alpha gene expression in HaCaT keratinocytes after ultraviolet B irradiation. *Clin Exp Dermatol* 38: 185-8, 2013.
- 35
36 235. Moresco RM, Lavazza T, Belloli S, Lecchi M, Pezzola A, Todde S, Matarrese M, Carpinelli A, Turolla E, Zimarino V, Popoli P, Malgaroli A, Fazio F. Quinolinic acid induced neurodegeneration in the striatum: a combined in vivo and in vitro analysis of receptor changes and microglia activation. *Eur J Nucl Med Mol Imaging* 35: 704-15, 2008.
- 37
38 236. Mori V, Amici A, Mazzola F, Di Stefano M, Conforti L, Magni G, Ruggieri S, Raffaelli N, Orsomando G. Metabolic profiling of alternative NAD biosynthetic routes in mouse tissues. *PLoS One* 9: e113939, 2014.
- 39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

- 1
2
3 237. Morigi M, Perico L, Rota C, Longaretti L, Conti S, Rottoli D, Novelli R, Remuzzi G, Benigni A. Sirtuin 3-dependent mitochondrial dynamic improvements protect against acute kidney injury. *J Clin Invest* 125: 715-26, 2015.
- 4
5
6 238. Moroni F, Carpenedo R, Chiarugi A. Kynurenine hydroxylase and kynureninase inhibitors as tools to study the role of kynurenine metabolites in the central nervous system. *Adv Exp Med Biol* 398: 203-10, 1996.
- 7
8
9 239. Mostoslavsky R, Chua KF, Lombard DB, Pang WW, Fischer MR, Gellon L, Liu P, Mostoslavsky G, Franco S, Murphy MM, Mills KD, Patel P, Hsu JT, Hong AL, Ford E, Cheng HL, Kennedy C, Nunez N, Bronson R, Frendewey D, Auerbach W, Valenzuela D, Karow M, Hottiger MO, Hursting S, Barrett JC, Guarente L, Mulligan R, Demple B, Yancopoulos GD, Alt FW. Genomic instability and aging-like phenotype in the absence of mammalian SIRT6. *Cell* 124: 315-29, 2006.
- 10
11
12 240. Myint AM, Kim YK. Network beyond IDO in psychiatric disorders: revisiting neurodegeneration hypothesis. *Prog Neuropsychopharmacol Biol Psychiatry* 48: 304-13, 2014.
- 13
14
15 241. Nahalkova J. Novel protein-protein interactions of TPPII, p53, and SIRT7. *Mol Cell Biochem* 409: 13-22, 2015.
- 16
17
18 242. Nakagawa T, Lomb DJ, Haigis MC, Guarente L. SIRT5 Deacetylates carbamoyl phosphate synthetase 1 and regulates the urea cycle. *Cell* 137: 560-70, 2009.
- 19
20
21 243. Nakahata Y, Bessho Y. The Circadian NAD⁺ Metabolism: Impact on Chromatin Remodeling and Aging. *Biomed Res Int* 2016: 3208429, 2016.
- 22
23
24 244. Nakamichi R, Miranda EP, Lobo SM, Tristao VR, Dalboni MA, Quinto BM, Batista MC. Action of nicotinic acid on the reversion of hypoxic-inflammatory link on 3T3-L1 adipocytes. *Lipids Health Dis* 15: 91, 2016.
- 25
26
27 245. Nematollahi A, Sun G, Jayawickrama GS, Church WB. Kynurenine Aminotransferase Isozyme Inhibitors: A Review. *Int J Mol Sci* 17, 2016.
- 28
29
30 246. Nemoto S, Fergusson MM, Finkel T. SIRT1 functionally interacts with the metabolic regulator and transcriptional coactivator PGC-1 α . *J Biol Chem* 280: 16456-60, 2005.
- 31
32
33 247. Niere M, Kernstock S, Koch-Nolte F, Ziegler M. Functional localization of two poly(ADP-ribose)-degrading enzymes to the mitochondrial matrix. *Mol Cell Biol* 28: 814-24, 2008.
- 34
35
36 248. Nishida Y, Rardin MJ, Carrico C, He W, Sahu AK, Gut P, Najjar R, Fitch M, Hellerstein M, Gibson BW, Verdin E. SIRT5 Regulates both Cytosolic and Mitochondrial Protein Malonylation with Glycolysis as a Major Target. *Mol Cell* 59: 321-32, 2015.
- 37
38
39 249. Nogueiras R, Habegger KM, Chaudhary N, Finan B, Banks AS, Dietrich MO, Horvath TL, Sinclair DA, Pfluger PT, Tschöp MH. Sirtuin 1 and sirtuin 3: physiological modulators of metabolism. *Physiol Rev* 92: 1479-514, 2012.
- 40
41
42 250. O'Neill AB, Morgan SJ, Brioni JD. Histological and behavioral protection by (-)-nicotine against quinolinic acid-induced neurodegeneration in the hippocampus. *Neurobiol Learn Mem* 69: 46-64, 1998.
- 43
44
45 251. Oblong JE. The evolving role of the NAD⁺/nicotinamide metabolome in skin homeostasis, cellular bioenergetics, and aging. *DNA Repair (Amst)* 23: 59-63, 2014.
- 46
47
48 252. Obrenovitch TP. Quinolinic acid accumulation during neuroinflammation. Does it imply excitotoxicity? *Ann N Y Acad Sci* 939: 1-10, 2001.
- 49
50
51 253. Obrenovitch TP, Urenjak J. Accumulation of quinolinic acid with neuroinflammation: does it mean excitotoxicity? *Adv Exp Med Biol* 527: 147-54, 2003.
- 52
53
54 254. Ohashi K, Kawai S, Murata K. Secretion of quinolinic acid, an intermediate in the kynurenine pathway, for utilization in NAD⁺ biosynthesis in the yeast *Saccharomyces cerevisiae*. *Eukaryot Cell* 12: 648-53, 2013.
- 55
56
57 255. Oliver E. [Diets of cereals & other vegetable proteins & cutaneous pellegra in our country]. *Rev Esp Enferm Apar Dig Nutr* 17: 68-73, 1958.
- 58
59
60

- 1
2
3 256. Olvera-Garcia G, Espinosa E, Sieg SF, Lederman MM. Cytomegalovirus-specific responses of
4 CD38(+) memory T cells are skewed towards IFN-gamma and dissociated from CD154 in HIV-
5 1 infection. *AIDS* 28: 311-6, 2014.
- 6 257. Palcic MM, Antoun M, Tanizawa K, Soda K, Floss HG. Stereochemistry of the kynureninase
7 reaction. *J Biol Chem* 260: 5248-51, 1985.
- 8 258. Penberthy WT. Nicotinic acid-mediated activation of both membrane and nuclear receptors
9 towards therapeutic glucocorticoid mimetics for treating multiple sclerosis. *PPAR Res* 2009:
10 853707, 2009.
- 11 259. Penberthy WT, Tsunoda I. The importance of NAD in multiple sclerosis. *Curr Pharm Des* 15:
12 64-99, 2009.
- 13 260. Peng Q, Jia SH, Parodo J, Ai Y, Marshall JC. Pre-B cell colony enhancing factor induces Nampt-
14 dependent translocation of the insulin receptor out of lipid microdomains in A549 lung
15 epithelial cells. *Am J Physiol Endocrinol Metab* 308: E324-33, 2015.
- 16 261. Peters JC. Tryptophan nutrition and metabolism: an overview. *Adv Exp Med Biol* 294: 345-58,
17 1991.
- 18 262. Phillips RS. Structure and mechanism of kynureninase. *Arch Biochem Biophys* 544: 69-74,
19 2014.
- 20 263. Pissios P. Nicotinamide N-Methyltransferase: More Than a Vitamin B3 Clearance Enzyme.
21 *Trends Endocrinol Metab* 28: 340-353, 2017.
- 22 264. Puri BK, Hirsch SR, Easton T, Richardson AJ. A volumetric biochemical niacin flush-based
23 index that noninvasively detects fatty acid deficiency in schizophrenia. *Progress in Neuro-*
24 *Psychopharmacology & Biological Psychiatry* 26: 49-52, 2002.
- 25 265. Qin W, Yang T, Ho L, Zhao Z, Wang J, Chen L, Zhao W, Thiyagarajan M, MacGrogan D,
26 Rodgers JT, Puigserver P, Sadoshima J, Deng H, Pedrini S, Gandy S, Sauve AA, Pasinetti GM.
27 Neuronal SIRT1 activation as a novel mechanism underlying the prevention of Alzheimer
28 disease amyloid neuropathology by calorie restriction. *J Biol Chem* 281: 21745-54, 2006.
- 29 266. Qiu X, Brown K, Hirschey MD, Verdin E, Chen D. Calorie restriction reduces oxidative stress
30 by SIRT3-mediated SOD2 activation. *Cell Metab* 12: 662-7, 2010.
- 31 267. Rahman A, Ting K, Cullen KM, Braidy N, Brew BJ, Guillemin GJ. The excitotoxin quinolinic acid
32 induces tau phosphorylation in human neurons. *PLoS One* 4: e6344, 2009.
- 33 268. Rajakumar SV, Lu B, Crikis S, Robson SC, d'Apice AJ, Cowan PJ, Dwyer KM. Deficiency or
34 inhibition of CD73 protects in mild kidney ischemia-reperfusion injury. *Transplantation* 90:
35 1260-4, 2010.
- 36 269. Rajamohan SB, Pillai VB, Gupta M, Sundaresan NR, Birukov KG, Samant S, Hottiger MO,
37 Gupta MP. SIRT1 promotes cell survival under stress by deacetylation-dependent
38 deactivation of poly(ADP-ribose) polymerase 1. *Mol Cell Biol* 29: 4116-29, 2009.
- 39 270. Ramautar A, Mabandla M, Blackburn J, Daniels WM. Inhibition of HIV-1 tat-induced
40 transactivation and apoptosis by the divalent metal chelators, fusaric acid and picolinic acid-
41 implications for HIV-1 dementia. *Neurosci Res* 74: 59-63, 2012.
- 42 271. Rankin PW, Jacobson EL, Benjamin RC, Moss J, Jacobson MK. Quantitative studies of
43 inhibitors of ADP-ribosylation in vitro and in vivo. *J Biol Chem* 264: 4312-7, 1989.
- 44 272. Ratajczak J, Joffraud M, Trammell SA, Ras R, Canela N, Boutant M, Kulkarni SS, Rodrigues M,
45 Redpath P, Migaud ME, Auwerx J, Yanes O, Brenner C, Canto C. NRK1 controls nicotinamide
46 mononucleotide and nicotinamide riboside metabolism in mammalian cells. *Nat Commun* 7:
47 13103, 2016.
- 48 273. Rawling JM, ApSimon MM, Kirkland JB. Lung poly(ADP-ribose) and NAD⁺ concentrations
49 during hyperoxia and niacin deficiency in the Fischer-344 rat. *Free Radic Biol Med* 20: 865-
50 71, 1996.
- 51 274. Rawling JM, Jackson TM, Driscoll ER, Kirkland JB. Dietary niacin deficiency lowers tissue
52 poly(ADP-ribose) and NAD⁺ concentrations in Fischer-344 rats. *J Nutr* 124: 1597-603, 1994.
- 53
54
55
56
57
58
59
60

- 1
2
3 275. Revollo JR, Korner A, Mills KF, Satoh A, Wang T, Garten A, Dasgupta B, Sasaki Y, Wolberger C,
4 Townsend RR, Milbrandt J, Kiess W, Imai S. Nampt/PBEF/Visfatin regulates insulin secretion
5 in beta cells as a systemic NAD biosynthetic enzyme. *Cell Metab* 6: 363-75, 2007.
- 6 276. Rodgers JT, Lerin C, Haas W, Gygi SP, Spiegelman BM, Puigserver P. Nutrient control of
7 glucose homeostasis through a complex of PGC-1 alpha and SIRT1. *Nature* 434: 113-118,
8 2005.
- 9 277. Rodgers JT, Lerin C, Haas W, Gygi SP, Spiegelman BM, Puigserver P. Nutrient control of
10 glucose homeostasis through a complex of PGC-1alpha and SIRT1. *Nature* 434: 113-8, 2005.
- 11 278. Roessler C, Tuting C, Meleshin M, Steegborn C, Schutkowski M. A Novel Continuous Assay for
12 the Deacylase Sirtuin 5 and Other Deacetylases. *J Med Chem* 58: 7217-23, 2015.
- 13 279. Rojas A, Stratford MR, Bentzen SM, Denekamp J. Is sensitization with nicotinamide and
14 carbogen dependent on nicotinamide concentration at the time of irradiation? *Int J Radiat*
15 *Biol* 80: 499-506, 2004.
- 16 280. Romashkan SV, Das SK, Villareal DT, Ravussin E, Redman LM, Rochon J, Bhapkar M, Kraus
17 WE, Group CS. Safety of two-year caloric restriction in non-obese healthy individuals.
18 *Oncotarget* 7: 19124-33, 2016.
- 19 281. Rongvaux A, Shea RJ, Mulks MH, Gigot D, Urbain J, Leo O, Andris F. Pre-B-cell colony-
20 enhancing factor, whose expression is up-regulated in activated lymphocytes, is a
21 nicotinamide phosphoribosyltransferase, a cytosolic enzyme involved in NAD biosynthesis.
22 *Eur J Immunol* 32: 3225-34, 2002.
- 23 282. Rothgiesser KM, Erener S, Waibel S, Luscher B, Hottiger MO. SIRT2 regulates NF-kappaB
24 dependent gene expression through deacetylation of p65 Lys310. *J Cell Sci* 123: 4251-8,
25 2010.
- 26 283. Ruddock MW, Hirst DG. Nicotinamide relaxes vascular smooth muscle by inhibiting myosin
27 light chain kinase-dependent signaling pathways: implications for anticancer efficacy. *Oncol*
28 *Res* 14: 483-9, 2004.
- 29 284. Sablina AA, Budanov AV, Ilyinskaya GV, Agapova LS, Kravchenko JE, Chumakov PM. The
30 antioxidant function of the p53 tumor suppressor. *Nat Med* 11: 1306-13, 2005.
- 31 285. Sakiyama Y. [Purine nucleoside phosphorylase deficiency]. *Ryoikibetsu Shokogun Shirizu*: 43-
32 5, 2000.
- 33 286. Sanchez WY, Obispo C, Ryan E, Grice JE, Roberts MS. Changes in the redox state and
34 endogenous fluorescence of in vivo human skin due to intrinsic and photo-aging, measured
35 by multiphoton tomography with fluorescence lifetime imaging. *J Biomed Opt* 18: 061217,
36 2013.
- 37 287. Sartini D, Santarelli A, Rossi V, Goteri G, Rubini C, Ciavarella D, Lo Muzio L, Emanuelli M.
38 Nicotinamide N-methyltransferase upregulation inversely correlates with lymph node
39 metastasis in oral squamous cell carcinoma. *Mol Med* 13: 415-21, 2007.
- 40 288. Sasaki Y, Araki T, Milbrandt J. Stimulation of nicotinamide adenine dinucleotide biosynthetic
41 pathways delays axonal degeneration after axotomy. *J Neurosci* 26: 8484-91, 2006.
- 42 289. Sasaki Y, Vohra BP, Lund FE, Milbrandt J. Nicotinamide mononucleotide adenylyl transferase-
43 mediated axonal protection requires enzymatic activity but not increased levels of neuronal
44 nicotinamide adenine dinucleotide. *J Neurosci* 29: 5525-35, 2009.
- 45 290. Satoh A, Brace CS, Rensing N, Cliften P, Wozniak DF, Herzog ED, Yamada KA, Imai S. Sirt1
46 extends life span and delays aging in mice through the regulation of Nk2 homeobox 1 in the
47 DMH and LH. *Cell Metab* 18: 416-30, 2013.
- 48 291. Sauer AV, Brigida I, Carriglio N, Aiuti A. Autoimmune dysregulation and purine metabolism in
49 adenosine deaminase deficiency. *Front Immunol* 3: 265, 2012.
- 50 292. Sauve AA, Moir RD, Schramm VL, Willis IM. Chemical activation of Sir2-dependent silencing
51 by relief of nicotinamide inhibition. *Mol Cell* 17: 595-601, 2005.
- 52 293. Scheibye-Knudsen M, Mitchell SJ, Fang EF, Iyama T, Ward T, Wang J, Dunn CA, Singh N, Veith
53 S, Hasan-Olive MM, Mangerich A, Wilson MA, Mattson MP, Bergersen LH, Cogger VC,
54
55
56
57
58
59
60

- Warren A, Le Couteur DG, Moaddel R, Wilson DM, 3rd, Croteau DL, de Cabo R, Bohr VA. A high-fat diet and NAD(+) activate Sirt1 to rescue premature aging in cockayne syndrome. *Cell Metab* 20: 840-55, 2014.
294. Schlicker C, Gertz M, Papatheodorou P, Kachholz B, Becker CF, Steegborn C. Substrates and regulation mechanisms for the human mitochondrial sirtuins Sirt3 and Sirt5. *J Mol Biol* 382: 790-801, 2008.
295. Schreiber V, Dantzer F, Ame JC, de Murcia G. Poly(ADP-ribose): novel functions for an old molecule. *Nat Rev Mol Cell Biol* 7: 517-28, 2006.
296. Schweiger M, Hennig K, Lerner F, Niere M, Hirsch-Kauffmann M, Specht T, Weise C, Oei SL, Ziegler M. Characterization of recombinant human nicotinamide mononucleotide adenylyl transferase (NMNAT), a nuclear enzyme essential for NAD synthesis. *FEBS Lett* 492: 95-100, 2001.
297. Seman M, Adriouch S, Scheuplein F, Krebs C, Freese D, Glowacki G, Deterre P, Haag F, Koch-Nolte F. NAD-induced T cell death: ADP-ribosylation of cell surface proteins by ART2 activates the cytolytic P2X7 purinoceptor. *Immunity* 19: 571-82, 2003.
298. Semba RD. The discovery of the vitamins. *Int J Vitam Nutr Res* 82: 310-5, 2012.
299. Semba RD. The historical evolution of thought regarding multiple micronutrient nutrition. *J Nutr* 142: 143S-56S, 2012.
300. Sharma A, Diecke S, Zhang WY, Lan F, He C, Mordwinkin NM, Chua KF, Wu JC. The role of SIRT6 protein in aging and reprogramming of human induced pluripotent stem cells. *J Biol Chem* 288: 18439-47, 2013.
301. Shetty PK, Galeffi F, Turner DA. Nicotinamide pre-treatment ameliorates NAD(H) hyperoxidation and improves neuronal function after severe hypoxia. *Neurobiol Dis* 62: 469-78, 2014.
302. Shi H, Sun N, Mayevsky A, Zhang Z, Luo Q. Preclinical evidence of mitochondrial nicotinamide adenine dinucleotide as an effective alarm parameter under hypoxia. *J Biomed Opt* 19: 17005, 2014.
303. Shi W, Hegeman MA, van Dartel DA, Tang J, Suarez M, Swarts H, van der Hee B, Arola L, Keijer J. Effects of a wide range of dietary nicotinamide riboside (NR) concentrations on metabolic flexibility and white adipose tissue (WAT) of mice fed a mildly obesogenic diet. *Mol Nutr Food Res*, 2017.
304. Shibata K. True Niacin Deficiency in Quinolinic Acid Phosphoribosyltransferase (QPRT) Knockout Mice. *J Nutr Sci Vitaminol (Tokyo)* 61 Suppl: S145-7, 2015.
305. Shibata K, Fukuwatari T. Large amounts of picolinic acid are lethal but small amounts increase the conversion of tryptophan-nicotinamide in rats. *J Nutr Sci Vitaminol (Tokyo)* 60: 334-9, 2014.
306. Shibata K, Mushiage M, Kondo T, Hayakawa T, Tsuge H. Effects of vitamin B6 deficiency on the conversion ratio of tryptophan to niacin. *Biosci Biotechnol Biochem* 59: 2060-3, 1995.
307. Shibata K, Ohno T, Sano M, Fukuwatari T. The urinary ratio of 3-hydroxykynurenine/3-hydroxyanthranilic acid is an index to predicting the adverse effects of D-tryptophan in rats. *J Nutr Sci Vitaminol (Tokyo)* 60: 261-8, 2014.
308. Siegel MP, Kruse SE, Knowels G, Salmon A, Beyer R, Xie H, Van Remmen H, Smith SR, Marcinek DJ. Reduced coupling of oxidative phosphorylation in vivo precedes electron transport chain defects due to mild oxidative stress in mice. *PLoS One* 6: e26963, 2011.
309. Sinclair DA, Guarente L. Extrachromosomal rDNA circles--a cause of aging in yeast. *Cell* 91: 1033-42, 1997.
310. Sociali G, Raffaghello L, Magnone M, Zamporlini F, Emionite L, Sturla L, Bianchi G, Vigliarolo T, Nahimana A, Nencioni A, Raffaelli N, Bruzzone S. Antitumor effect of combined NAMPT and CD73 inhibition in an ovarian cancer model. *Oncotarget* 7: 2968-84, 2016.

- 1
2
3 311. Someya S, Yu W, Hallows WC, Xu J, Vann JM, Leeuwenburgh C, Tanokura M, Denu JM, Prolla
4 TA. Sirt3 mediates reduction of oxidative damage and prevention of age-related hearing loss
5 under caloric restriction. *Cell* 143: 802-12, 2010.
- 6 312. Song J, Keppler BD, Wise RR, Bent AF. PARP2 Is the Predominant Poly(ADP-Ribose)
7 Polymerase in Arabidopsis DNA Damage and Immune Responses. *PLoS Genet* 11: e1005200,
8 2015.
- 9 313. Sono M. Enzyme kinetic and spectroscopic studies of inhibitor and effector interactions with
10 indoleamine 2,3-dioxygenase. 2. Evidence for the existence of another binding site in the
11 enzyme for indole derivative effectors. *Biochemistry* 28: 5400-7, 1989.
- 12 314. Soudijn W, van Wijngaarden I, Ijzerman AP. Nicotinic acid receptor subtypes and their
13 ligands. *Med Res Rev* 27: 417-33, 2007.
- 14 315. Spaans SK, Weusthuis RA, van der Oost J, Kengen SW. NADPH-generating systems in bacteria
15 and archaea. *Front Microbiol* 6: 742, 2015.
- 16 316. Spinnler R, Gorski T, Stolz K, Schuster S, Garten A, Beck-Sickinger AG, Engelse MA, de Koning
17 EJ, Korner A, Kiess W, Maedler K. The adipocytokine Nampt and its product NMN have no
18 effect on beta-cell survival but potentiate glucose stimulated insulin secretion. *PLoS One* 8:
19 e54106, 2013.
- 20 317. Stratford MR, Dennis MF, Hoskin P, Phillips H, Hodgkiss RJ, Rojas A. Nicotinamide
21 pharmacokinetics in humans: effect of gastric acid inhibition, comparison of rectal vs oral
22 administration and the use of saliva for drug monitoring. *Br J Cancer* 74: 16-21, 1996.
- 23 318. Sun S, Cheng S, Zhu Y, Zhang P, Liu N, Xu T, Sun C, Lv Y. Identification of PRKDC (Protein
24 Kinase, DNA-Activated, Catalytic Polypeptide) as an essential gene for colorectal cancer
25 (CRCs) cells. *Gene* 584: 90-6, 2016.
- 26 319. Sundaresan NR, Gupta M, Kim G, Rajamohan SB, Isbatan A, Gupta MP. Sirt3 blocks the
27 cardiac hypertrophic response by augmenting Foxo3a-dependent antioxidant defense
28 mechanisms in mice. *J Clin Invest* 119: 2758-71, 2009.
- 29 320. Surjana D, Halliday GM, Damian DL. Role of nicotinamide in DNA damage, mutagenesis, and
30 DNA repair. *J Nucleic Acids* 2010, 2010.
- 31 321. Surjana D, Halliday GM, Martin AJ, Moloney FJ, Damian DL. Oral nicotinamide reduces actinic
32 keratoses in phase II double-blinded randomized controlled trials. *J Invest Dermatol* 132:
33 1497-500, 2012.
- 34 322. Suzuki K, Koike T. Mammalian Sir2-related protein (SIRT) 2-mediated modulation of
35 resistance to axonal degeneration in slow Wallerian degeneration mice: a crucial role of
36 tubulin deacetylation. *Neuroscience* 147: 599-612, 2007.
- 37 323. Szkudelski T, Zywert A, Szkudelska K. Metabolic disturbances and defects in insulin secretion
38 in rats with streptozotocin-nicotinamide-induced diabetes. *Physiol Res* 62: 663-70, 2013.
- 39 324. Takahashi R. [Anti-aging studies on the senescence accelerated mouse (SAM) strains].
40 *Yakugaku Zasshi* 130: 11-8, 2010.
- 41 325. Tang K, Sham H, Hui E, Kirkland JB. Niacin deficiency causes oxidative stress in rat bone
42 marrow cells but not through decreased NADPH or glutathione status. *J Nutr Biochem* 19:
43 746-53, 2008.
- 44 326. Tang KS, Suh SW, Alano CC, Shao Z, Hunt WT, Swanson RA, Anderson CM. Astrocytic
45 poly(ADP-ribose) polymerase-1 activation leads to bioenergetic depletion and inhibition of
46 glutamate uptake capacity. *Glia* 58: 446-57, 2010.
- 47 327. Taniguchi A. [PNP (purine nucleoside phosphorylase) deficiency]. *Nihon Rinsho* 61 Suppl 1:
48 372-6, 2003.
- 49 328. Tanno O, Ota Y, Kitamura N, Katsube T, Inoue S. Nicotinamide increases biosynthesis of
50 ceramides as well as other stratum corneum lipids to improve the epidermal permeability
51 barrier. *Br J Dermatol* 143: 524-31, 2000.
- 52 329. Tao R, Coleman MC, Pennington JD, Ozden O, Park SH, Jiang H, Kim HS, Flynn CR, Hill S,
53 Hayes McDonald W, Olivier AK, Spitz DR, Gius D. Sirt3-mediated deacetylation of
54
55
56
57
58
59
60

- 1
2
3 evolutionarily conserved lysine 122 regulates MnSOD activity in response to stress. *Mol Cell*
4 40: 893-904, 2010.
- 5 330. Tao R, Kim SH, Honbo N, Karliner JS, Alano CC. Minocycline protects cardiac myocytes
6 against simulated ischemia-reperfusion injury by inhibiting poly(ADP-ribose) polymerase-1. *J*
7 *Cardiovasc Pharmacol* 56: 659-68, 2010.
- 8 331. Tempel W, Rabeh WM, Bogan KL, Belenky P, Wojcik M, Seidle HF, Nedyalkova L, Yang T,
9 Sauve AA, Park HW, Brenner C. Nicotinamide riboside kinase structures reveal new pathways
10 to NAD+. *PLoS Biol* 5: e263, 2007.
- 11 332. Terada N, Kinoshita K, Taguchi S, Tokuda Y. Wernicke encephalopathy and pellagra in an
12 alcoholic and malnourished patient. *BMJ Case Rep* 2015, 2015.
- 13 333. Ting KK, Brew BJ, Guillemin GJ. Effect of quinolinic acid on human astrocytes morphology
14 and functions: implications in Alzheimer's disease. *J Neuroinflammation* 6: 36, 2009.
- 15 334. Tong DL, Zhang DX, Xiang F, Teng M, Jiang XP, Hou JM, Zhang Q, Huang YS. Nicotinamide
16 pretreatment protects cardiomyocytes against hypoxia-induced cell death by improving
17 mitochondrial stress. *Pharmacology* 90: 11-8, 2012.
- 18 335. Trammell SA, Brenner C. Targeted, LCMS-based Metabolomics for Quantitative
19 Measurement of NAD(+) Metabolites. *Comput Struct Biotechnol J* 4: e201301012, 2013.
- 20 336. Trammell SA, Brenner C. NNMT: A Bad Actor in Fat Makes Good in Liver. *Cell Metab* 22: 200-
21 1, 2015.
- 22 337. Trammell SA, Schmidt MS, Weidemann BJ, Redpath P, Jaksch F, Dellinger RW, Li Z, Abel ED,
23 Migaud ME, Brenner C. Nicotinamide riboside is uniquely and orally bioavailable in mice and
24 humans. *Nat Commun* 7: 12948, 2016.
- 25 338. Trammell SA, Yu L, Redpath P, Migaud ME, Brenner C. Nicotinamide Riboside Is a Major
26 NAD+ Precursor Vitamin in Cow Milk. *J Nutr* 146: 957-63, 2016.
- 27 339. Tran MT, Zsengeller ZK, Berg AH, Khankin EV, Bhasin MK, Kim W, Clish CB, Stillman IE,
28 Karumanchi SA, Rhee EP, Parikh SM. PGC1alpha drives NAD biosynthesis linking oxidative
29 metabolism to renal protection. *Nature* 531: 528-32, 2016.
- 30 340. Tummla KS, Gomes AL, Yilmaz M, Grana O, Bakiri L, Ruppen I, Ximenez-Embun P,
31 Sheshappanavar V, Rodriguez-Justo M, Pisano DG, Wagner EF, Djouder N. Inhibition of de
32 novo NAD(+) synthesis by oncogenic URI causes liver tumorigenesis through DNA damage.
33 *Cancer Cell* 26: 826-39, 2014.
- 34 341. Turunc Bayrakdar E, Uyanikgil Y, Kanit L, Koylu E, Yalcin A. Nicotinamide treatment reduces
35 the levels of oxidative stress, apoptosis, and PARP-1 activity in Abeta(1-42)-induced rat
36 model of Alzheimer's disease. *Free Radic Res* 48: 146-58, 2014.
- 37 342. Ugur S, Ulu R, Dogukan A, Gurel A, Yigit IP, Gozel N, Aygen B, Ilhan N. The renoprotective
38 effect of curcumin in cisplatin-induced nephrotoxicity. *Ren Fail* 37: 332-6, 2015.
- 39 343. Vaccari CS, Nagamia S, Thoenes M, Oguchi A, Hammoud R, Khan BV. Efficacy of controlled-
40 release niacin in treatment of metabolic syndrome: Correlation to surrogate markers of
41 atherosclerosis, vascular reactivity, and inflammation. *J Clin Lipidol* 1: 605-13, 2007.
- 42 344. Vakhrusheva O, Smolka C, Gajawada P, Kostin S, Boettger T, Kubin T, Braun T, Bober E. Sirt7
43 increases stress resistance of cardiomyocytes and prevents apoptosis and inflammatory
44 cardiomyopathy in mice. *Circ Res* 102: 703-10, 2008.
- 45 345. van der Hoorn JW, de Haan W, Berbee JF, Havekes LM, Jukema JW, Rensen PC, Princen HM.
46 Niacin increases HDL by reducing hepatic expression and plasma levels of cholesteryl ester
47 transfer protein in APOE*3Leiden.CETP mice. *Arterioscler Thromb Vasc Biol* 28: 2016-22,
48 2008.
- 49 346. Vaquero A, Sternglanz R, Reinberg D. NAD+-dependent deacetylation of H4 lysine 16 by class
50 III HDACs. *Oncogene* 26: 5505-20, 2007.
- 51 347. Vazquez BN, Thackray JK, Serrano L. Sirtuins and DNA damage repair: SIRT7 comes to play.
52 *Nucleus* 8: 107-115, 2017.
- 53
54
55
56
57
58
59
60

- 1
2
3 348. Verdin E. NAD(+) in aging, metabolism, and neurodegeneration. *Science* 350: 1208-1213,
4 2015.
- 5 349. Vohra BP, Sasaki Y, Miller BR, Chang J, DiAntonio A, Milbrandt J. Amyloid precursor protein
6 cleavage-dependent and -independent axonal degeneration programs share a common
7 nicotinamide mononucleotide adenyltransferase 1-sensitive pathway. *J Neurosci* 30:
8 13729-38, 2010.
- 9 350. Wang F, Nguyen M, Qin FX, Tong Q. SIRT2 deacetylates FOXO3a in response to oxidative
10 stress and caloric restriction. *Aging Cell* 6: 505-14, 2007.
- 11 351. Wang F, Zhang WP. [Research progress on nicotinamide phosphoribosyl transferase involved
12 in aging and age-related diseases]. *Zhejiang Da Xue Xue Bao Yi Xue Ban* 40: 680-4, 2011.
- 13 352. Wang H, Liang X, Luo G, Ding M, Liang Q. Protection effect of nicotinamide on
14 cardiomyoblast hypoxia/re-oxygenation injury: study of cellular mitochondrial metabolism.
15 *Mol Biosyst* 12: 2257-64, 2016.
- 16 353. Wang P, Du H, Zhang RY, Guan YF, Xu TY, Xu QY, Su DF, Miao CY. Circulating and local
17 visfatin/Nampt/PBEF levels in spontaneously hypertensive rats, stroke-prone spontaneously
18 hypertensive rats and Wistar-Kyoto rats. *J Physiol Sci* 60: 317-24, 2010.
- 19 354. Wang P, Miao CY. NAMPT as a Therapeutic Target against Stroke. *Trends Pharmacol Sci* 36:
20 891-905, 2015.
- 21 355. Wang P, Xu TY, Guan YF, Su DF, Fan GR, Miao CY. Perivascular adipose tissue-derived visfatin
22 is a vascular smooth muscle cell growth factor: role of nicotinamide mononucleotide.
23 *Cardiovasc Res* 81: 370-80, 2009.
- 24 356. Wang X, Hu X, Yang Y, Takata T, Sakurai T. Nicotinamide mononucleotide protects against
25 beta-amyloid oligomer-induced cognitive impairment and neuronal death. *Brain Res* 1643:
26 1-9, 2016.
- 27 357. Warburg O, Christian W. Pyridine, the hydrogen transferring element of fermentation
28 enzymes. (Pyridine-nucleotide.). *Biochemische Zeitschrift* 287: 291-328, 1936.
- 29 358. Wardman P. Hypoxic cell radiosensitizers--chemical aspects and in vitro models.
30 *Strahlentherapie Sonderb* 75: 20-6, 1978.
- 31 359. Wardman P. Chemical radiosensitizers for use in radiotherapy. *Clin Oncol (R Coll Radiol)* 19:
32 397-417, 2007.
- 33 360. Wei CC, Kong YY, Li GQ, Guan YF, Wang P, Miao CY. Nicotinamide mononucleotide
34 attenuates brain injury after intracerebral hemorrhage by activating Nrf2/HO-1 signaling
35 pathway. *Sci Rep* 7: 717, 2017.
- 36 361. Wei M, Fabrizio P, Hu J, Ge H, Cheng C, Li L, Longo VD. Life span extension by calorie
37 restriction depends on Rim15 and transcription factors downstream of Ras/PKA, Tor, and
38 Sch9. *PLoS Genet* 4: e13, 2008.
- 39 362. Weidele K, Beneke S, Burkle A. The NAD+ precursor nicotinic acid improves genomic
40 integrity in human peripheral blood mononuclear cells after X-irradiation. *DNA Repair (Amst)*
41 52: 12-23, 2017.
- 42 363. Whitacre CM, Hashimoto H, Tsai ML, Chatterjee S, Berger SJ, Berger NA. Involvement of
43 NAD-poly(ADP-ribose) metabolism in p53 regulation and its consequences. *Cancer Res* 55:
44 3697-701, 1995.
- 45 364. Wiley CA. Quinolinic acid and neurodegeneration in AIDS. *J Neurovirol* 1: 328-9, 1995.
- 46 365. Williams AC, Hill LJ, Ramsden DB. Nicotinamide, NAD(P)(H), and Methyl-Group Homeostasis
47 Evolved and Became a Determinant of Ageing Diseases: Hypotheses and Lessons from
48 Pellagra. *Curr Gerontol Geriatr Res* 2012: 302875, 2012.
- 49 366. Williams AC, Ramsden DB. Autotoxicity, methylation and a road to the prevention of
50 Parkinson's disease. *J Clin Neurosci* 12: 6-11, 2005.
- 51 367. Wojcik M, Seidle HF, Bieganowski P, Brenner C. Glutamine-dependent NAD+ synthetase.
52 How a two-domain, three-substrate enzyme avoids waste. *J Biol Chem* 281: 33395-402,
53 2006.
- 54
55
56
57
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59
60

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2
3 368. Wood JD, Peesker SJ. The effect on GABA metabolism in brain of isonicotinic acid hydrazide
4 and pyridoxine as a function of time after administration. *J Neurochem* 19: 1527-37, 1972.
- 5 369. Wu W, Nicolazzo JA, Wen L, Chung R, Stankovic R, Bao SS, Lim CK, Brew BJ, Cullen KM,
6 Guillemin GJ. Expression of tryptophan 2,3-dioxygenase and production of kynurenine
7 pathway metabolites in triple transgenic mice and human Alzheimer's disease brain. *PLoS*
8 *One* 8: e59749, 2013.
- 9 370. Yang H, Zuo XZ, Tian C, He DL, Yi WJ, Chen Z, Zhang PW, Ding SB, Ying CJ. Green Tea
10 Polyphenols Attenuate High-Fat Diet-Induced Renal Oxidative Stress through SIRT3-
11 Dependent Deacetylation. *Biomed Environ Sci* 28: 455-9, 2015.
- 12 371. Yang SJ, Choi JM, Kim L, Park SE, Rhee EJ, Lee WY, Oh KW, Park SW, Park CY. Nicotinamide
13 improves glucose metabolism and affects the hepatic NAD-sirtuin pathway in a rodent
14 model of obesity and type 2 diabetes. *J Nutr Biochem* 25: 66-72, 2014.
- 15 372. Yao Z, Yang W, Gao Z, Jia P. Nicotinamide mononucleotide inhibits JNK activation to reverse
16 Alzheimer disease. *Neurosci Lett* 647: 133-140, 2017.
- 17 373. Yin F, Boveris A, Cadenas E. Mitochondrial energy metabolism and redox signaling in brain
18 aging and neurodegeneration. *Antioxid Redox Signal* 20: 353-71, 2014.
- 19 374. Yoon J, Lee KJ, Oh GS, Kim GH, Kim SW. Regulation of Nampt expression by transcriptional
20 coactivator NCOA6 in pancreatic beta-cells. *Biochem Biophys Res Commun* 487: 600-606,
21 2017.
- 22 375. Yoshino J, Baur JA, Imai SI. NAD(+) Intermediates: The Biology and Therapeutic Potential of
23 NMN and NR. *Cell Metab*, 2017.
- 24 376. Yoshino J, Mills KF, Yoon MJ, Imai S. Nicotinamide mononucleotide, a key NAD(+)
25 intermediate, treats the pathophysiology of diet- and age-induced diabetes in mice. *Cell*
26 *Metab* 14: 528-36, 2011.
- 27 377. Young GS, Choleris E, Lund FE, Kirkland JB. Decreased cADPR and increased NAD+ in the
28 Cd38-/- mouse. *Biochem Biophys Res Commun* 346: 188-92, 2006.
- 29 378. Young GS, Jacobson EL, Kirkland JB. Water maze performance in young male Long-Evans rats
30 is inversely affected by dietary intakes of niacin and may be linked to levels of the NAD+
31 metabolite cADPR. *J Nutr* 137: 1050-7, 2007.
- 32 379. Yu J, Sadhukhan S, Noriega LG, Moullan N, He B, Weiss RS, Lin H, Schoonjans K, Auwerx J.
33 Metabolic characterization of a Sirt5 deficient mouse model. *Sci Rep* 3: 2806, 2013.
- 34 380. Zeng J, Libien J, Shaik F, Wolk J, Hernandez AI. Nucleolar PARP-1 Expression Is Decreased in
35 Alzheimer's Disease: Consequences for Epigenetic Regulation of rDNA and Cognition. *Neural*
36 *Plast* 2016: 8987928, 2016.
- 37 381. Zhai RG, Cao Y, Hiesinger PR, Zhou Y, Mehta SQ, Schulze KL, Verstreken P, Bellen HJ.
38 Drosophila NMNAT maintains neural integrity independent of its NAD synthesis activity.
39 *PLoS Biol* 4: e416, 2006.
- 40 382. Zhai RG, Zhang F, Hiesinger PR, Cao Y, Haueter CM, Bellen HJ. NAD synthase NMNAT acts as
41 a chaperone to protect against neurodegeneration. *Nature* 452: 887-91, 2008.
- 42 383. Zhang B, Muller-Steffner H, Schuber F, Potter BVL. Nicotinamide 2-fluoroadenine
43 dinucleotide unmasks the NAD(+) glycohydrolase activity of Aplysia californica adenosine 5'-
44 diphosphate ribosyl cyclase. *Biochemistry* 46: 4100-4109, 2007.
- 45 384. Zhong L, D'Urso A, Toiber D, Sebastian C, Henry RE, Vadysirisack DD, Guimaraes A, Marinelli
46 B, Wikstrom JD, Nir T, Clish CB, Vaitheesvaran B, Iliopoulos O, Kurland I, Dor Y, Weissleder R,
47 Shirihai OS, Ellisen LW, Espinosa JM, Mostoslavsky R. The histone deacetylase Sirt6 regulates
48 glucose homeostasis via Hif1alpha. *Cell* 140: 280-93, 2010.
- 49 385. Zhong L, Yeh TY, Hao J, Pourtabatabaei N, Mahata SK, Shao J, Chessler SD, Chi NW.
50 Nutritional energy stimulates NAD+ production to promote tankyrase-mediated PARsylation
51 in insulinoma cells. *PLoS One* 10: e0122948, 2015.
- 52 386. Zhou SS, Li D, Sun WP, Guo M, Lun YZ, Zhou YM, Xiao FC, Jing LX, Sun SX, Zhang LB, Luo N,
53 Bian FN, Zou W, Dong LB, Zhao ZG, Li SF, Gong XJ, Yu ZG, Sun CB, Zheng CL, Jiang DJ, Li ZN.
54
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56
57
58
59
60

- Nicotinamide overload may play a role in the development of type 2 diabetes. *World J Gastroenterol* 15: 5674-84, 2009.
387. Zhou SS, Li D, Zhou Y. Letter to the Editor: High Serum N(1)-Methylnicotinamide in Obesity and Diabetes: A Consequence of Excess Nicotinamide? *J Clin Endocrinol Metab* 100: L78-9, 2015.
388. Zhou Y, Wang L, Yang F, Lin X, Zhang S, Zhao ZK. Determining the extremes of the cellular NAD(H) level by using an Escherichia coli NAD(+)-auxotrophic mutant. *Appl Environ Microbiol* 77: 6133-40, 2011.
389. Zhu XH, Lu M, Lee BY, Ugurbil K, Chen W. In vivo NAD assay reveals the intracellular NAD contents and redox state in healthy human brain and their age dependences. *Proc Natl Acad Sci U S A* 112: 2876-81, 2015.
390. Zhuo L, Fu B, Bai X, Zhang B, Wu L, Cui J, Cui S, Wei R, Chen X, Cai G. NAD blocks high glucose induced mesangial hypertrophy via activation of the sirtuins-AMPK-mTOR pathway. *Cell Physiol Biochem* 27: 681-90, 2011.
391. Zykova TA, Zhu F, Zhai X, Ma WY, Ermakova SP, Lee KW, Bode AM, Dong Z. Resveratrol directly targets COX-2 to inhibit carcinogenesis. *Mol Carcinog* 47: 797-805, 2008.

List of Figures

Figure 1. Putative relationship between changes in tryptophan catabolism and de novo NAD⁺ synthesis in ADC neuropathology. Immune activated oxidative L-tryptophan catabolism can contribute positively to the maintenance of cell viability through increased metabolism of NAD⁺ in astrocytes and mononuclear phagocytes. However, chronic activation of this pathway may also enhance neuronal excitotoxicity through the production of QUIN and possibly 3-HK.

Figure 2. The NAD⁺ metabolome. L-Tryptophan (L-TRYP), nicotinic acid (NA), nicotinamide (NAM), nicotinamide mononucleotide (NMN), nicotinamide riboside (NR) and nicotinic acid riboside (NAR) can be used as precursors for NAD⁺ synthesis. L-TRYP is catabolised to N-formylkynurenine (N-f-KYN) by indoleamine 2,3-dioxygenase (IDO) or tryptophan 2,3-dioxygenase (TDO) (a). N-f-KYN is catabolised by arylformidase to form kynurenine (KYN) (b). Kynurenine aminotransferases (KATs) catabolise KYN to form kynurenic acid (KA) (c). Kynurenine 3-hydroxylase uses KYN as a substrate to form 3-hydroxykynurenine (3-HK) (d). Kynureninase then forms 3-hydroxyanthranilic acid (3-HAA) (e) which is converted to 2-amino-3-carboxymuconate semialdehyde (not shown) by 3-hydroxyanthranilic acid oxygenase (3-HAAO) (f). This product is then converted to picolinic acid by picolinic acid carboxylase (g). Alternatively, the semialdehyde undergoes spontaneous condensation and rearrangement to form quinolinic acid (QUIN) (h), which

forms nicotinic acid mononucleotide (NAMN) by quinolinic acid phosphoribosyltransferase (QPRT) **(i)**. NAMN undergoes adenylation by nicotinamide mononucleotide adenylyltransferases (NMNAT1-3) to form nicotinic acid adenine dinucleotide (NAAD) **(u)**, which forms NAD⁺ by glutamine-dependent NAD⁺ synthetases **(m)**. NA is used by the Preiss-Handler pathway. NAMN is formed by nicotinic acid phosphoribosyltransferase (NAPRT) following addition of 5-phosphoribose group from 5-phosphoribosyl-1-pyrophosphate to NA **(l)**. Nicotinamide phosphoribosyltransferase (NAMPT) forms NMN by addition of phosphoribose moiety to NAM **(p)**. NMN is then converted to NAD⁺ via the catalytic activity of NMNAT1-3 **(u)**. NAM is also produced as a by-product of NAD-dependent enzymes eg. PARPs, sirtuins, and CD38 **(n)**. NAM can also be converted to NA by bacterial nicotinamidases **(o)**. NR is phosphorylated to form NMN by nicotinamide riboside kinases (NRK1/NRK2) **(j)** which is then subsequently converted to NAD⁺ by NMNAT1-3. NAR can also be used to form NAMN by NRK1/NRK2 **(j)** or NA by purine nucleoside phosphorylase **(k)**. NAM is methylated by nicotinamide N-methyltransferase (NNMT) to N-methylnicotinamide (MeNAM) and modulates the efficiency of NAD-dependent biological processes **(q)**. NAD⁺ can be reduced to form NADH **(t)**. NAD⁺ can also undergo phosphorylation to NADP⁺ **(r)** and then further reduction to NADPH **(s)**.

Figure 3. Concomitant induction of IDO and free radical generation by IFN- γ . Chronic immune activation of macrophages and astrocytes will result in increased reactive oxygen and nitrogen species and elevates glutamate levels (in the absence of efficient uptake into astrocytes). A possible relationship exists between IFN- γ stimulated free radical production and IDO induction, leading to increased *de novo* synthesis of NAD⁺.

Figure 4. Cofactors required for QPRT activity and NAD⁺ synthesis. PRPP is important for the regulation of QPRT activity.

Figure 5. Cellular roles of NAD⁺. The mechanisms of degradation of NAD⁺ including CD38, PARPs, and sirtuins. NAD⁺ can be phosphorylated to NADP⁺. There are also oxidation reactions of NAD⁺ to NADH and NADP⁺ to NADH. CD38 is an NAD-dependent enzyme which leads to the production of cADPR from NAD⁺ and NADP⁺

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2
3 respectively. Cytosolic cADPR target to ryanodine receptors on endoplasmic reticulum, and
4 transient receptor potential mucolipin 1 on lysosomes, regulating intracellular calcium
5 signalling from the endoplasmic reticulum and lysosome-mediated intracellular calcium
6 signalling.
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10 **Figure 6 (A).** PARP and PARG enzymatic activity. PARP breaks down NAD⁺ to NAM and
11 an ADP-ribosyl product degradation of ADP-ribose polymers occurs relatively rapidly
12 through the action of poly(ADP-ribose) glycohydrolase. **(B)** Relationship between DNA
13 damage, PARP activation, and NAD⁺ depletion. Under normal physiological conditions,
14 PARP activation leads to repair of damaged DNA. However, increased PARP activity
15 resulting in decreased NAD⁺ has been shown to decrease ATP as well as cause cell lysis and
16 death (45,203) (Figure 6B).
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24 **Figure 7.** Stoichiometry of CD38-mediated Ca²⁺ mobilising, and NADase activities. CD38
25 require NAD⁺ to produce ADP ribose (ADPR) and hydrolyse the secondary messenger
26 signalling molecule, cyclic-ADP-ribose (cADPR) which helps mediate intracellular calcium
27 transients.
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34 **Figure 8.** Schematic representation of CD38-mediated intracellular Ca²⁺ secondary
35 messenger signalling. , CD38 is also an NADase which primarily regulates intracellular
36 levels of NAD⁺ and its physiological processes. CD38 also catalyses a base exchange
37 between NADP and NA, leading to the formation of nicotinic acid adenine dinucleotide
38 phosphate (NAADP), which is also used as a hydrolytic substrate.
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45 **Figure 9.** Sirtuin enzymatic activity. NAM is rendered as a by-product of sirtuin-mediated
46 deacetylation. Deacetylation occurs when the modified lysine side chain is coupled to the
47 cleavage of the glycosidic bonds in NAD⁺, leading to the generation of the deacetylated
48 lysine, acetylated ADP-ribose, and NAM as by-products.
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54 **Figure 10.** Functions of NAD-dependent sirtuins and relevant transcription factors. Sirtuin-
55 mediated deacetylation affects numerous target enzymes and transcription factors relevant to
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3 ageing and disease. Importantly, sirtuin activities stimulate oxidative phosphorylation
4 (OXPHOS) while yet unknown acetylation mechanisms serve to inhibit oxidative
5 phosphorylation (anti-OXPHOS).
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11 **Figure 11.** Modulation of NAD⁺ and NAD-dependent pathways by caloric restriction in
12 mice and humans. Caloric restriction has been shown to increase neuronal SIRT1 activity in
13 humans. In mice, hepatic total NAD⁺ levels increased in fasted mice, and these changes were
14 accompanied by increased SIRT1 activation, PGC1 α deacetylation, and increased
15 mitochondrial biogenesis.
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22 **Figure 12.** Chemical structure of NAD⁺ precursors.
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27 **Figure 13.** Mechanisms of action of nicotinic acid (NA) in dyslipidemia. The lipid lowering
28 effects of NA are thought to be mediated by binding of NA to the cell surface of a G-protein
29 coupled receptor known as HM74A or GPR109A. This association in adipocytes suppresses
30 triglyceride lipolysis, culminating in the reduction of circulating fatty acids, and reduced liver
31 VLDL formation and circulating LDL-cholesterol.
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38 **Figure 14.** Schematic representation of the molecular mechanism of skin flushing following
39 treatment with nicotinic acid (NA). NA mediated stimulation of HM74A in some skin
40 immune cells results in the conversion of the omega-6 metabolite arachidonic acid (AA) into
41 prostaglandin E₂, stimulating vasodilation of skin capillaries, causing skin flush.
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48 **Figure 15.** Mechanisms of action of nicotinamide (NAM) and its effect on the NAD⁺
49 metabolome. NAM also serves as a natural feedback inhibitor for NAD-dependent enzymes.
50 For example, PARP, sirtuin and CD38 activities are proportionately inhibited as NAM
51 concentrations increase, and this has been postulated as the mechanism for the anti-diabetic
52 effects of NAM in humans. While NAD⁺ levels are still elevated, the important NAD-
53 dependent functions (e.g. SIRT1 activity) are inhibited.
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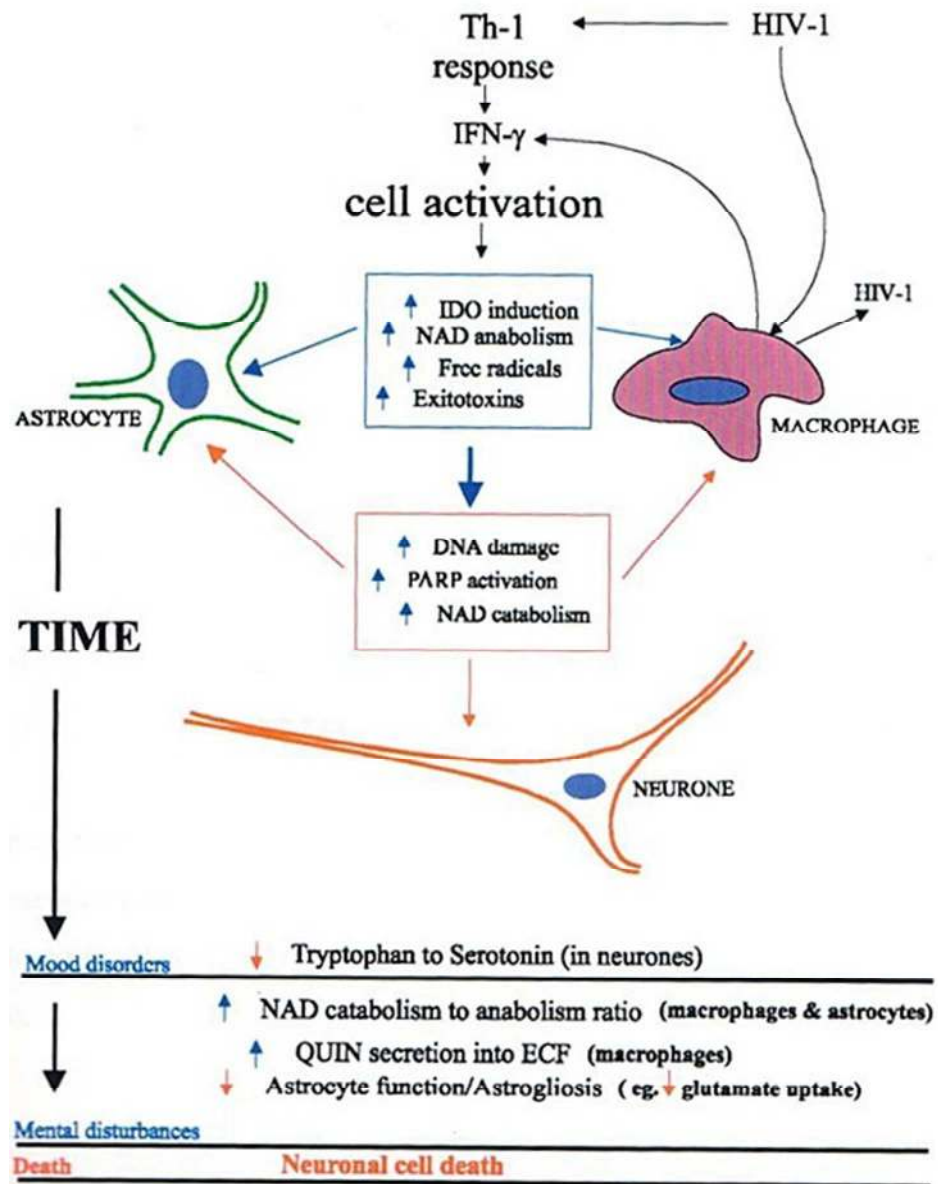
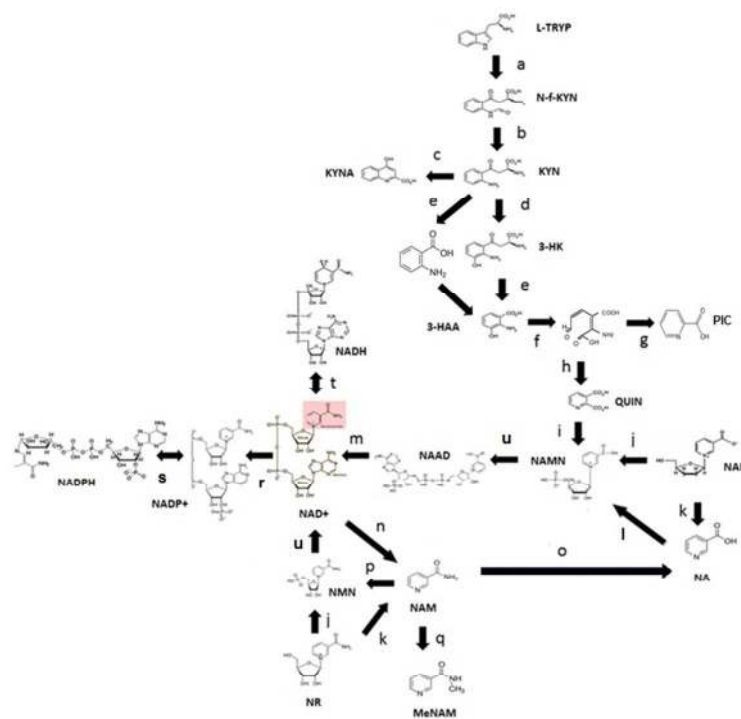


Figure 1. Putative relationship between changes in tryptophan catabolism and de novo NAD⁺ synthesis in ADC neuropathology.

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Figure 2. The NAD⁺ metabolome.

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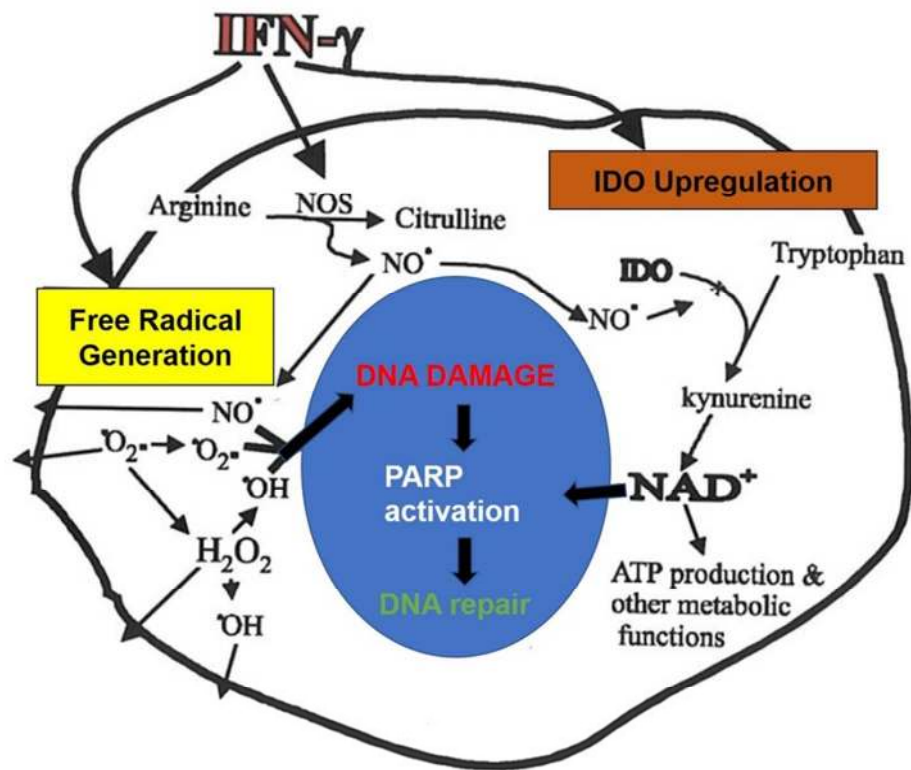


Figure 3. Concomitant induction of IDO and free radical generation by IFN- γ .

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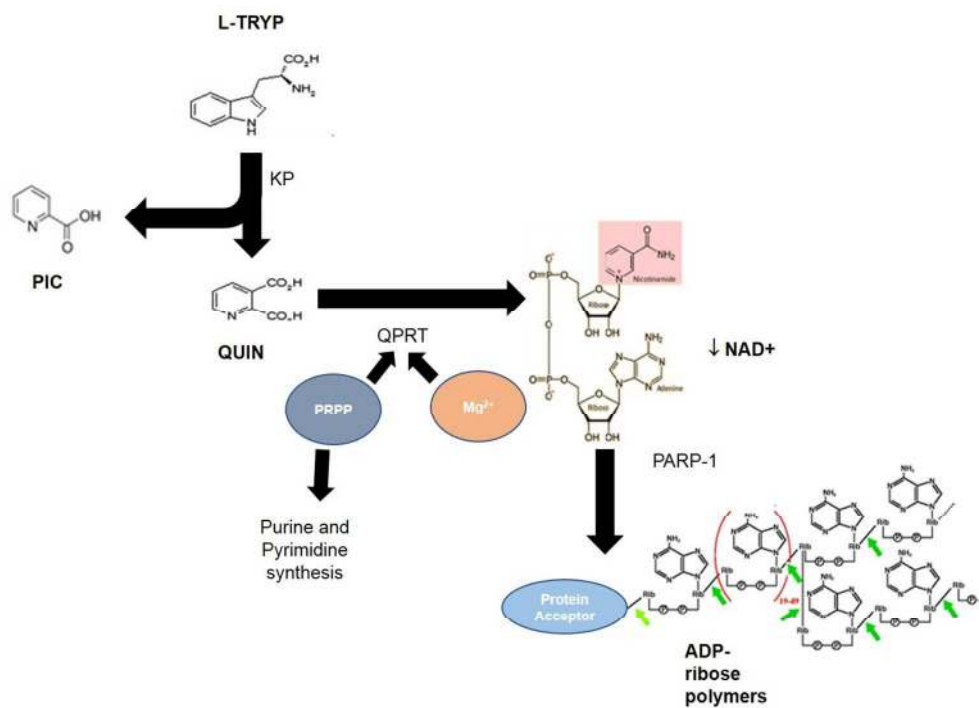


Figure 4. Cofactors required for QPRT activity and NAD⁺ synthesis.

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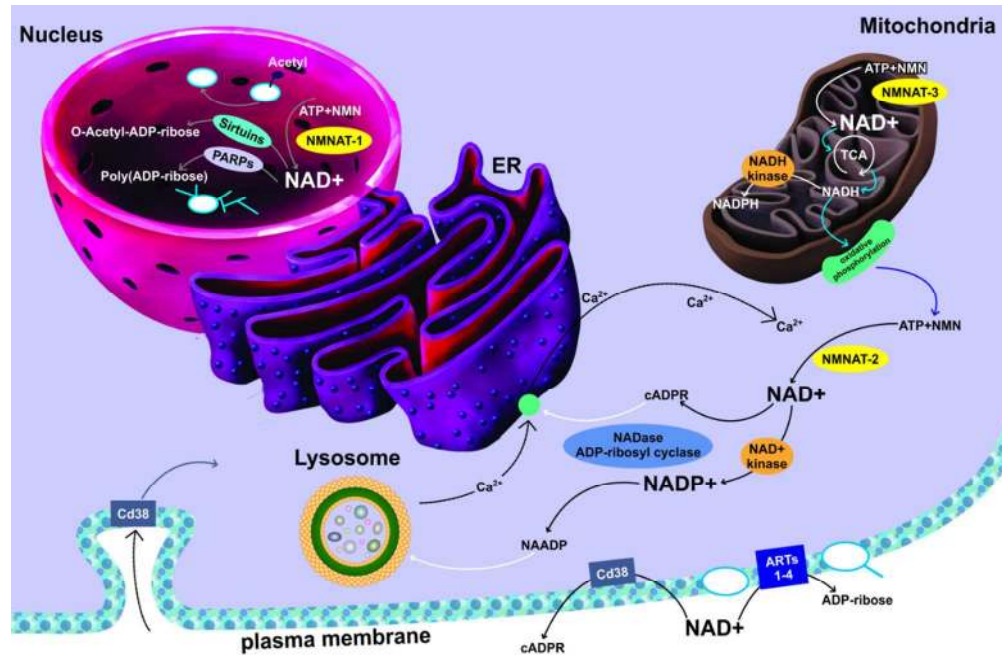


Figure 5. Cellular roles of NAD+.

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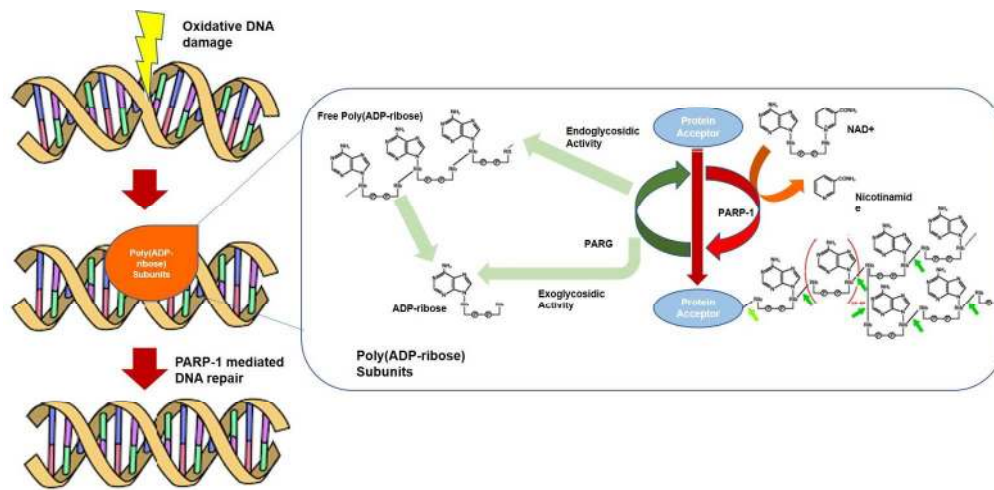


Figure 6 (A). PARP and PARG enzymatic activity.

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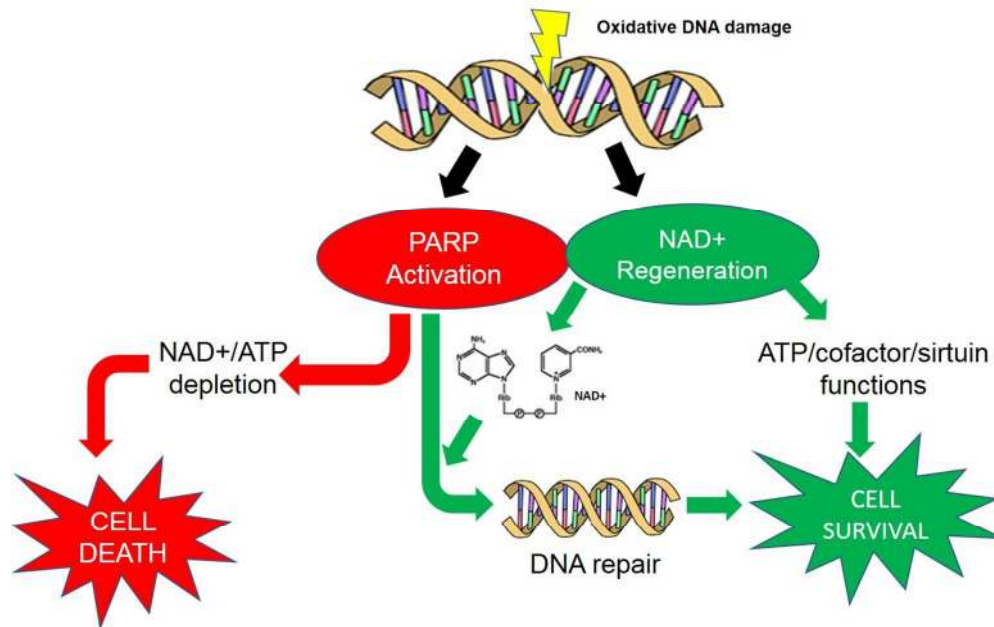


Figure 6 (B) Relationship between DNA damage, PARP activation, and NAD+ depletion.

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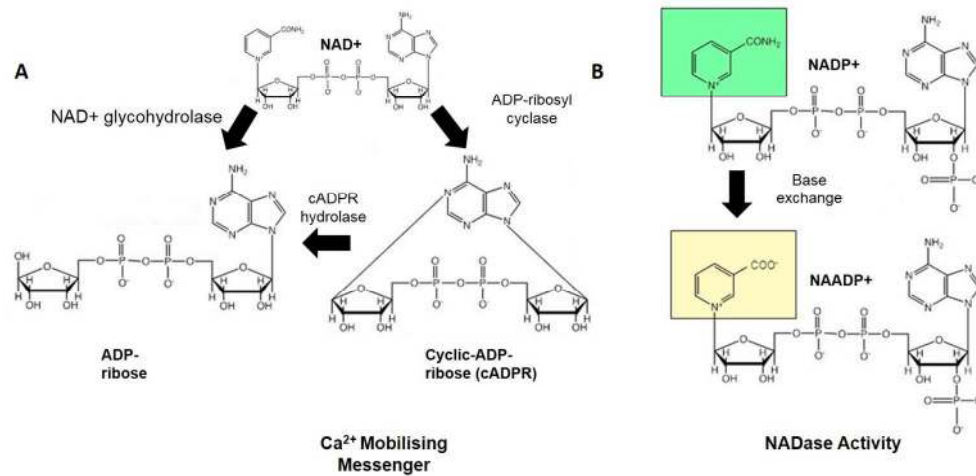


Figure 7. Stoichiometry of CD38-mediated Ca²⁺ mobilising, and NADase activities.

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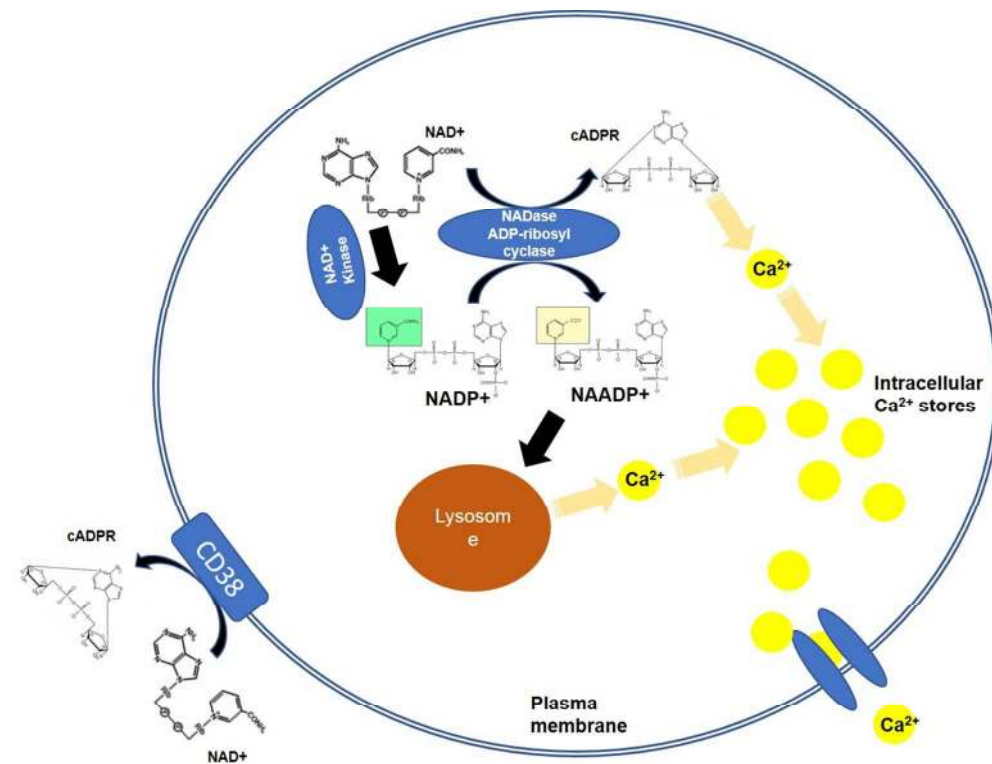


Figure 8. Schematic representation of CD38-mediated intracellular Ca²⁺ secondary messenger signalling

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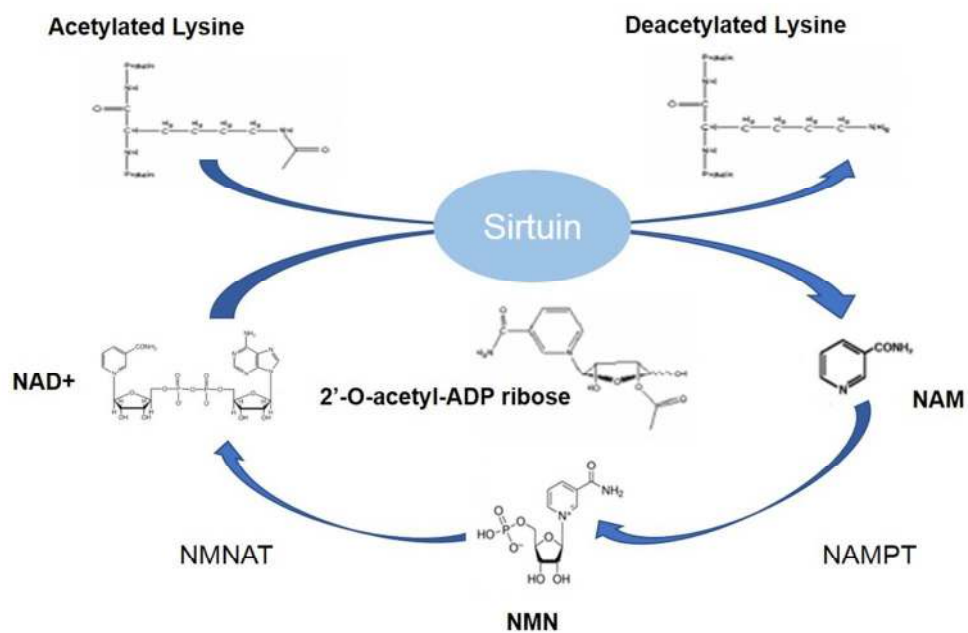
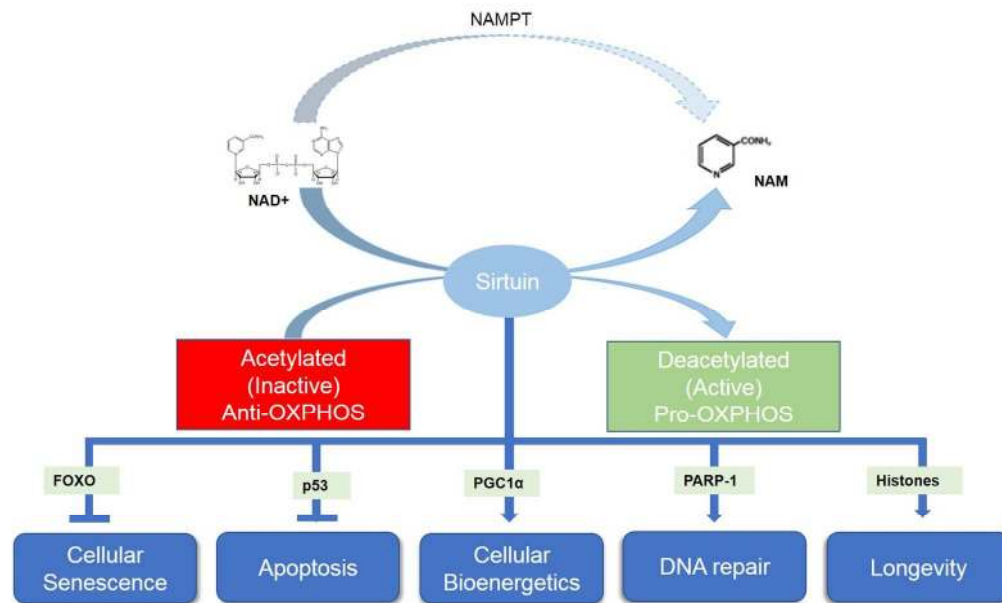


Figure 9. Sirtuin enzymatic activity.

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27 Figure 10. Functions of NAD-dependent sirtuins and relevant transcription factors.

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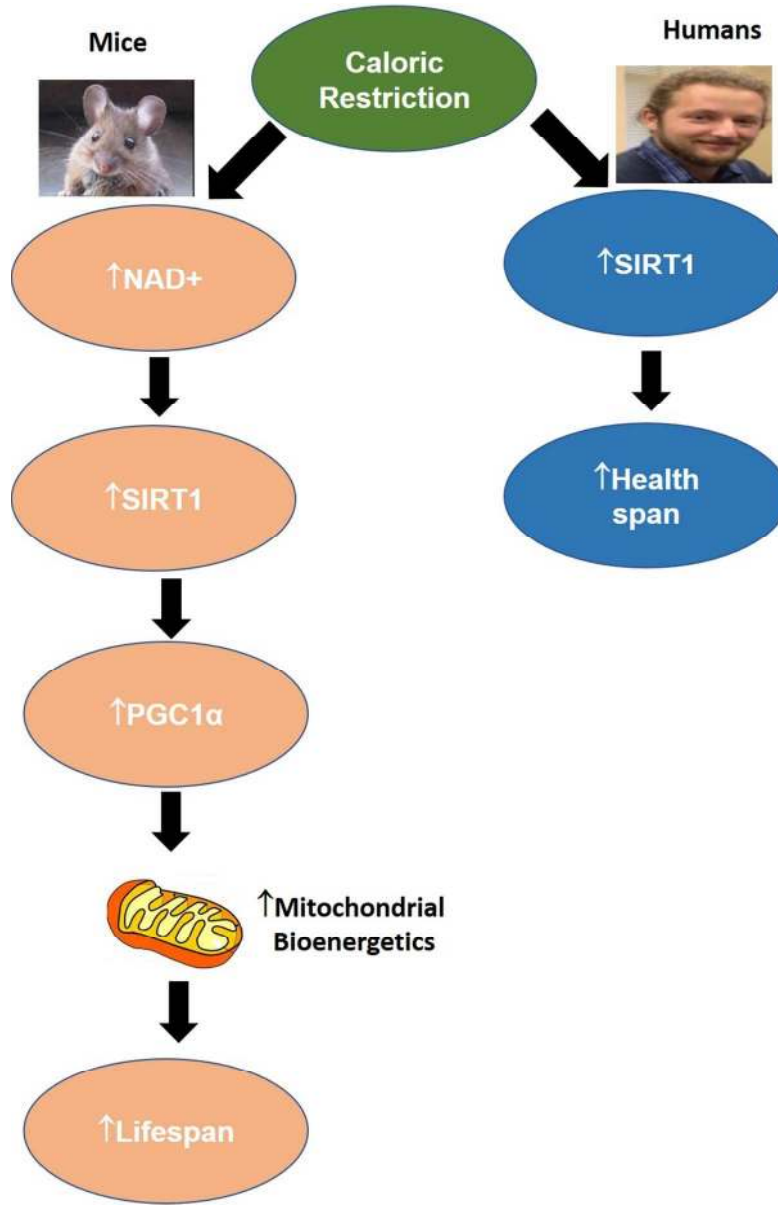


Figure 11. Modulation of NAD⁺ and NAD-dependent pathways by caloric restriction in mice and humans.

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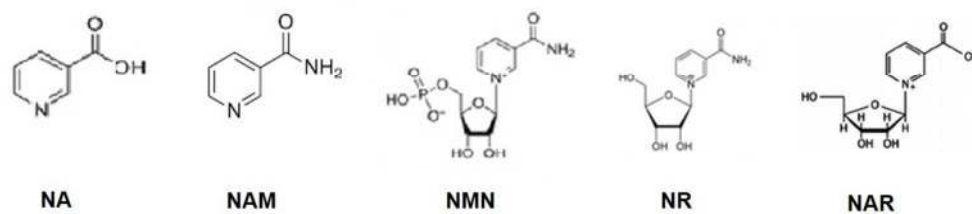


Figure 12. Chemical structure of NAD+ precursors.

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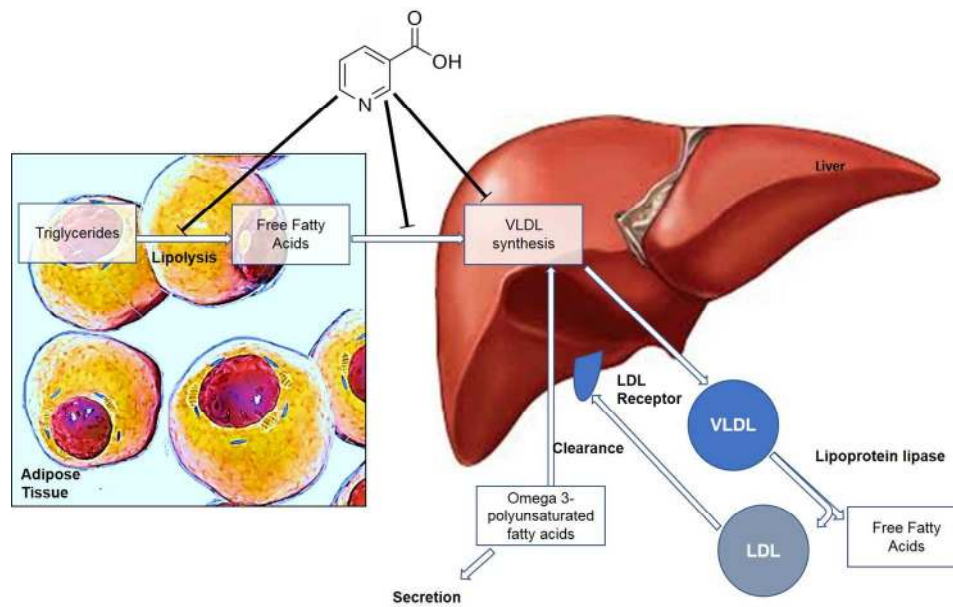


Figure 13. Mechanisms of action of nicotinic acid (NA) in dyslipidemia.

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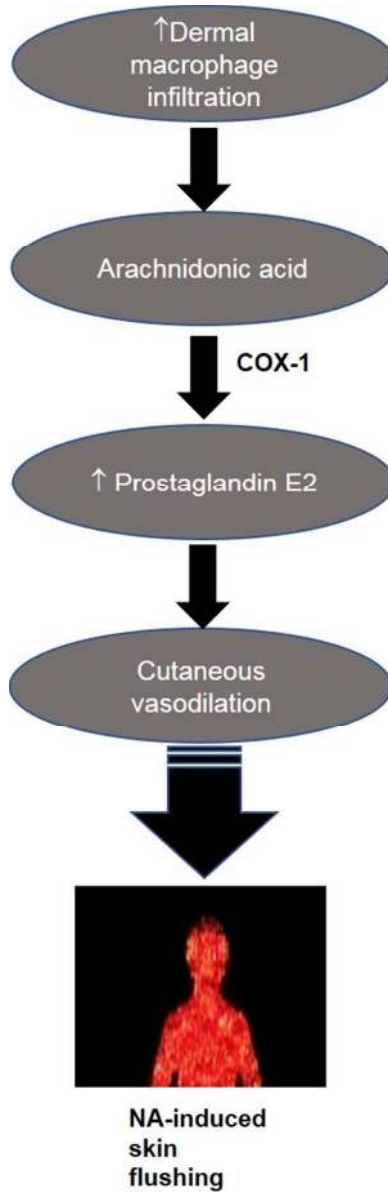


Figure 14. Schematic representation of the molecular mechanism of skin flushing following treatment with nicotinic acid (NA).

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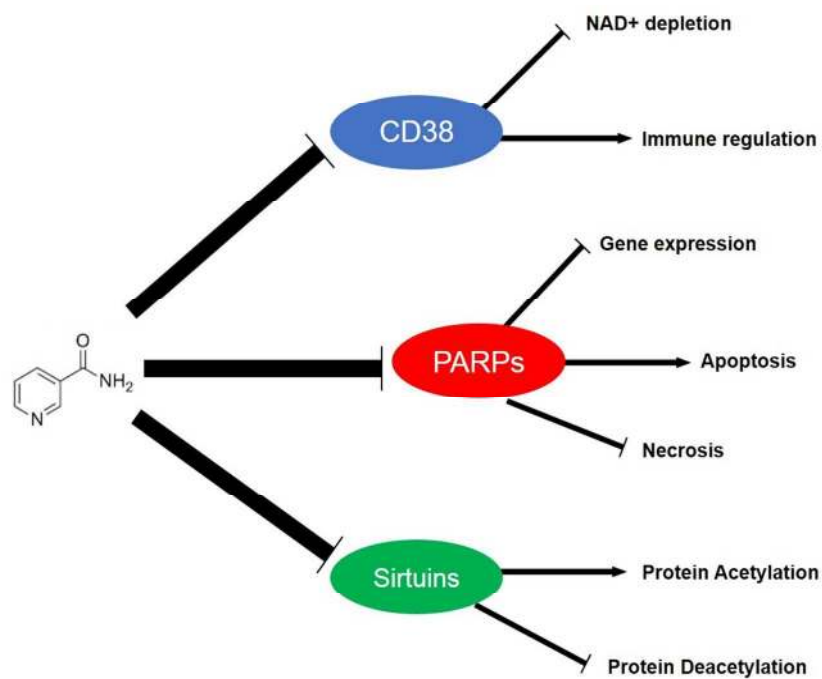


Figure 15. Mechanisms of action of nicotinamide (NAM) and its effect on the NAD⁺ metabolome.

339x232mm (96 x 96 DPI)