The FEBS Journal

FEBS PRESS science publishing by scientists

REVIEW ARTICLE

Nicotinamide pathways as the root cause of sepsis – an evolutionary perspective on macrophage energetic shifts

Melinda S. Suchard[1,2] and Dana M. Savulescu[1]

1 Chemical Pathology, School of Pathology, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa
2 Centre for Vaccines and Immunology, National Institute for Communicable Diseases, a division of the National Health Laboratory Service, Johannesburg, South Africa

Keywords
COVID-19; IDO; immunometabolism; kynurenine; NAD; niacin; SARS-CoV-2; tryptophan; vitamin B3; Warburg

Correspondence
M. S. Suchard, Chemical Pathology, School of Pathology, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa
Tel: +2711 386 6000
E-mail: melindas@nicd.ac.za

(Received 7 October 2020, revised 7 February 2021, accepted 8 March 2021)

doi:10.1111/febs.15807

Divergent pathways of macrophage metabolism occur during infection, notably switching between oxidative phosphorylation and aerobic glycolysis (Warburg-like metabolism). Concurrently, macrophages shift between alternate and classical activation. A key enzyme upregulated in alternatively activated macrophages is indoleamine 2,3-dioxygenase, which converts tryptophan to kynurenine for *de novo* synthesis of nicotinamide. Nicotinamide can be used to replenish cellular NAD^+ supplies. We hypothesize that an insufficient cellular NAD^+ supply is the root cause of metabolic shifts in macrophages. We assert that manipulation of nicotinamide pathways may correct deleterious immune responses. We propose evaluation of nicotinamide (Vitamin B3) and analogues, including isoniazid, nicotinamide mononucleotide and nicotinamide riboside, as potential therapy for infectious causes of sepsis, including COVID-19.

Introduction

Sepsis, not to be confused with 'septicaemia' (bloodstream infection), is life-threatening organ dysfunction condition caused by the body's own dysregulated response to infection [1]. Sepsis can be triggered by diverse pathogens and noninfectious insults. Why some people succumb to sepsis from triggers such as influenza or SARS-CoV-2 infection, while others have mild or asymptomatic infection, are unknown. Heightened susceptibility with old age is common with many infections [2,3], as is the association with comorbidities often described as immunosuppressive, including type 2 diabetes [3–7]. While many eyes focus on pathogen itself, a closer look at host immune and metabolic responses to diverse pathogens is critical.

Avenues of investigation from both ageing research and immunology are converging attention on a pathway, which connects immune responses with ageing [8–13]. Nicotinamide adenine dinucleotide (NAD) in its oxidized (NAD^+) and reduced (NADH) forms plays an essential role in energy metabolism in every eukaryotic cell [14,15]. Ageing research has shown that NAD metabolism is crucial in determining 'healthy ageing' [16]. Declining NAD^+ levels and reduced NAD^+ : NADH ratios are associated with age-related declines in cellular functions [17]. NAD-degrading enzymes including poly (ADP-ribose) polymerases (PARPs), sirtuins and CD38 have similarly been linked with ageing. Sirtuins are NAD^+-dependent proteins involved in longevity

Abbreviations
AIDS, acquired immune deficiency syndrome; DNA, deoxyribonucleic acid; HIV, human immunodeficiency virus; IDO, indoleamine 2,3-dioxygenase; IL, interleukin; NAD, nicotinamide adenine dinucleotide; NAMPT, nicotinamide phosphoribosyltransferase; PARP, poly(ADP-ribose) polymerase; RNA, ribonucleic acid; TNF-α, tumour necrosis factor alpha.

associated with caloric restriction [18]. Similarly, advances in immunology have shown that *de novo* production of nicotinamide, a precursor of NAD^+, is a central pathway upregulated in many infectious diseases [8,19]. In the macrophage, a central mediator of immune responses, *de novo* NAD^+ synthesis was required to maintain an 'anti-inflammatory homeostatic state with robust phagocytic capacity' [8].

We propose that sepsis due to SARS-CoV-2 or other viral or bacterial infections is caused by pathogens interfering with host NAD metabolism. Elderly individuals, or those with pre-existing diseases, may already have certain aberrations in their NAD metabolic pathways that predispose them to more severe disease [20]. We will review recent understanding of metabolic shifts in cells of the immune system, particularly macrophages, and link macrophage metabolic shifts to intracellular NAD concentration as a focal point. We use an evolutionary perspective to motivate that alterations in NAD metabolism may be causal rather than merely correlated with infectious disease.

Nicotinamide and macrophage phenotypes

In humans, nicotinamide is acquired from dietary sources as vitamin B3 and subsequently recycled through existing nicotinamide pools. Alternatively, nicotinamide may be synthesized *de novo* from the amino acid tryptophan. The rate-limiting enzyme for *de novo* nicotinamide synthesis is indoleamine 2,3-dioxygenase (IDO), a haem-containing intracellular enzyme found predominantly in cells of the macrophage/monocyte lineage.

Macrophages have long been described as able to become activated in one of two ways, simplistically thought of as 'pro-inflammatory' and 'anti-inflammatory' or termed by 'M1' and 'M2' nomenclature. Technicalities and disputes regarding nomenclature abound [21], but the principle holds that macrophages have at least two divergent ways of becoming activated, rather than the stereotypical pro-inflammatory textbook version. M1 macrophages secrete pro-inflammatory cytokines such as TNF alpha and interleukin 1-β, while M2 macrophages secrete cytokines such as interleukin-10, which have immune suppressive functions and play a role in wound healing.

Key enzyme activities differentiate between M1 and M2 macrophages. From arginine as a substrate, M1 macrophages use nitric oxide synthase to make nitric oxide, while M2 macrophages use arginase to catabolize arginine to urea [22]. Most strikingly, M2 macrophages express IDO, which diverts tryptophan to *de novo* synthesis of nicotinamide rather than towards serotonin synthesis [23]. IDO-mediated tryptophan catabolism in turn depresses T-cell proliferation and stimulates regulatory T cells, which are cells with immune suppressive function [24].

Counterintuitively, IDO is induced by pro-inflammatory cytokines such as interferon-γ [25]. The IDO-catalysed *de novo* nicotinamide synthesis pathway is therefore upregulated during diverse infectious conditions as a response to pro-inflammatory cytokines [26]. We can infer that the IDO pathway may be part of a negative feedback loop to dampen inflammation.

Immunometabolism in macrophages

Recent work has led to the burgeoning field of immunometabolism [19,27,28]. Not only do M1 and M2 macrophages differ in the cytokines produced and enzymes activated, but also they differ in their glucose metabolism [29,30]. Immune cells, including macrophages, lymphocytes and neutrophils, can switch between the 'resting' state, during which the cell undergoes glycolysis, Krebs cycle and oxidative phosphorylation, and an activated state in which aerobic glycolysis (also known as Warburg-like metabolism) occurs [29,30]. During Warburg-like metabolism, glycolysis (aerobic glycolysis) is completed without further completion of a traditional Krebs cycle and electron transport chain. Warburg-like metabolism, originally identified as a hallmark of cancer cells, is often associated with cell division and cell activation [30–32]. When infected with pathogens, macrophages shift their metabolism to a Warburg-like metabolism, with potential pathogen-specific variations [19,33]; for example, *Legionella pnuemophila*, *Chlamydia trachomatis* and *Mycobacterium tuberculosis* may impact metabolic pathways at different points, all upregulating aerobic glycolysis [19]. In T and B lymphocytes, shifting to Warburg-like metabolism is associated with lymphocyte proliferation [34].

Evolutionary perspective on immunometabolism

To elucidate how macrophage or lymphocyte metabolic shifts correlate with infection by 'foreign' microorganisms, an evolutionary perspective may be of value. All eukaryotic cells require NAD and NADH for metabolism [15,35]. This commonality stems from the origin itself of eukaryotic life, when two prokaryotes (an α-proteobacterium and an archaeon) fused, after which the α-proteobacterium became the mitochondrion [36].

Mitochondrial function within the eukaryotic cell largely revolves around a hydrogen-dependent symbiosis, mediated through NAD and NADH [37]. Mitochondria control not only the life but also the death of the cell, including initiation of programmed cell death pathways [38]. Viewed in this light, the switch to aerobic glycolysis (Warburg-like metabolism) during times of immune activation is indicative of a change in mitochondrial function, almost as if the cell reverts to a more prokaryotic form of metabolism, becoming temporarily independent of its need for symbiotic mitochondrial metabolism.

More recently in evolutionary terms, focusing our attention on human reproductive fitness and immune tolerance may illuminate triggers for IDO production. In addition to macrophages, IDO is expressed in placental tissue and is essential for mammalian immune tolerance of an allogeneic, but not syngeneic fetus [39]. During sperm–ovum fusion, the ovum actively targets and destroys male mitochondria, a process known as mitophagy or allophagy [40,41]. The mechanism of sensing which mitochondria are paternal remains unelucidated. Mitochondrial contents are reminiscent of their bacterial origins, differing from nuclear or cytoplasmic contents by the presence of unmethylated CpG motifs in mitochondrial DNA [42], possession of unique lipids including cardiolipin [43] and synthesis of double-stranded RNA intermediates [44], which can trigger innate immune responses if released into the cytoplasm or circulation [45]. Mitochondria themselves harbour receptors, such as the mitochondrial antiviral signalling protein (MAVS), that can be triggered by double-stranded RNA [46]. It is plausible that mitochondrial sensing of double-stranded RNA, or other mitochondrial patterns, is a mechanism that has been conserved through evolution, stemming from similar functions during mitophagy at conception. Mitochondrial switching between oxidative phosphorylation and Warburg-like metabolism may be the response of the mitochondria to sensing foreign mitochondrial patterns within the cell, both during reproduction and during infection. We hypothesize that mitochondrial sensing of foreign double-stranded RNA or other mitochondrial identifiers increases the cellular requirement for NAD^+, thereby prompting increased tryptophan conversion to nicotinamide via IDO activation. Such a requirement for increased *de novo* nicotinamide production could explain the requirement for IDO activity in materno-fetal tolerance at the placenta. Intersection of NAD pathways with mitochondrial metabolism points in the direction of mitochondrial metabolic shifts correlating with altered cellular NAD requirements.

NAD requirements of the macrophage may prompt metabolic shifts

In M2 macrophages, a fully functional Krebs cycle and oxidative phosphorylation within the mitochondrion corresponds to increased IDO activity within the cytoplasm (Fig. 1). In classically activated macrophages, however, a shift that can be triggered during diverse infections, mitochondria undergo only limited function [19,33]. Sensing of double-stranded RNA is known to trigger conserved mitochondrial response pathways [47–49]. We hypothesize that mitochondrial response to double-stranded RNA precipitates an acute cellular NAD insufficiency. A detectable increase in IDO activity would be a measure of the body's attempt to compensate for insufficient nicotinamide. Elevated IDO activity for *de novo* nicotinamide synthesis would be termed an initial M2 shift. If *de novo* nicotinamide synthesis failed to meet cellular NAD requirements, the macrophage may shift to classical activation, speculatively due to a lower NAD requirement.

A closer look at pathways intersecting with NAD

Cameron and colleagues showed *in vitro* that during Warburg-like metabolism, reactive oxygen species were upregulated, DNA damage occurred and PARP enzymes were upregulated, depleting NAD^+. M1 macrophage activation occurred simultaneously with NAD^+ depletion and upregulation of the NAD salvage enzyme, nicotinamide phosphoribosyltransferase (NAMPT) [50]. The authors attributed early depletion of NAD^+ to PARP activity, but noted that sirtuins and CD38 may also have been involved with NAD^+ depletion. Their work illustrated that NAD depletion was a key feature of M1 activation, but multiple hypotheses may apply as to the initial cause of NAD^+ depletion and PARP activation [50].

Zhang *et al* investigated low-dose compared with high-dose endotoxin responses *in vitro*. They showed that low-dose endotoxin triggered upregulation of NAMPT, resulting in NAD salvage from nicotinamide, while high-dose endotoxin caused a switch to *de novo* nicotinamide synthesis from tryptophan via IDO [51]. NAD synthesis (via NAMPT or IDO) affected nuclear NAD^+ pools, which could affect transcription of key inflammatory genes.

NAD^+ is released from cells during early inflammation and functions as an extracellular signalling molecule, resulting in death of naive $CD4^+$ and $CD8^+$ T cells while relatively sparing activated and memory cells [52]. The ectoenzyme CD38, an activation marker on T and B

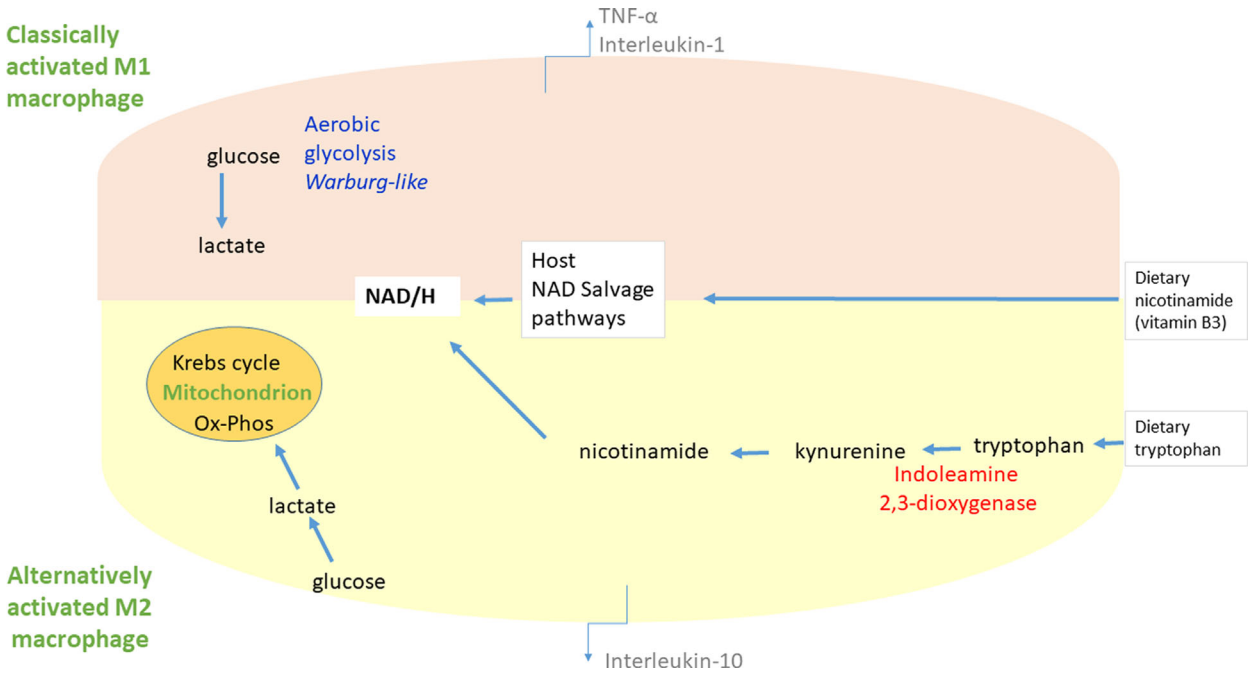

Fig. 1. Increased IDO activity towards *de novo* nicotinamide synthesis is a hallmark of alternatively activated macrophages. Classically activated macrophages secrete pro-inflammatory cytokines such as tumour necrosis factor-α (TNF-α) and interleukin-1. Alternatively activated macrophages secrete anti-inflammatory cytokines such as interleukin-10 [21]. In terms of mitochondrial function, classically activated (M1) macrophages undergo aerobic glycolysis (Warburg-like metabolism) without completion of the Krebs cycle or oxidative phosphorylation, despite the presence of oxygen. Alternatively activated (M2) macrophages undergo glycolysis followed by the Krebs cycle and electron transport chain [19]. IDO upregulation in alternatively activated macrophages may be an attempt to compensate for insufficient NAD production through salvage pathways and dietary uptake.

lymphocytes, functions as an NADase, regulating extracellular concentrations of NAD^+ [52]. CD38 can also be upregulated on airway smooth muscle cells in response to inflammatory cytokines [53]; thus, the NADase activity of CD38 is not limited to immune cells [54].

NAD^+ also plays a key role in autophagy through partnering with sirtuins, which are NAD-dependent deacetylases [55]. Autophagy relates to degradation of intracellular organelles, often in response to nutrient stresses [55]. Sirtuins regulate autophagy [55], as well as circadian rhythms in the cell [56]. The intersection of mitochondrial, cytoplasmic, nuclear and extracellular NAD pools is critical for key cellular functions including DNA repair (via PARP enzymes), autophagy (via sirtuins) and activation or suppression of surrounding lymphocytes (via CD38) [57].

Interaction of infectious organisms with *de novo* nicotinamide synthesis

Interestingly, IDO activity is elevated in human sepsis and severe inflammatory response syndrome (SIRS), with higher values predicting mortality [58]. Patients with sepsis of diverse origins have increased kynurenine and decreased tryptophan plasma concentrations [59–61]. Kynurenine/tryptophan ratios are higher in patients with severe sepsis than in those with mild sepsis, and were inversely related to microvascular reactivity [59]. Kynurenine has blood vessel-relaxing properties [62], and it has been suggested that IDO activity links together immune dysregulation and loss of microvascular reactivity in sepsis [59].

Many bacteria use NAD and NADH in their own metabolism. As prokaryotes, bacteria have their own unique pathways of NAD synthesis. Commensal and pathogenic flora form a complex cycle of NAD synthesis and consumption, which may regulate the pathogen–host balance [19,63]. As an example, tuberculosis-necrotizing toxin, secreted by Mycobacterium tuberculosis, with homologues in many bacterial and fungal pathogens, hydrolyses NAD^+ and results in cellular death by necroptosis [64]. Mycobacterium tuberculosis can also synthesize niacin [65], leading to postulates that latent Mycobacterium tuberculosis has beneficial effects for the human host by increasing nicotinamide availability [63,66].

Viruses cannot synthesize their own NAD or NADH but usurp host metabolism to engage with the

host NAD pathways; for example, acute and chronic viral infections affect IDO activity and nicotinamide pathways [67–69]. Cytomegalovirus, influenza virus, herpesviruses 1 and 2, hepatitis B, hepatitis C and human immunodeficiency virus (HIV) interact with the IDO pathway [67–69]. IDO activity is elevated during HIV infection, resulting in raised kynurenine/tryptophan ratios. IDO activity represents an independent marker of disease progression to acquired immunodeficiency syndrome (AIDS), whereas antiretrovirals decrease the kynurenine/tryptophan ratio [69]. Notably, acute viral infection with influenza virus induces IDO activity [70]. Importantly, in the Human Influenza INSIGHT FLU 003 Plus study, increased IDO activity was associated with poor clinical outcome (death, transfer to intensive care or requiring mechanical ventilation) [71].

Patients with severe disease from SARS-CoV-2 infection (coronavirus disease 2019 or COVID-19) often have lymphopenia together with high white cell counts, suggesting involvement of myeloid cells in disease pathogenesis [72]. Lung pathology in COVID-19 has been ascribed to hyperinflammatory syndrome or cytokine release occurring in the later phases of the illness [72], with some descriptions akin to macrophage activation syndrome [73,74]. The replication of SARS-CoV-2, similar to many other viruses [75], comprises a double-stranded RNA step, which may be sensed by mitochondrial antiviral mechanisms [76]. SARS-CoV-2 has been shown experimentally to upregulate PARP enzymes, which degrade NAD^+ [77]. Higher titres of SARS-CoV-2 differentially regulated various PARP enzymes and downregulated quinolinic acid phosphoribosyltransferase, an enzyme required for *de novo* nicotinamide synthesis from tryptophan, while upregulating NAMPT, which utilizes nicotinamide to synthesize NAD [77]. In a transcriptomics analysis, SARS-CoV-2 downregulated nuclear-encoded mitochondrial genes coding for complex 1 of the mitochondrial electron transport chain [78]. Thus, evidence exists to suggest that SARS-CoV-2 interacts with host NAD^+ metabolism. Intriguingly, loss of smell and taste, symptoms associated with COVID-19, was recognized in the 1930s as pellagra symptoms, amenable to niacin treatment [79].

The link between infection, NAD^+ consumption and metabolic shifts in macrophages

Shifts in macrophage metabolism during infection are only partially understood, and opposing explanations may fit the observations described. Our preferred hypothesis is that viral infection initially triggers increased consumption of NAD^+ or decreased availability of NAD^+ for the human host. The IDO pathway is then upregulated to replenish NAD^+ supplies (an initial M2 shift), and when capacity of the *de novo* pathway is exceeded, a relative deficiency of NAD^+ may force a shift to M1 phenotype. Alternative explanations have not been excluded; for example, infection may initially trigger a shift to an M1 phenotype, speculatively leading to decreased NAD^+ availability, after which IDO is upregulated in order to meet the need for *de novo* synthesis, which would be seen as a secondary M2 shift. Further work will elucidate these intricacies, but the understanding that pathogens induce shifts in human metabolism leads to practical host-directed interventions. Kynurenine biosynthesis via IDO upregulation is a signature of infection with a wide range of pathogens [68]. Increased kynurenine biosynthesis implies that diverse microbes trigger a convergent host response of increased *de novo* nicotinamide synthesis, likely secondary to an increased NAD^+ requirement. Interventions targeted at the IDO-catalysed pathway may therefore ameliorate severe illness, despite diverse aetiologies.

Novel therapies for sepsis

If the primary pathology is competition for depleted NAD^+ stores, the most rational intervention would be to increase NAD^+ supply. The leading candidate for investigation for treatment of sepsis, including COVID-19, should therefore be nicotinamide (Vitamin B3) and related compounds, in agreement with other authors [20,77,80–85]. Indeed, in animal models, vitamin B3 ameliorates polymicrobial sepsis [86], lung ischaemia–reperfusion injury [87] or experimentally induced lung fibrosis [88]. In animals, nicotinamide mononucleotide preserves mitochondrial function and promotes survival from haemorrhagic shock [89]. Importantly, in a mouse model of SARS-CoV-2 infection, nicotinamide administration reduced inflammatory cell aggregates, emboli and cell death [90] We will focus further on the experience with administration of vitamin B3 and related compounds to humans.

Nicotinamide has been used for a multitude of clinical indications, including lung diseases, as outlined below. The stalwart of antituberculosis therapy, isoniazid, was initially developed as a nicotinamide analogue, but showed superior performance compared with nicotinamide for clinical outcome in Tuberculosis [91]. Indeed, pyrazinamide – another antituberculosis agent – has downstream metabolites, which convert nicotinamide to NAD^+ [10]. Thus, at least some of the efficacy of isoniazid and pyrazinamide for treatment of sepsis caused by

tuberculosis may be due to interactions of the drugs with NAD synthesis and salvage [63].

There is extensive prior experience with nicotinamide in human trials for conditions other than infections [92–98]. Nicotinamide forms part of certain cancer treatment regimens [93,94]. The recommended daily dose of nicotinamide is 14–16 mg, and nutritional supplementation dosages are usually below 35 $mg{\cdot}day^{-1}$. The upper recommended limit for adults is, however, 900 $mg{\cdot}day^{-1}$ [99], and doses used for treatment of malignancy are approximately 3 $g{\cdot}day^{-1}$. Impaired oxidative burst activity of neutrophils in type 2 diabetics was improved by nicotinamide supplementation at 50 $mg{\cdot}kg^{-1}{\cdot}day^{-1}$ [100]. Dosage of nicotinamide for clinical trials should thus span physiological and higher ranges for thorough assessment of clinical impact.

In addition to experience with nicotinamide, three related compounds interact at various points in NAD metabolism and are options for human therapeutic trials for sepsis. Niacin is a clinically licensed therapy for hypercholesterolaemia; however, its use may be limited by side effects such as flushing. Ageing experts recommend an alternate product, nicotinamide mononucleotide, as their drug of choice for prevention of age-related declines, with multiple examples in animal models [101,102] and safety data in humans [103]. Nicotinamide mononucleotide may have superior properties to nicotinamide in terms of its activity and side effect profile [102]. Importantly, dramatic clinical improvement was reported in a case series of nine severe COVID-19 patients treated with a nicotinamide mononucleotide cocktail (nicotinamide mononucleotide, betaine, sodium chloride and zinc sulfate) [85]. A third compound, nicotinamide riboside, has shown safety in human trials and ability to raise whole blood NAD^+ levels [104]. Some have suggested that for treatment of COVID-19, administration of nicotinamide-related compounds should be accompanied by an inhibitor of PARP enzymes, which degrade NAD^+ [83].

Conclusion

In summary, we advocate adjusting our understanding of pathogenesis of infectious illness away from a microbe-oriented view, such that the microbe is seen as 'causing' the disease, towards a host NAD–metabolism-oriented view, where the microbe is seen as triggering an evolutionarily conserved response that shifts NAD metabolism. In certain contexts, such as during reproduction, shifts in NAD metabolism may be beneficial for the organism. In the context of disease, such shifts are associated with adverse consequences. Understanding interactions of various bacterial and viral pathogens with the NAD pathway will guide us further. While biochemical studies will ultimately yield mechanistic explanations, a more direct approach is to investigate vitamin B3-related compounds in human trials for sepsis.

The *de novo* nicotinamide synthesis pathway, catalysed by IDO, poses a therapeutically malleable pathway integrally linked to host mitochondrial metabolism and to immune tolerance. Understanding the relationship between pathogenic infection and macrophage metabolism, and identifying how to diagnose and direct flux through the *de novo* nicotinamide synthesis pathway, should lead towards host-directed therapy for sepsis. Host–microbe competition and interaction for limited intracellular NAD^+ supplies are the lens through which we should view mitochondrial metabolic shifts within cells of the immune system.

In conclusion, we strongly suggest that vitamin B3 be investigated as a therapy for sepsis, including that caused by COVID-19, ideally as a single agent at high dose rather than within a multivitamin, which will not allow accumulation of efficacy data. If nicotinamide is found to be ineffective, related compounds including isoniazid, niacin, nicotinamide riboside and nicotinamide mononucleotide are alternate prospects that impact NAD pathways at different entry points of the NAD cycle and warrant further investigation.

Acknowledgements

MSS acknowledges Pierre Durand of the University of the Witwatersrand for formative discussions on evolutionary medicine and critical reading of the manuscript. This work was partially funded by the Strategic Health Innovations Partnerships Unit of the South African Medical Research Council with funds from the South African Department of Science and Technology.

Conflict of interest

The authors declare no conflict of interest.

Author contributions

MSS conceptualized and wrote the piece. DMS edited the manuscript and gave critical comment.

References

1 Marik PE & Taeb AM (2017) SIRS, qSOFA and new sepsis definition. *J Thorac Dis* **9**, 943–945.
2 Thompson WW, Shay DK, Weintraub E, Cox N, Anderson LJ & Fukuda K (2003) Mortality associated

with influenza and respiratory syncytial virus in the United States. *J Am Med Assoc* **289**, 179.
3 Ma CM, Yin FZ & Nakajima K (2019) The mortality in infectious inpatients with type 2 diabetes compared with non-diabetic population: infection in type 2 diabetes. *Medicine (Baltimore)* **98**, e16025.
4 Beumer MC, Koch RM, van Beuningen D, OudeLashof AM, van de Veerdonk FL, Kolwijck E, van der Hoeven JG, Bergmans DC & Hoedemaekers CWE (2019) Influenza virus and factors that are associated with ICU admission, pulmonary co-infections and ICU mortality. *J Crit Care* **50**, 59–65.
5 Kim EJ, Ha KH, Kim DJ & Choi YH (2019) Diabetes and the risk of infection: a national cohort study. *Diabetes Metab J* **43**, 804.
6 Bertoni AG, Saydah S & Brancati FL (2001) Diabetes and the risk of infection-related mortality in the U.S. *Diabetes Care* **24**, 1044–1049.
7 Zador Z, Landry A, Cusimano MD & Geifman N (2019) Multimorbidity states associated with higher mortality rates in organ dysfunction and sepsis: a data-driven analysis in critical care. *Crit Care* **23**, 247.
8 Minhas PS, Liu L, Moon PK, Joshi AU, Dove C, Mhatre S, Contrepois K, Wang Q, Lee BA, Coronado M *et al*. (2019) Macrophage de novo NAD+ synthesis specifies immune function in aging and inflammation. *Nat Immunol* **20**, 50–63.
9 Kasperski A & Kasperska R (2018) Bioenergetics of life, disease and death phenomena. *Theory Biosci* **137**, 155–168.
10 Rajman L, Chwalek K & Sinclair DA (2018) Therapeutic potential of NAD-boosting molecules: the in vivo evidence. *Cell Metab* **27**, 529–547.
11 Okabe K, Yaku K, Tobe K & Nakagawa T (2019) Implications of altered NAD metabolism in metabolic disorders. *J Biomed Sci* **26**, 34.
12 Massudi H, Grant R, Guillemin GJ & Braidy N (2012) NAD + metabolism and oxidative stress: the golden nucleotide on a crown of thorns. *Redox Rep* **17**, 28–46. https://doi.org/10.1179/1351000212Y.0000000001
13 Sas K, Szabó E & Vécsei L (2018) Mitochondria, oxidative stress and the kynurenine system, with a focus on ageing and neuroprotection. *Molecules* **23**, 191.
14 Pétriacq P, de Bont L, Tcherkez G & Gakière B (2013) NAD: not just a pawn on the board of plant-pathogen interactions. *Plant Signal Behav* **8**, e22477.
15 Ternes CM & Schönknecht G (2014) Gene transfers shaped the evolution of de novo NAD+ biosynthesis in eukaryotes. *Genome Biol Evol* **6**, 2335–2349.
16 Mitchell SJ, Bernier M, Aon MA, Cortassa S, Kim EY, Fang EF, Palacios HH, Ali A, Navas-Enamorado I, Di Francesco A *et al*. (2018) Nicotinamide improves aspects of healthspan, but not lifespan, in mice. *Cell Metab* **27**, 667–676.e4.
17 Gomes AP, Price NL, Ling AJY, Moslehi JJ, Montgomery MK, Rajman L, White JP, Teodoro JS, Wrann CD, Hubbard BP *et al*. (2013) Declining NAD+ induces a pseudohypoxic state disrupting nuclear-mitochondrial communication during aging. *Cell* **155**, 1624–1638.
18 Guarente L & Picard F (2005) Calorie restriction – the SIR2 connection. *Cell* **120**, 473–482.
19 Escoll P & Buchrieser C (2018) Metabolic reprogramming of host cells upon bacterial infection: why shift to a Warburg-like metabolism? *FEBS J* **285**, 2146–2160.
20 Miller R, Wentzel AR & Richards GA (2020) COVID-19: NAD+ deficiency may predispose the aged, obese and type2 diabetics to mortality through its effect on SIRT1 activity. *Med Hypotheses* **144**, 110044.
21 Martinez FO & Gordon S (2014) The M1 and M2 paradigm of macrophage activation: time for reassessment. *F1000Prime Rep* **6**, 13.
22 Munder M (2009) Arginase: an emerging key player in the mammalian immune system. *Br J Pharmacol* **158**, 638–651.
23 Wang XF, Wang HS, Wang H, Zhang F, Wang KF, Guo Q, Zhang G, Cai SH & Du J (2014) The role of indoleamine 2,3-dioxygenase (IDO) in immune tolerance: focus on macrophage polarization of THP-1 cells. *Cell Immunol* **289**, 42–48.
24 Savage NDL, de Boer T, Walburg KV, Joosten SA, van Meijgaarden K, Geluk A & Ottenhoff THM (2008) Human anti-inflammatory macrophages induce Foxp3 + GITR + CD25 + regulatory T cells, which suppress via membrane-bound TGFβ-1. *J Immunol* **181**, 2220–2226.
25 Dai W & Gupta SL (1990) Regulation of indoleamine 2,3-dioxygenase gene expression in human fibroblasts by interferon-γ. Upstream control region discriminates between interferon-γ and interferon-α. *J Biol Chem* **265**, 19871–19877.
26 Grant RS (2018) Indoleamine 2,3-dioxygenase activity increases NAD+ production in IFN-γ-stimulated human primary mononuclear cells. *Int J Tryptophan Res*. https://doi.org/10.1177/1178646917751636
27 Diskin C & Pålsson-McDermott EM (2018) Metabolic modulation in macrophage effector function. *Front Immunol* **9**. https://doi.org/10.3389/fimmu.2018.00270
28 O'Neill LAJ, Kishton RJ & Rathmell J (2016) A guide to immunometabolism for immunologists. *Nat Rev Immunol* **16**, 553–565.
29 Galván-Peña S & O'Neill LAJ (2014) Metabolic reprogramming in macrophage polarization. *Front Immunol*. https://doi.org/10.3389/fimmu.2014.00420
30 Tang CY & Mauro C (2017) Similarities in the metabolic reprogramming of immune system and endothelium. *Front Immunol* **8**, 837.
31 Palmer CS, Ostrowski M, Balderson B, Christian N & Crowe SM (2015) Glucose metabolism regulates T cell

activation, differentiation, and functions. *Front Immunol* **6**, 1.
32 Warburg O (1956) On the origin of cancer cells. *Science*, **123**, 309–314. https://doi.org/10.1126/science.123.3191.309
33 Ramond E, Jamet A, Coureuil M & Charbit A (2019) Pivotal role of mitochondria in macrophage response to bacterial pathogens. *Front Immunol* **10**, 2461.
34 Abdel-Haleem AM, Lewis NE, Jamshidi N, Mineta K, Gao X & Gojobori T (2017) The emerging facets of non-cancerous Warburg effect. *Front Endocrinol (Lausanne)* **8**, 279.
35 Rongvaux A, Andris F, Van Gool F & Leo O (2003) Reconstructing eukaryotic NAD metabolism. *BioEssays* **25**, 683–690.
36 Sagan L (1967) On the origin of mitosing cells. *J Theor Biol* **14**, 225–274.
37 Martin W & Müller M (1998) The hydrogen hypothesis for the first eukaryote. *Nature* **392**, 37–41.
38 Frade JM & Michaelidis TM (1997) Origin of eukaryotic programmed cell death: a consequence of aerobic metabolism? *BioEssays* **19**, 827–832.
39 Munn DH, Zhou M, Attwood JT, Bondarev I, Conway SJ, Marshall B, Brown C & Mellor AL (1998) Prevention of allogeneic fetal rejection by tryptophan catabolism. *Science* **281**, 1191–1193.
40 Sato K & Sato M (2017) Multiple ways to prevent transmission of paternal mitochondrial DNA for maternal inheritance in animals. *J Biochem* **162**, 247–253.
41 Rodger CE, McWilliams TG & Ganley IG (2018) Mammalian mitophagy – from in vitro molecules to in vivo models. *FEBS J* **285**, 1185–1202.
42 Zhang Q, Itagaki K & Hauser CJ (2010) Mitochondrial DNA is released by shock and activates neutrophils via P38 map kinase. *Shock* **34**, 55–59.
43 Dudek J (2017) Role of cardiolipin in mitochondrial signaling pathways. *Front Cell Dev Biol* **5**, 90.
44 Kim Y, Park J, Kim S, Kim MA, Kang MG, Kwak C, Kang M, Kim B, Rhee HW & Kim VN (2018) PKR senses nuclear and mitochondrial signals by interacting with endogenous double-stranded RNAs. *Mol Cell* **71**, 1051–1063.e6. https://doi.org/10.1016/j.molcel.2018.07.029
45 Dhir A, Dhir S, Borowski LS, Jimenez L, Teitell M, Rötig A, Crow YJ, Rice GI, Duffy D, Tamby C *et al.* (2018) Mitochondrial double-stranded RNA triggers antiviral signalling in humans. *Nature* **560**, 238–242.
46 Refolo G, Vescovo T, Piacentini M, Fimia GM & Ciccosanti F (2020) Mitochondrial interactome: a focus on antiviral signaling pathways. *Front Cell Dev Biol* **8**, 8.
47 Wang Y, Yuan S, Jia X, Ge Y, Ling T, Nie M, Lan X, Chen S & Xu A (2019) Mitochondria-localised ZNFX1 functions as a dsRNA sensor to initiate antiviral responses through MAVS. *Nat Cell Biol* **21**, 1346–1356.
48 Dutta S, Das N & Mukherjee P (2020) Picking up a fight: fine tuning mitochondrial innate immune defenses against RNA viruses. *Front Microbiol* **11**, 1990.
49 Sander LE & Garaude J (2018) The mitochondrial respiratory chain: a metabolic rheostat of innate immune cell-mediated antibacterial responses. *Mitochondrion* **41**, 28–36.
50 Cameron AM, Castoldi A, Sanin DE, Flachsmann LJ, Field CS, Puleston DJ, Kyle RL, Patterson AE, Hässler F, Buescher JM *et al.* (2019) Inflammatory macrophage dependence on NAD + salvage is a consequence of reactive oxygen species–mediated DNA damage. *Nat Immunol* **20**, 420–432.
51 Zhang J, Tao J, Ling Y, Li F, Zhu X, Xu L, Wang M, Zhang S, McCall CE & Liu TF (2019) Switch of NAD salvage to de novo biosynthesis sustains SIRT1-RelB-dependent inflammatory tolerance. *Front Immunol* **10**, 2358.
52 Adriouch S, Hubert S, Pechberty S, Koch-Nolte F, Haag F & Seman M (2007) NAD + released during inflammation participates in T cell homeostasis by inducing ART2-mediated death of naive T cells in vivo. *J Immunol* **179**, 186–194. https://doi.org/10.4049/jimmunol.179.1.186
53 Tirumurugaan KG, Jude JA, Kang BN, Panettieri RA, Walseth TF & Kannan MS (2007) TNF-α induced CD38 expression in human airway smooth muscle cells: role of MAP kinases and transcription factors NF-κB and AP-1. *Am J Physiol Lung Cell Mol Physiol* **292**, L1385–L1395.
54 Chini EN, Chini CCS, Espindola Netto JM, de Oliveira GC & van Schooten W (2018) The pharmacology of CD38/NADase: an emerging target in cancer and diseases of aging. *Trends Pharmacol Sci* **39**, 424–436.
55 In HL, Cao L, Mostoslavsky R, Lombard DB, Liu J, Bruns NE, Tsokos M, Alt FW & Finkel T (2008) A role for the NAD-dependent deacetylase Sirt1 in the regulation of autophagy. *Proc Natl Acad Sci USA* **105**, 3374–3379.
56 Masri S (2015) Sirtuin-dependent clock control: new advances in metabolism, aging and cancer. *Curr Opin Clin Nutr Metab Care* **18**, 521–527.
57 Schultz MB & Sinclair DA (2016) Why NAD+ declines during aging: it's destroyed. *Cell Metab* **23**, 965–966.
58 Schmidt SV & Schultze JL (2014) New insights into IDO biology in bacterial and viral infections. *Front Immunol* **5**, 1–12.
59 Darcy CJ, Davis JS, Woodberry T, McNeil YR, Stephens DP, Yeo TW & Anstey NM (2011) An observational cohort study of the kynurenine to tryptophan ratio in sepsis: association with impaired immune and microvascular function. *PLoS One* **6**, e21185.
60 Wirthgen E & Hoeflich A (2015) Endotoxin-induced tryptophan degradation along the kynurenine pathway:

the role of indolamine 2,3-dioxygenase and aryl hydrocarbon receptor-mediated immunosuppressive effects in endotoxin tolerance and cancer and its implications for immunoparalysis. *J Amino Acids* **2015**, 1–13.

61 Zeden JP, Fusch G, Holtfreter B, Schefold JC, Reinke P, Domanska G, Haas JP, Gruendling M, Westerholt A & Schuett C (2010) Excessive tryptophan catabolism along the kynurenine pathway precedes ongoing sepsis in critically ill patients. *Anaesth Intensive Care* **38**, 307–316.

62 Changsirivathanathamrong D, Wang Y, Rajbhandari D, Maghzal GJ, Mak WM, Woolfe C, Duflou J, Gebski V, Dos Remedios CG, Celermajer DS *et al.* (2011) Tryptophan metabolism to kynurenine is a potential novel contributor to hypotension in human sepsis. *Crit Care Med* **39**, 2678–2683.

63 Suchard MS, Adu-Gyamfi CG, Cumming BM & Savulescu DM (2020) Evolutionary views of tuberculosis: indoleamine 2,3-dioxygenase catalyzed nicotinamide synthesis reflects shifts in macrophage metabolism: indoleamine 2,3-dioxygenase reflects altered macrophage metabolism during tuberculosis pathogenesis. *BioEssays* **42**, 1–10.

64 Pajuelo D, Gonzalez-Juarbe N, Tak U, Sun J, Orihuela CJ & Niederweis M (2018) NAD+ depletion triggers macrophage necroptosis, a cell death pathway exploited by *Mycobacterium tuberculosis*. *Cell Rep* **24**, 429–440.

65 Young WD, Maslansky A, Lefar MS & Kronish DP (1970) Development of a paper strip test for detection of niacin produced by mycobacteria. *Appl Microbiol* **20**, 939–945.

66 Williams AC & Dunbar RIM (2013) Big brains, meat, tuberculosis, and the nicotinamide switches: co-evolutionary relationships with modern repercussions? *Int J Tryptophan Res*. https://doi.org/10.4137/IJTR.S12838

67 Keshavarz M, Solaymani-Mohammadi F, Namdari H, Arjeini Y, Mousavi MJ & Rezaei F (2020) Metabolic host response and therapeutic approaches to influenza infection. *Cell Mol Biol Lett* **25**, 15.

68 Gaelings L, Söderholm S, Bugai A, Fu Y, Nandania J, Schepens B, Lorey MB, Tynell J, Vande Ginste L, Le Goffic R *et al.* (2017) Regulation of kynurenine biosynthesis during influenza virus infection. *FEBS J* **284**, 222–236.

69 Mehraj V & Routy J-P (2015) Tryptophan catabolism in chronic viral infections: handling uninvited guests. *Int J Tryptophan Res* **8**, IJTR.S26862.

70 van der Sluijs KF, Nijhuis M, Levels JHM, Florquin S, Mellor AL, Jansen HM, van der Poll T & Lutter R (2006) Influenza-induced expression of indoleamine 2,3-dioxygenase enhances interleukin-10 production and bacterial outgrowth during secondary *Pneumococcal pneumonia*. *J Infect Dis* **193**, 214–222.

71 Pett SL, Kunisaki KM, Wentworth D, Griffin TJ, Kalomenidis I, Nahra R, Montejano Sanchez R, Hodgson SW, Ruxrungtham K, Dwyer D *et al.* (2018) Increased indoleamine-2,3-dioxygenase activity is associated with poor clinical outcome in adults hospitalized with influenza in the INSIGHT FLU003Plus study. *Open Forum Infect Dis* **5**, ofx228. https://doi.org/10.1093/ofid/ofx228

72 Shi Y, Wang Y, Shao C, Huang J, Gan J, Huang X, Bucci E, Piacentini M, Ippolito G & Melino G (2020) COVID-19 infection: the perspectives on immune responses. *Cell Death Differ* **27**, 1451–1454.

73 McGonagle D, Sharif K, O'Regan A & Bridgewood C (2020) The role of cytokines including interleukin-6 in COVID-19 induced pneumonia and macrophage activation syndrome-like disease. *Autoimmun Rev* **19**, 102537.

74 Jamilloux Y, Henry T, Belot A, Viel S, Fauter M, El Jammal T, Walzer T, François B & Sève P (2020) Should we stimulate or suppress immune responses in COVID-19? Cytokine and anti-cytokine interventions. *Autoimmun Rev* **19**, 102567.

75 Weber F, Wagner V, Rasmussen SB, Hartmann R & Paludan SR (2006) Double-stranded RNA is produced by positive-strand RNA viruses and DNA viruses but not in detectable amounts by negative-strand RNA viruses. *J Virol* **80**, 5059–5064.

76 Aaby P, Benn CS, Flanagan KL, Klein SL, Kollmann TR, Lynn DJ & Shann F (2020) The non-specific and sex-differential effects of vaccines. *Nat Rev Immunol* **20**, 464–470.

77 Heer CD, Sanderson DJ, Voth LS, Alhammad YMO, Schmidt MS, Trammell SAJ, Perlman S, Cohen MS, Fehr AR & Brenner C (2020) Coronavirus infection and PARP expression dysregulate the NAD metabolome: an actionable component of innate immunity. *J Biol Chem*, **295**: 17986–17996. https://doi.org/10.1074/jbc.RA120.015138.

78 Miller B, Silverstein A, Flores M, Cao K, Kumagai H, Mehta HH, Yen K, Kim SJ & Cohen P (2021) Host mitochondrial transcriptome response to SARS-CoV-2 in multiple cell models and clinical samples. *Sci Rep* **11**, 3.

79 Green RF (1971) Subclinical pellagra and idiopathic hypogeusia. *JAMA* **218**, 1303.

80 Omran HM & Almaliki MS (2020) Influence of NAD+ as an ageing-related immunomodulator on COVID 19 infection: a hypothesis. *J Infect Public Health* **13**, 1196–1201.

81 Gebicki J & Wieczorkowska M (2020) COVID-19 infection: mitohormetic concept of immune response. *Cell Death Discov* **6**, 60. https://doi.org/10.1038/s41420-020-00297-9

82 Gharote MA (2020) Role of poly (ADP) ribose polymerase-1 inhibition by nicotinamide as a possible additive treatment to modulate host immune response and prevention of cytokine storm in COVID-19. *Indian J Med Sci* **72**, 25–28.
83 Kouhpayeh S, Shariati L, Boshtam M, Rahimmanesh I, Mirian M, Zeinalian M, Salari-jazi A, Khanahmad N, Damavandi MS, Sadeghi P *et al.* (2020) The molecular story of COVID-19; NAD+ depletion addresses all questions in this infection. *Preprints* 2020030346. https://doi.org/10.20944/preprints202003.0346.v1
84 Badawy AAB (2020) Immunotherapy of COVID-19 with poly (ADP-ribose) polymerase inhibitors: starting with nicotinamide. *Biosci Rep* **40**, BSR20202856. https://doi.org/10.1042/BSR20202856
85 Huizenga R (2020) Dramatic cytokine storm reversal with an over the counter NMN cocktail. https://ssrn.com/abstract=3581388 or http://dx.doi.org/10.2139/ssrn.3581388
86 Yuan H, Wan J, Li L, Ge P, Li H & Zhang L (2012) Therapeutic benefits of the group B3 vitamin nicotinamide in mice with lethal endotoxemia and polymicrobial sepsis. *Pharmacol Res* **65**, 328–337.
87 Su CF, Liu DD, Kao SJ & Chen HI (2007) Nicotinamide abrogates acute lung injury caused by ischaemia/reperfusion. *Eur Respir J* **30**, 199–204.
88 Nagai A, Matsumiya H, Hayashi M, Yasui S, Okamoto H & Konno K (1994) Effects of nicotinamide and niacin on bleomycin-induced acute injury and subsequent fibrosis in hamster lungs. *Exp Lung Res* **20**, 263–281.
89 Sims CA, Guan Y, Mukherjee S, Singh K, Botolin P, Davila A & Baur JA (2018) Nicotinamide mononucleotide preserves mitochondrial function and increases survival in hemorrhagic shock. *JCI insight* **3**, e120182.
90 Jiang Y, Deng Y, Ma T, Pang H, Hu Z, Qin C & Xu Z. Treatment of SARS-CoV-2 induced pneumonia with NAD + in a mouse model. 1–12. https://doi.org/10.21203/rs.3.rs-96999/v1
91 Murray MF (2003) Nicotinamide: an oral antimicrobial agent with activity against both *Mycobacterium tuberculosis* and human immunodeficiency virus. *Clin Infect Dis* **36**, 453–460.
92 Cheng SC, Young DO, Huang Y, Delmez JA & Coyne DW (2008) A randomized, double-blind, placebo-controlled trial of niacinamide for reduction of phosphorus in hemodialysis patients. *Clin J Am Soc Nephrol* **3**, 1131–1138.
93 Nikas IP, Paschou SA & Ryu HS (2020) The role of nicotinamide in cancer chemoprevention and therapy. *Biomolecules* **10**, 1–20.
94 Janssens GO, Rademakers SE, Terhaard CH, Doornaert PA, Bijl HP, Van Den Ende P, Chin A, Takes RP, De Bree R, Hoogsteen IJ *et al.* (2014) Improved recurrence-free survival with ARCON for anemic patients with laryngeal cancer. *Clin Cancer Res* **20**, 1345–1354.
95 Walocko FM, Eber AE, Keri JE, AL-Harbi MA & Nouri K (2017) The role of nicotinamide in acne treatment. *Dermatol Ther* **30**, e12481.
96 Rennie G, Chen AC, Dhillon H, Vardy J & Damian DL (2015) Nicotinamide and neurocognitive function. *Nutr Neurosci* **18**, 193–200.
97 Tsujita N, Akamatsu Y, Nishida MM, Hayashi T & Moritani T (2019) Effect of tryptophan, vitamin b6, and nicotinamide-containing supplement loading between meals on mood and autonomic nervous system activity in young adults with subclinical depression: a randomized, double-blind, and placebo-controlled study. *J Nutr Sci Vitaminol (Tokyo)* **65**, 507–514.
98 Knip M, Douek IF, Moore WPT, Gillmor HA, McLean AEM, Bingley PJ & Gale EAM (2000) Safety of high-dose nicotinamide: a review. *Diabetologia* **43**, 1337–1345.
99 Nutritional Health and Medical Research Council Australia (NHMRC) (2006) Nutrient reference values for Australia and New Zealand. https://www.nhmrc.gov.au/about-us/publications/nutrient-reference-values-australia-and-new-zealand-including-recommended-dietary-intakes
100 Osar Z, Samanci T, Demirel GY, Damci T & Ilkova H (2004) Nicotinamide effects oxidative burst activity of neutrophils in patients with poorly controlled type 2 diabetes mellitus. *Exp Diabesity Res* **5**, 155–162.
101 Tarantini S, Valcarcel-Ares MN, Toth P, Yabluchanskiy A, Kiss T, Ballabh P, Farkas E, Baur J, Sinclair D, Csiszar A *et al.* (2020) Nicotinamide mononucleotide (NMN) supplementation rescues cerebromicrovascular endothelial function and neurovascular coupling responses and improves cognitive function in aged mice. *FASEB J* **24**, 101192.
102 Poddar SK, Sifat AE, Haque S, Nahid NA, Chowdhury S & Mehedi I (2019) Nicotinamide mononucleotide: exploration of diverse therapeutic applications of a potential molecule. *Biomolecules* **9**, 34.
103 Irie J, Inagaki E, Fujita M, Nakaya H, Mitsuishi M, Yamaguchi S, Yamashita K, Shigaki S, Ono T, Yukioka H *et al.* (2020) Effect of oral administration of nicotinamide mononucleotide on clinical parameters and nicotinamide metabolite levels in healthy Japanese men. *Endocr J* **67**, 153–160.
104 Conze D, Brenner C & Kruger CL (2019) Safety and metabolism of long-term administration of NIAGEN (nicotinamide riboside chloride) in a randomized, double-blind, placebo-controlled clinical trial of healthy overweight adults. *Sci Rep* **9**, 9772.