White Paper: NAD+

WHAT IS NAD+?

Nicotinamide adenine dinucleotide (NAD+) is a vital coenzyme in numerous metabolic processes. It is endogenously produced via several biosynthetic pathways utilising a number of precursor compounds.[1,2]

NAD+ is responsible for hydrogen transfer in REDOX reactions that drive ATP synthesis via glycolytic and fatty acid pathways. Hydrogen transfer via NAD+ also oversees the biological recycling of vitamins such as K2 and C.[12,13] It also carries out this function in the cytosol and in the mitochondrial citric acid cycle and the electron transport chain.[1,3] NAD+ is required by enzymes involved in genome transcription, replication and integrity, calcium signalling, cellular stress adaptation and oxidation response.[1,3] Recent research has discovered that NAD+ plays a role in DNA ligation (joining) and RNA capping.[3] NAD+ therefore has influence over energy production, the metabolism and maintenance of other biological nutrients, the physiological stress response, DNA repair and epigenetic adaptation.

As NAD+ is in a constant cycle of synthesis and breakdown, its metabolism is tightly regulated to maintain balance.[4]

Whilst much is made of the role of various nutrients in the *synthesis* of healthy proteins, macromolecules and tissues, NAD+ has a role in *post-synthesis* maintenance, adaptation and protection of fundamental biological proteins, macromolecules, and cells, giving it a very unique and valuable role in human health.[3]

NAD+ SYNTHESIS

NAD+ biosynthesis can occur via one of 4 pathways and utilises 4 precursors, 3 of which are vitamin B3 analogues (NAM, NR and NA).[1,2,4] Table 1 includes a key to abbreviations

Precursors:[1]

1. Nicotinamide (NAM)
2. Nicotinic Acid (NA)
3. Nicotinamide riboside (NR)
4. Tryptophan

Pathways:[1,2,4]

1. Salvage pathway utilising NA, NR and NAM
2. Preiss-Handler pathway utilising NA
3. *De novo* pathway utilising Tryptophan
4. NRK1/2 pathway utilising NR

The **salvage pathway** is the preferred pathway in mammals. In this 3-step pathway, the precursor (either NR, NA, or NAM) is converted into an intermediate substrate known as nicotinamide mononucleotide (NMN) under the influence of the enzyme nicotinamide phosphoribosyltransferase (NAMPT). The subsequent enzymatic conversion of NMN to NAD+ is facilitated by nicotinamide mononucleotide adenylyltransferase (NMNAT aka NMN transferase).[1]

NAD+ produced via this pathway, can be converted back to NAM by enzymes such as the sirtuins, PARPs and cADPR (which are discussed in more detail further on in this paper). This NAM can then go back through the salvage pathway to produce more NAD+.[1]

The **Preiss-Handler Pathway** converts dietary NA into nicotinate mononucleotide (NAMN) via nicotinic acid phosphoribosyltransferase (NAPRT). NAMN is then converted to Nicotinic acid adenine dinucleotide (NAAD) via NMNAT. NAAD is then amidated (converted to an amide) to produce NAD+ via NAD synthase which uses glutamine as a nitrogen donor.[1,3]

De novo synthesis enzymatically converts tryptophan to NAMN via quinolinate phosphoribosyltransferase (QPRT). NAMN is then converted to NAD+ (via NMNAT).[1]

Table 1: Key

NAD+	Nicotinamide adenine dinucleotide (oxidised form- hydrogen acceptor)
NADH	Nicotinamide adenine dinucleotide + hydrogen (reduced form – hydrogen donor)
NADP+	Nicotinamide adenine dinucleotide phosphate (oxidised form – hydrogen acceptor)
NADPH	Nicotinamide adenine dinucleotide phosphate + hydrogen (reduced form – hydrogen donor)
NR	Nicotinamide riboside
NA	Nicotinic acid
NAM	Nicotinamide
NMN	Nicotinamide mononucleotide (the phosphorylated form of NR)
NAMN	Nicotinate mononucleotide
NAAD	Nicotinic acid adenine dinucleotide
NAMPT	Nicotinamide phosphoribosyltransferase
NMNAT	Nicotinamide mononucleotide adenylyltransferase
NAPRT	Nicotinic acid phosphoribosyltransferase
QPRT	Quinolinate phosphoribosyltransferase

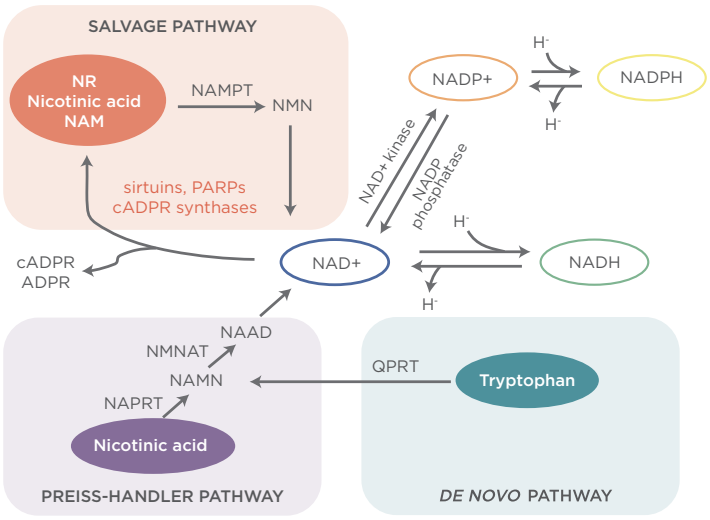

Figure 1: NAD+ biosynthesis via pathways 1, 2 and 3.[1]

Anabolism and catabolism of NAD+. NAD+ is synthesised from four precursors including nicotinamide (NAM), tryptophan, nicotinic acid, and nicotinamide riboside (NR) through three pathways (salvage pathway, *de novo* pathway, and Preiss-Handler pathway). In salvage pathway, the precursors are converted into an intermediate called nicotinamide mononucleotide (NMN) through nicotinamide phosphoribosyltransferase (NAMPT). Then NMN is converted into NAD+ via nicotinamide mononucleotide adenylyltransferase (NMNAT). NAD+ generated by this pathway is consumed by multiple enzymes including sirtuins, PARPs, and cADPR synthases, to generate NAM, and reused in salvage pathway. In Preisse-Handler pathway, nicotinic acid is converted into nicotinate mononucleotide (NAMN) through nicotinic acid phosphoribosyltransferase (NAPRT), then NAMN is converted into nicotinic acid adenine dinucleotide (NAAD) through NMN transferase (NMNAT), further generate NAD+. In *de novo* pathway, tryptophan is converted into NAMN via quinolinate phosphoribosyltransferase (QPRT), and then into NAD+ via the Preiss-Handler pathway. In redox reactions, NAD+ can be phophorylated to NADP+ by NAD+ kinase. NADP+ can be dephosphorylated to NAD+ by NADP phosphatase. Both the oxidised forms (NAD+ and NADP+) serve as hydride acceptors to generate their reduced forms (NADH and NADPH).

The **NRK1/2 pathway** (Figure 2) is similar to the salvage pathway; however, it only uses NR as a precursor, and utilises a different enzyme in the initial phase of the conversion. NRK1/2 (nicotinamide riboside kinase 1/2) enables the phosphorylation of NR to NMN (rather than NAMPT) which is then converted to NAD+ by NMNAT.[4] This pathway is preferred by tissues with high energy outputs such as the heart, as the NRK enzymes require only 1-2 ATP molecules, whereas the NAMPT enzymes require 3-4.[2]

NAD+ AND ATP SYNTHESIS

NAD+ is a cofactor (hydrogen carrier) in REDOX processes involved in mitochondrial energy metabolism and ATP synthesis.[1] NAD+ accepts hydrogen ions during reduction reactions to become NADH. NADH is subsequently utilised in intracellular processes in the cytosol and mitochondria that generate ATP by donating hydrogen ions in oxidation reactions. In other words, NAD+ can be seen as a H+ acceptor and NADH a H+ donator, participating in the conversion of key intermediate compounds and critical energy production processes in the Kreb's cycle, and maintaining REDOX homeostasis.[1,3,4]

NAD+ can also be phosphorylated to NADP+ via an enzyme called NAD+ kinase in a reversible reaction. Around 10% of cellular NAD+ is phosphorylated.[3] NADP+ can also accept hydrogen ions to become NADPH and participate in REDOX reactions that drive ATP, lipid and nucleic acid synthesis, and antioxidant processes.[1,4]

NAD+/NADH and NADP+/NADPH are active in the Citric Acid Cycle (CAC/TCA cycle) and Electron Transport Chain (ETC), as well as side chains that shuttle intermediates into and out of the entire Kreb's cycle.[3]

For every pair of electrons donated by NADH in the ETC, 2.5 ATP molecules are formed, and each day, the average adult male creates and burns approximately 80kg ATP, requiring as much as 30kg NADH to be generated and cycled through Kreb's.[4] Thus, maintenance of the NAD+ pool is vital for preserving optimal metabolic function.

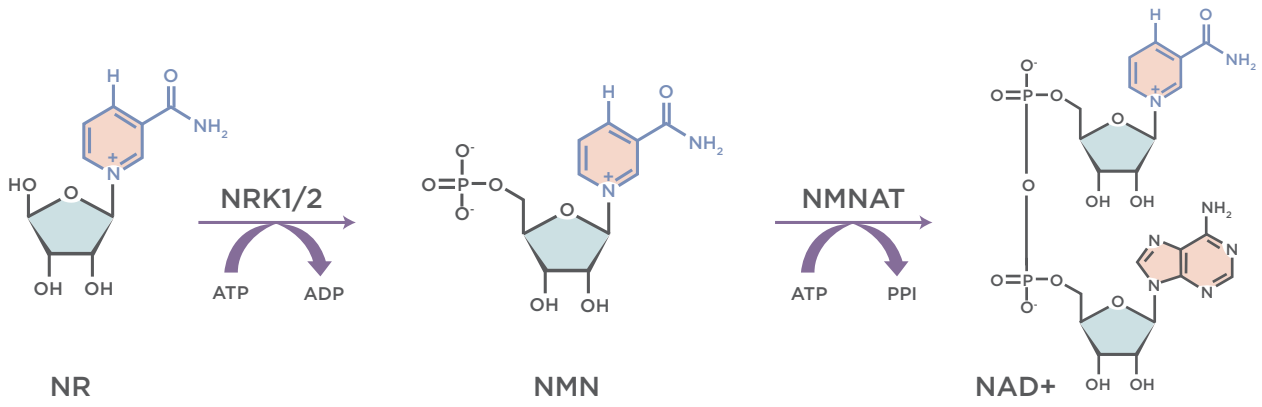

Figure 2: The NRK1/2 Pathway.[4]

NRK 1/2 mediated NAD+ biosynthesis pathway. Nicotinamide riboside (NR) is metabolised by nicotinamide riboside kinase (NRK 1/2) to nicotinamide mononucleotide (NMN) and subsequently converted to NAD+ by NMN-adenylyltransferase (NMNAT) activity).

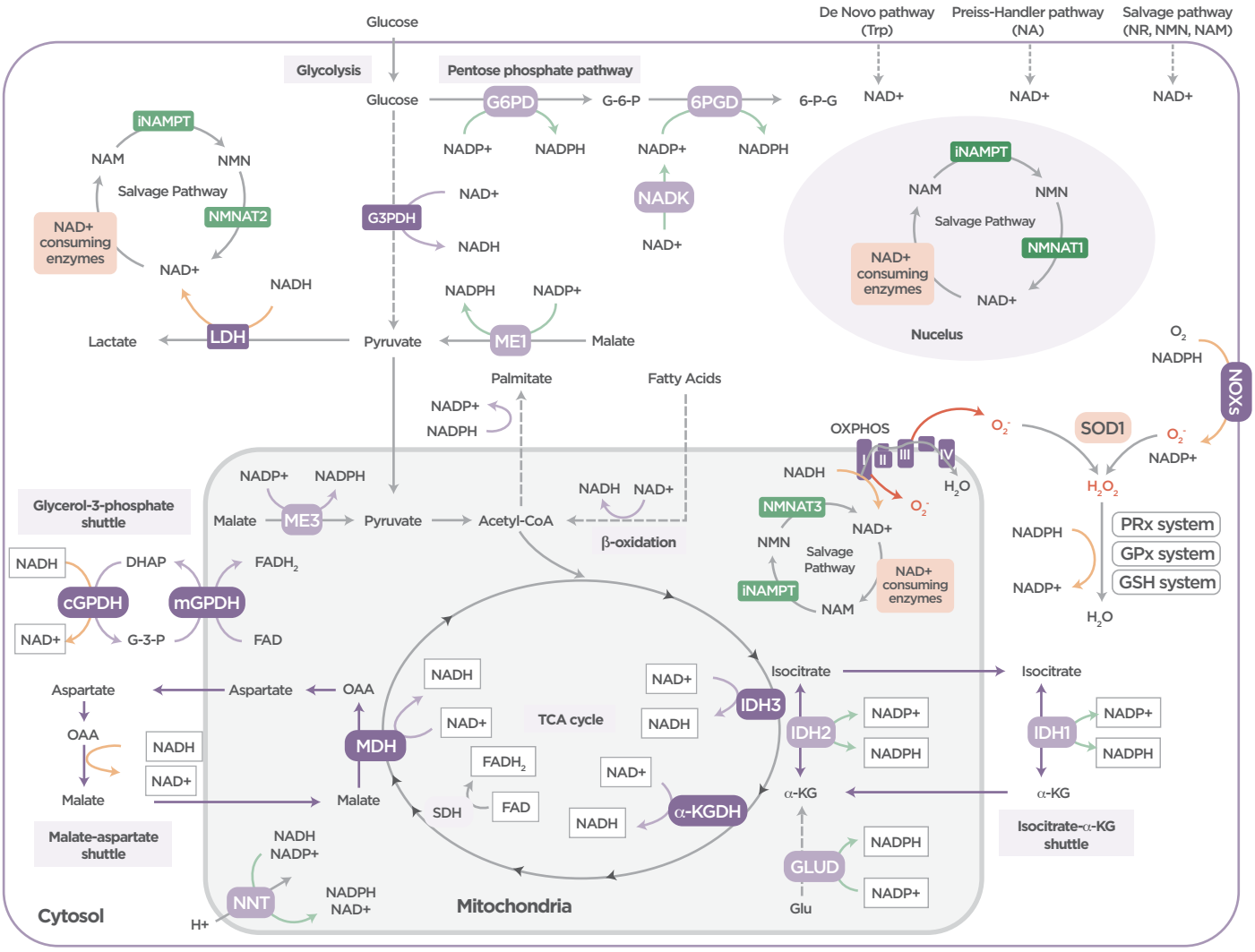

Figure 3: NAD+ utilisation in the cell and roles in energy production.[3]

Table 2: Krebs Cycle utilisation of NAD+/NADH and NADP+/NADPH[3]

COMPOUND	FUNCTION/INVOLVEMENT	LOCATION
NAD+/NADH	Conversion of glucose to pyruvate	Glycolytic pathway (cytosol)
	Hydrogen donor and acceptor	ETC (mitochondrial membrane)
	Conversion of aspartate to malate	Malate/aspartate shuttle (cytosol <-> mitochondria)
	Conversion of fatty acids to acetyl-Co-A	Citric Acid Cycle (fatty acid entrance into the CAC/TCA cycle)
	Conversion of pyruvate to lactic acid	Cytosol
NADP+/NADPH	Conversion of malate to pyruvate	Citric acid cycle (mitochondria)
	Conversion of isocitrate to α-ketoglutarate	Isocitrate/α-KG shuttle (cytosol <-> mitochondria)
	Conversion of glucose to 6-phosphogluconate	Pentose phosphate pathway (cytosol)

NAD+ BREAKDOWN & UTILISATION: NAD+ AS AN ENZYMATIC CO-FACTOR

Outside of its activity in mitochondrial REDOX systems and ATP synthesis, NAD+ acts as a co-substrate for a number of other enzymes that mediate the aging process and stress response[2] including the sirtuins, poly (ADP-ribose) polymerases (PARPS) and cyclic ADP-ribose (cADPR) synthases (such as CD38, CD73, CD157).[1,2,3] These enzymes "consume" NAD+ and contribute to its biological breakdown to NAM which cycles back into the salvage pathway to produce more NAD+.[3]

Sirtuins

The sirtuins (silent information regulator 2 (Sir 2) proteins)[11] are NAD+ reliant deacetylating anti-aging and regulating enzymes that can be found in the nucleus, mitochondria, and cytoplasm of cells.[11] They are considered to be the regulators of aging and longevity and are known as the "master switchers of metabolism"[2] and "key metabolic sensors for body homeostasis"[10] owing to their ability to regulate metabolic energy output and create adaptations to the mitochondrial response of cells to oxidative damage resulting from pathogenic or physiological stress. Under these conditions, sirtuins (particularly SIRT1 and SIRT3) can influence mitochondrial biogenesis and activity leading to more efficient ATP production, helping to modulate the stress response, repair DNA, and oversee chromatin remodelling.[2,3,4,10]

These functions give the sirtuins a role in several biological systems, and an area of focus in recent research is their role in human fertility. They play a key role in gonadal function, gametogenesis and post-fertilisation management, acting as critical antioxidant stewards in oocytes, granulosa cells and embryos. They also modulate the function of male germ cells, Sertoli cells and Leydig cells, thereby influencing spermatogenesis.[10,11] Table 3 gives a summary of the roles SIRT enzymes play in human reproductive health.

Table 3: Sirtuins in human reproductive health[10,11]

SIRT 1	• Regulates proliferation and apoptosis in granulosa cells • Oversees production of competent oocytes • Protects oocytes from premature aging • Facilitates spermatogenesis • Protects spermatozoa membranes from oxidative damage • May play a role in embryo implantation and development • May promote steroid hormone production and upregulate ovarian hormone secretion
SIRT 2	• Involved in oocyte meiosis (spindle assembly and chromosome alignment) • Protects oocytes from premature aging • Involved in cell division and embryo development
SIRT 3	• Promotes luteinisation and folliculogenesis • Promotes progesterone secretion • Improves health of oocytes (antioxidant) • Protects oocytes from premature aging • Protects early embryos against stress and supports embryo development • Protects spermatozoa membranes from oxidative damage
SIRT 6	• Oversees meiotic progression of oocytes • Follicular development/folliculogenesis

Table 4: Sirtuin functions outside of reproductive health [2,10]

SIRTUIN	LOCATION	FUNCTION
SIRT1	Cytosol and nucleus	Involved in glucose metabolism and nerve and vascular health. Oversees the balance between DNA repair, cell senescence and apoptosis.
SIRT2	Cytosol and nucleus	Manages adipose tissue development and function, blood glucose balance and myelination
SIRT3	Cytosol, nucleus & mitochondria	Functions in ATP homeostasis, detoxification of ROS in the mitochondria, DNA repair and apoptosis inhibition
SIRT4	Mitochondria	Functions in glucose metabolism and insulin secretion
SIRT5	Cytosol, nucleus & mitochondria	Involved in the urea cycle, ketone production, nitrogenous waste removal and detoxification of ROS
SIRT6	Nucleus	Plays a role in DNA repair, telomerase protection, genome stability, energy production and cholesterol balance
SIRT7	Nucleus (nucleolus)	Involved in the management of cardiac protection

PARPs

PARPs are members of the ADP-ribosyltransferase group of enzymes that facilitate the transference of ADP-ribose from NAD+ to protein compounds inducing the ADP-ribosylation (PARylation) of DNA, RNA, and other proteins. This can result in adaptations to cellular responses to oxidative stimuli, particularly when DNA has been damaged.[3,4] PARylation at damaged DNA sites attracts proteins critical for repair. During this process and due to the removal of ADP-ribose from NAD+, PARPs consume NAD+ in large amounts when active. As such, continued PARP activation may deplete intracellular NAD+ levels.[4]

cADPR synthases

cADPRSs are enzymes that also consume large amounts of NAD+. These enzymes are involved in calcium metabolism and immune function, and also seem to be involved in aging processes.[4,9] Examples of these are CD38 and CD157. CD38 expression increases as we age (which is a potential cause of the age-related decline in NAD+ levels), and interestingly, studies on CD38 knock out mice (where CD38 is reduced) showed heightened SIRT3-dependent mitochondrial function and reduced age-related NAD+ decline, indicating that NAD+ is shunted towards SIT functions in the absence of CC38. [9]

CD38 is expressed by all immune cells and its activity is intensified in circumstances when inflammation is triggered.[9] Chronic inflammation in aging (termed "Inflammaging") is one of the main drivers of age-related conditions. Persistent activation of the immune system becomes metabolically expensive in terms of the amount of energy required to fuel sustained stimulation. It has recently been found that pro-inflammatory M1 macrophages show increased NADase activity and increased CD38 expression which can both drive degradation of NR and NMN and lead to NAD+ depletion. This highlights the particularly close relationship between metabolism and immune function and indicates a requirement for NAD+ replenishment under conditions of sustained immune activation.[9]

NADP+/NADPH - AN ANTIOXIDANT ROLE

NADPH is required for oxidative reactions facilitated by enzymes such as NAPDH oxidases (NOXs), nitric oxide synthase (NOS) and cytochrome P-450. Furthermore, NADPH provides the H+ ions necessary for the thioredoxin and glutathione systems to perform their antioxidant (reducing) activity optimally, and quench destructive reactive oxygen species (ROS).[3]

NADH and NADPH are also involved in the maintenance of the antioxidant functions of vitamin C. Ascorbic acid can reduce harmful ROS by forming a one-electron donor known as an "ascorbate free radical" (AFR). The AFR is recycled (converted) back to ascorbic acid via NADH and NADPH dependent reductases.[13]

PHARMACOKINETICS AND THE NAD+ POOL

Enzymes involved in the various production pathways seem have specific tissue affinity, and therefore accumulation of NAD+ in particular tissues is dependent upon which pathway has been utilised for synthesis. For instance, the enzymes utilised in the *de novo* synthesis pathways are expressed mainly in the tissues of the liver and kidney, therefore dietary tryptophan content affects liver and kidney NAD+ levels etc.[4]

Further to this, NAD+ tends to be compartmentalised into different parts of the cell, since it can cross some membranes (e.g., cytosol/nucleus) and not others (e.g., cytosol/mitochondria). This allows intracellular NAD+ pools to act almost independently of one another, a phenomenon which is particularly important when the cytosol pool becomes depleted, as the mitochondrial pool can continue to activate the ETC, maintaining the synthesis and supply of ATP. The various shuttles discussed above however, allow a certain amount of "cross-talk" between compartments to maintain electron balance in the cell, so long-term cytosolic NAD+ depletion will eventually affect the mitochondrial pool leading to impaired ATP synthesis and energy production and eventually cause the death of the cell.[4]

Different tissues also contain different NAD+ compartment pool sizes depending on their REDOX activity and energy requirements. For example, the mitochondrial NAD+ pools of cardiac and skeletal muscle are higher than the mitochondrial NAD+ pools of hepatocytes which have greater NAD+ concentrations in the cytosol.[4]

SUPPLEMENTING WITH NAD+ PRECURSORS TO TARGET THE SALVAGE PATHWAYS

Maintaining the homeostasis between NAD+ production and breakdown is vital for the health of the cell, and NAD+ levels are known to decline with age.[9] These declining levels can be associated with the development of age-related disorders and replenishment via supplementation may slow the progression of these conditions.[5,9]
NAD+ pool replenishment seems to be best and most ubiquitously achieved when the Vitamin B3 precursors are prioritised, as is evidenced by the fact that most tissues (including macrophages) are dependent on the NAMPT (salvage) pathway for NAD+ replenishment.[4,9]

Among the B3 class of precursor, NR is the biologically preferred intermediate to use as there is a diminished risk of side effects that may come from supplementing with either NA or NAM (for example NA supplementation may induce flushing at doses greater than 50mg/day, and NAM can inhibit SIRT activity at high doses).[2] NR has been found to be superior to NA and NAM at increasing hepatic NAD+ levels, and superior to NMN at increasing NAD+ levels in muscle cells.[2] Also, as mentioned above, utilising the NAMPT enzyme is also a more energy intensive pathway as this conversion requires more ATP (3-4 molecules as opposed to 1-2 molecules utilised by NRK1/2).[2]

Supplementation with NR has shown to be safe at doses up to 2000mg daily for 12 weeks.[5] It is also more bioavailable than other precursors of the B3 class as assessed by increases in intracellular NAD+ after ingestion of all four compounds (NR, NAM, NA and NMN) in preclinical animal studies. One reason for this may be that it doesn't require conversion before entering a cell, unlike NA and NAM.[2]

NR supplementation at doses between 100 – 2000 mg[2,4,7,8] has been shown to enhance NAD+ levels and increase SIRT activity in a dose dependent manner, leading to improvements in mitochondrial number & function and ultimately energy production.[2,5,6]

Table 5a: When to consider NAD+ precursor supplementation – clinical presentations[2,3,9,10,11]

CLINICAL PRESENTATIONS	RATIONALE
Chronic fatigue	NAD+ optimises ATP production – cofactor in entire Krebs cycle. Improves REDOX balance and reduces inflammation
Fibromyalgia	NAD+ improves mitochondrial number and function in mitochondrial myopathy. Improves REDOX balance and reduces inflammation
Exercise intolerance	NAD+ optimises ATP production – cofactor in entire Krebs cycle
Poor exercise recovery	NAD+ used up during oxidative stress and inflammation
Fertility support	Antioxidative support from Sirtuin enzymes - role in gonadal function, gametogenesis, post-fertilisation management and embryo development
To provide support for healthy aging	NAD+ levels naturally decline as we age
Acute and chronic inflammatory conditions	Increased expression of CD38 requires NAD+ replenishment
Cardiovascular concerns	NR preferred as a precursor for cardiac tissue as NRK1/2 pathway is more economical energy wise. Mitochondrial dysfunction and REDOX imbalance a causative factor in CV dysfunction. NAD+ activates SIRTs (2 & 6) and maintains Ca^{2+} homeostasis via cADPR enzymes
Metabolic dysfunction	Hepatic NADP+/NADPH levels restored with supplementation, improving hepatic metabolism (hepatocyte replication and regeneration, reduction in lipid accumulation) and ATP content; NAD+ metabolism influences glucose tolerance and insulin sensitivity
Neurological dysfunction	NAD+ protects against oxidation, inflammation and DNA damage. Association between neuromuscular conditions and reduced NAD+

Table 5b: When to consider NAD+ precursor supplementation – causes of NAD+ depletion.[3,9]

Alcohol consumption
Stress
Situations that increase Krebs activity (exercise)
Aging
Inflammation
Immune system activation

References supplied on request.