translational science

The iNADequacy of renal cell metabolism: modulating NAD$^+$ biosynthetic pathways to forestall kidney diseases

Luca Perico[1] and Ariela Benigni[1]

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Since its discovery a century ago as a cofactor in the process of fermentation, nicotinamide adenine dinucleotide (NAD$^+$) has received considerable attention in biomedical research. Major breakthroughs in our understanding of the biological role of NAD$^+$ were provided in the 1930s by Otto Warburg, who significantly advanced our understanding of its chemistry and cellular functions. NAD$^+$ is a hydride acceptor that, along with its reduced form, NADH, is vital to the reduction-oxidation (redox) reactions in metabolic processes, including glycolysis and mitochondrial oxidative phosphorylation (Figure 1a).[1,2] Additional functions of NAD$^+$ have been recognized over the last 2 decades, with the identification of several proteins that consume NAD$^+$ to sustain their proper enzymatic activity (Figure 1b). Under physiological conditions, the available NAD$^+$ cellular pool is the result of a steady balance between consumption and generation, the latter carried out through either a *de novo* pathway from tryptophan or a salvage pathway recycling nicotinamide back to NAD$^+$ (Figure 1c). Alterations to this tight equilibrium decrease NAD$^+$ content, which has been associated with the development of a wide range of diseases.[1] In line with these findings, a large body of evidence has established that replenishing cellular NAD$^+$ is a beneficial therapeutic intervention in a variety of pathogenic processes, although the protective mechanisms remain elusive.

An elegant demonstration of the beneficial effect of NAD$^+$ restoration is offered by Poyan Mehr *et al.*,[3] who described the role of the bottleneck enzyme of NAD$^+$ *de novo* biosynthesis in mediating resistance to acute kidney injury (AKI). By using unbiased metabolomics screening in the urine of mice with ischemic AKI, the investigators identified 27 metabolites that increased more than 2-fold, including several sugars and amino acids.[3] They chose to focus on quinolinate, an intermediate in the *de novo* NAD$^+$ biosynthetic pathway from tryptophan (Figure 1c). One might wonder why the investigators concentrated on a metabolite that was not among the most highly upregulated, which included arachidonic acid whose epoxide metabolites are known to play a role in AKI.[4] Nonetheless, the investigators found that quinolinate accumulated in the kidneys of AKI mice, along with a decrease in NAD$^+$ content and mRNA expression of quinolinate phosphoribosyltransferase (QPRT),[3] the enzyme that converts quinolinate to nicotinic acid mononucleotide (NAMN). To confirm the causal role of QPRT downregulation in quinolinate accumulation and NAD$^+$ depletion, the investigators generated a mouse model with 1 allele deletion of QPRT (QPRT$^{+/-}$).[3] The investigators attributed the accumulation of quinolinate solely to an intrarenal process given that reduced renal QPRT resulted in increased quinolinate excretion in the urine, as well as reduced levels of NAD$^+$ in the kidney.[3] However, the increase in renal quinolinate content following AKI does not exclude the possibility that this compound could also have accumulated in and derived from other tissues. In experimental models of chronic kidney disease, the content of NAD$^+$ metabolites, including quinolinate, increases

[1]*Istituto di Ricerche Farmacologiche Mario Negri IRCCS, Bergamo, Italy*

Correspondence: *Ariela Benigni, Istituto di Ricerche Farmacologiche Mario Negri IRCCS, Centro Anna Maria Astori, Science and Technology Park Kilometro Rosso, Via Stezzano 87, 24126 Bergamo, Italy. E-mail: ariela.benigni@marionegri.it*

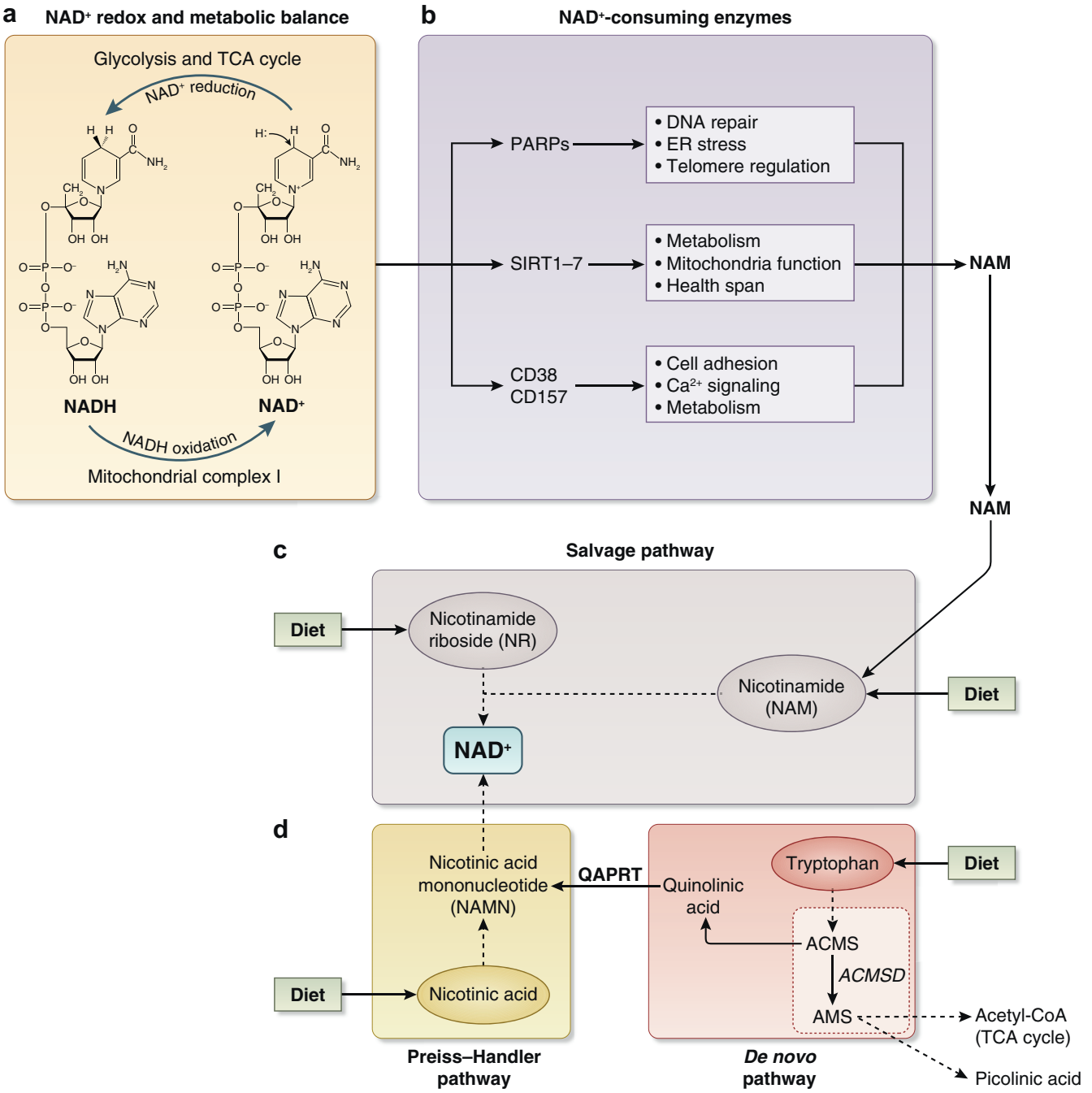

Figure 1 | NAD$^+$ biological functions and intracellular biosynthetic pathways. (**a**) In physiologic conditions, NAD$^+$ is reduced to NADH by several dehydrogenases involved in glycolysis and tricarboxylic acid (TCA) cycle. Conversely, NADH oxidation occurs in the first step of the mitochondrial oxidative phosphorylation mediated by complex I, which converts NADH to NAD$^+$. In all these enzymatic reactions, NAD$^+$ is not consumed and is steadily cycled between the redox pair NAD$^+$/NADH. (**b**) On the other hand, several intracellular proteins, including poly(ADP-ribose) polymerases (PARPs), sirtuins (SIRT1-7), and cADP-ribose synthases, consume NAD$^+$ during their enzymatic reactions and produce nicotinamide (NAM) as a by-product. (**c**) To restore NAD$^+$ levels, the salvage pathway converts NAM to NAD$^+$ by 2 enzymatic reactions. (**d**) On the other hand, NAD$^+$ can be generated by the *de novo* and the Preiss–Handler pathways that can produce NAD$^+$ starting from tryptophan. As an essential amino acid, tryptophan cannot be synthesized by the organism, but it is taken through daily food intake. Foods containing high levels of tryptophan include nuts, seeds, cheese, red meat, chicken, fish, oats, beans, lentils, and eggs. In the *de novo* pathway, tryptophan is converted to α-amino-β-carboxymuconate-ε-semialdehyde (ACMS) through multiple enzymatic reactions. Spontaneous cyclization of ACMS generates quinolinic acid, which is then converted to nicotinic acid mononucleotide (NAMN) by quinolinate phosphoribosyltransferase (QAPRT). In the dotted box, ACMS decarboxylase (ACMSD) can divert away ACMS from NAD$^+$ synthesis, which can be used in the TCA cycle or nonenzymatically converted to picolinic acid. Additionally, NAD$^+$ can be generated from nicotinamide riboside and nicotinic acid through the salvage and Preiss–Handler pathways, respectively. These 2 compounds are trace nutrients and thus do not exceed the micromolar range in foods.[2] As a result, nicotinamide riboside and nicotinic acid should be taken as supplements to achieve adequate circulating levels able to boost NAD$^+$ production across the different tissues.

significantly in the liver, lung, intestine, and spleen, in addition to the kidney.[1]

Indeed, a recent study identified the liver as the main site at which NAD^+ is converted from tryptophan and NAD^+ precursors are released into the bloodstream to reach other tissues.[5] In line with those findings, the investigators found that QPRT was also significantly reduced in the liver of $QPRT^{+/-}$ mice,[3] suggesting that the downregulation of QPRT in extrarenal tissues could contribute to the increase in urinary quinolinate and decreased NAD^+ in the kidney.

To assess the potential role of QPRT in the pathophysiology of AKI, the investigators demonstrated that $QPRT^{+/-}$ mice exhibited exacerbated AKI after an ischemic insult.[3] NAM supplementation preserved kidney function in $QPRT^{+/-}$ mice with AKI,[3] suggesting that boosting the NAD^+ salvage pathway via NAM supplementation could be a viable tool to overcome the impairment of the *de novo* pathway in AKI. Unfortunately, the investigators did not assess NAD^+ renal levels after treatment, making it impossible to assess whether NAM renoprotection was indeed dependent on replenishment of the NAD^+ pool.

To apply these experimental findings to humans, the investigators performed a nested case-control study to assess urinary levels of *de novo* pathway metabolites in patients with and without AKI following cardiac surgery or intensive care unit admission.[3] In these 2 cohorts, increased urinary quinolinate levels were associated with increased risk of incident AKI,[3] suggesting that urinary quinolinate could be a novel noninvasive biomarker for early diagnosis of AKI. This conclusion is tempered by the following considerations. First, these studies were performed in highly heterogeneous cohorts in which a broad array of AKI definitions and widely differing clinical settings may confound interpretation of the results. In addition, interpretation of metabolomics studies may be affected by other factors, including diet and gut microbiome status, which are more variable in human subjects.[6] Lastly, the current metabolomics platforms provide incomplete coverage of the human metabolome, making it difficult to assess the full extent of metabolic changes in the clinical setting.[6]

The investigators next tested the effects of oral nicotinamide in a phase I, placebo-controlled, randomized pilot study in adults undergoing cardiac surgery.[3] Although the primary end-point was a comparison of the circulating NAM levels in the different treatment arms, subjects receiving NAM also experienced less AKI, in a dose-independent manner, than did subjects in the placebo arm.[3] If these preliminary findings are confirmed in large-scale trials, this study will open the way for a novel treatment option to reduce the risk of AKI.

Overall, this study provides compelling evidence to support the role of the NAD^+ *de novo* pathway in AKI and offers proof-of-concept data confirming that supplementation of NAD^+ precursors could be an effective strategy to improve patient outcomes.[3] Despite the obvious translational implications of these findings, several factors must be taken into consideration before the therapeutic potential of NAD^+ precursor supplementation is explored in large-scale clinical trials. In particular, a recent study has reported that oral consumption of NAD^+ precursors might not be an efficient strategy to increase NAD^+ levels.[5] For this reason, developing more specifically targeted pharmacological approaches to boost NAD^+ in different organs should be the first goal.

To this end, Katsyuba *et al.*[7] recently developed a specific chemical inhibitor of α-amino-β-carboxymuconate-ε-semialdehyde decarboxylase (ACMSD), the enzyme that limits the flux of tryptophan to NAD^+ in the *de novo* pathway (Figure 1c, dotted box). The ACMSD inhibitor effectively increased NAD^+ levels in kidney and robustly protected mice in 2 different AKI models.[7] An alternative therapeutic approach could be the use of mesenchymal stromal cells (MSCs). Indeed, mice with cisplatin-induced AKI exhibited replenished renal levels of NAD^+ following injection with human umbilical cord-derived MSCs.[8] This protective effect on renal NAD^+ is ascribable to the ability of MSCs to activate both the *de novo* and the salvage pathway in proximal tubular epithelial cells.[8] These findings raise the intriguing possibility that tissue-specific NAD^+ manipulation is an achievable option that warrants further investigation as a potential therapeutic tool.

DISCLOSURE

All the authors declared no competing interests.

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genetics of kidney disease

The genomic landscape of CAKUT; you gain some, you lose some

Nine V.A.M. Knoers[1] and Kirsten Y. Renkema[2]

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Congenital anomalies of the kidney and urinary tract (CAKUT) collectively refer to a diverse group of structural malformations that result from perturbations in embryonic kidney and urinary tract development. CAKUT affects 3 to 6 per 1000 live births, constitutes the leading cause (~40%) of end-stage renal disease in childhood, and is a significant contributor to chronic kidney disease in adults.[1] The existence of syndromic phenotypes and familial clustering suggest a major genetic contribution to the etiology of CAKUT. In recent years, alterations in more than 50 genes have been shown to cause isolated or syndromic CAKUT, in an autosomal dominant or, less frequently, recessive model of inheritance. Mutations in these genes, however, only explain 10% to 20% of CAKUT cases.[2] Moreover, the clinical phenotype and severity of CAKUT can vary markedly among patients, both within and between families with the same underlying mutation, demonstrating the complex genotype-phenotype relationship in CAKUT.

A promising approach in CAKUT genetics is the analysis of copy number variations (CNVs), structural variations in the genome of an individual in the form of gains (duplications) or losses (deletions) of DNA fragments. CNVs range in size from 1 kilobase (kb) to several megabases (Mb), and CNVs smaller than 1 to 2 Mb are not identifiable by conventional chromosomal analysis (karyotyping). Array-based techniques including comparative genomic hybridization (CGH) arrays, single nucleotide polymorphism (SNP) arrays, and more recently, next-generation sequencing techniques allow the detection of these smaller CNVs.[3] CNVs are widespread in our genomes, are an important source of both normal and pathogenic genetic variation, and have been implicated in the etiology of a wide variety of human diseases, including CAKUT.[4] The pathogenicity of identified CNVs is not always clear. In general, a pathogenic effect is suggested when specific CNVs are absent from healthy individuals and when there is phenotypic resemblance among affected individuals with overlapping CNVs.[5] Based on these and other criteria, a growing list of rare recurrent CNVs of definite pathogenic significance, associated with well-characterized genetic

[1]*Department of Genetics, University Medical Centre Groningen, Groningen, the Netherlands; and* [2]*Department of Genetics, Center for Molecular Medicine, University Medical Center Utrecht, Utrecht, the Netherlands*

Correspondence: *Nine V.A.M. Knoers, University Medical Centre Groningen, Department of Genetics, Antonius Deusinglaan 1, P.O. Box 30 001, 9700 RB Groningen, the Netherlands. E-mail: v.v.a.m.knoers@umcg.nl*