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Editorial Comment

EDITORIAL COMMENT

Nicotinamide and acute kidney injury

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ABSTRACT

In a recent issue of *ckj*, Piedrafita *et al.* reported that urine tryptophan and kynurenine are reduced in cardiac bypass surgery patients that develop acute kidney injury (AKI), suggesting reduced activity of the kynurenine pathway of nicotinamide (NAM) adenine dinucleotide (NAD^+) synthesis from tryptophan. However, NAM supplementation aiming at repleting NAD^+ did not replete kidney NAD^+ and did not improve glomerular filtration or reduce histological injury in ischaemic–reperfusion kidney injury in mice. The lack of improvement of kidney injury is partially at odds with prior reports that did not study kidney NAD^+, glomerular filtration or histology in NAM-treated wild-type mice with AKI. We now present an overview of research on therapy with vitamin B3 vitamers and derivate molecules {niacin, Nicotinamide [NAM; niacinamide], NAM riboside [Nicotinamide riboside (NR)], Reduced nicotinamide riboside [NRH] and NAM mononucleotide} in kidney injury, including an overview of ongoing clinical trials, and discuss the potential explanations for diverging reports on the impact of these therapeutic approaches on pre-clinical acute and chronic kidney disease.

Keywords: acute kidney injury, chronic kidney disease, NAD, nicotinamide, treatment, vitamin B3

VITAMIN B3 AND NAD^+

Vitamin B3 is a water-soluble vitamin family that includes three vitamers, nicotinic acid (niacin), nicotinamide (NAM; niacinamide) and NAM riboside (NR). The three are precursors of NAM adenine dinucleotide (NAD^+) and NAD phosphate ($NADP^+$), which may be synthesized *de novo* from dietary vitamin B3 (niacin, NAM or NR) or from tryptophan, the latter via the kynurenine pathway (Figure 1) [1, 2]. Additionally, the salvage pathway re-uses NAM produced from NAD^+-utilizing enzymes and is the main source of NAD^+ in many cell types.

Dietary niacin requirements are expressed as 'niacin equivalent' (NE), in which 1 NE is equal to 1 mg of niacin, or 60 mg of tryptophan. The US recommended dietary allowance for niacin is 14–16 mg for adults. This dose is far below the doses of niacin used to treat hyperlipidaemia [3] (Figure 2). Niacin deficiency results in pellagra, characterized by a photosensitive pigmented dermatitis, diarrhoea and dementia. Pellagra may be caused by insufficient intake or absorption of vitamin B3 and tryptophan or by conditions in which tryptophan processing to niacin is compromised: carcinoid syndrome, in which tryptophan is predominantly metabolized to 5-OH tryptophan and serotonin; drugs that deplete pyridoxal phosphate (which enhances the metabolism of tryptophan into niacin) stores and SLC6A19 deficiency (Hartnup disease), in which tryptophan and other neutral amino acids are not absorbed in the gut and are additionally lost in urine [4].

Tryptophan is an essential amino acid in humans, meaning that it cannot be synthesized and must be obtained from the diet [5]. However, tryptophan metabolism may yield uraemic toxins

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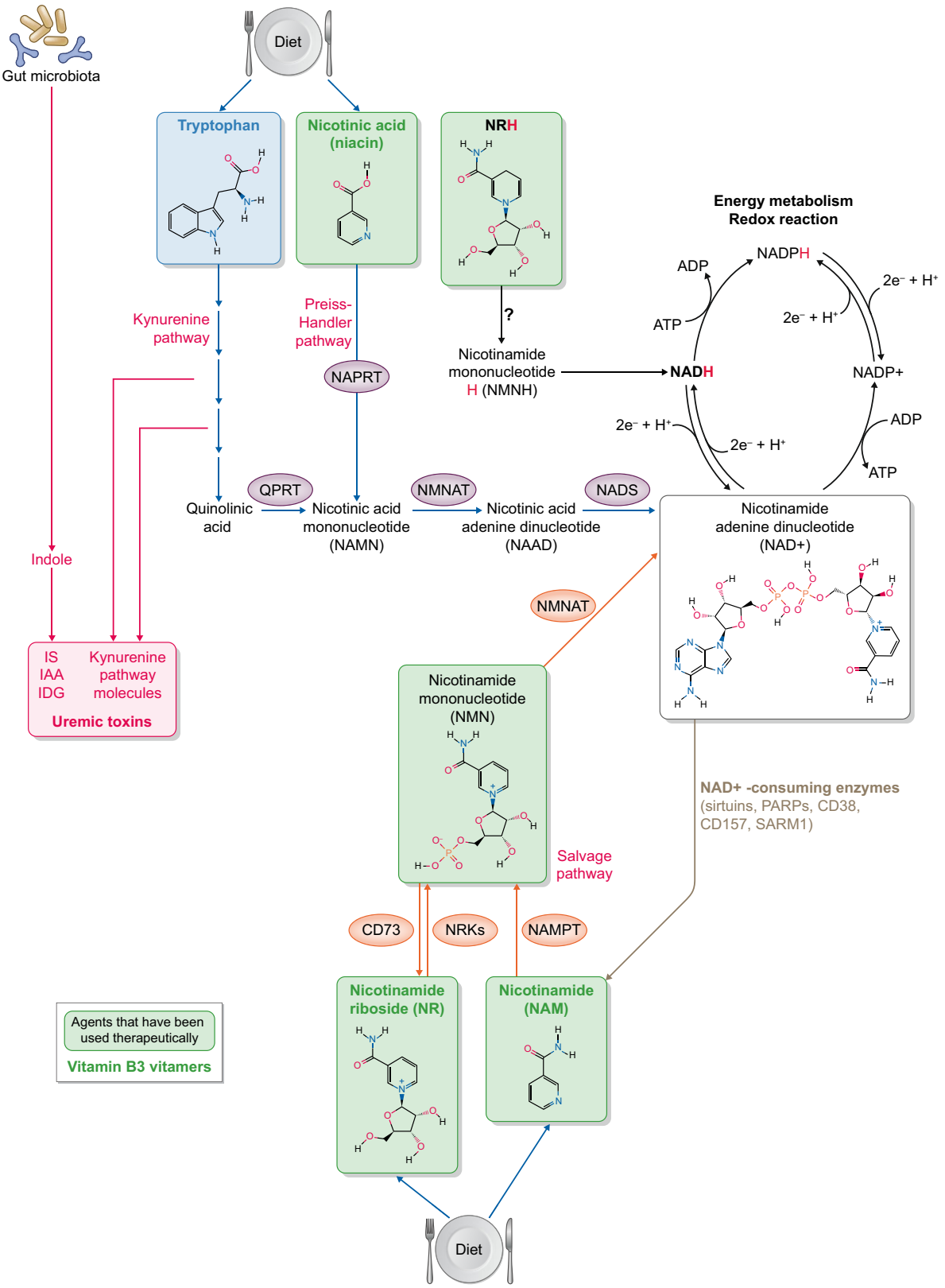

FIGURE 1: NAD^+ metabolic pathways. NAD^+ production from NAM or from NR consists of two steps, while production from niacin includes three enzymatic reactions and is known as the Preiss–Handler pathway. In contrast, NAD^+ synthesis from tryptophan through the kynurenine pathway requires six enzymatic steps to yield quinolinic acid, which is then transformed to NAMN that, in turn, is the precursor of NAD^+ in the Preiss–Handler pathway. NAD^+ levels can be decreased by enzyme such as CD38, CD157 and SARM1 (sterile alpha and Toll/interleukin-1 receptor motif-containing 1). CD38 also degrades NR and NMN [3]. Tryptophan may also be a source of uraemic toxins, which are either metabolites of the kynurenine pathway or of indole, which is generated by bacterial tryptophanase in the gut.

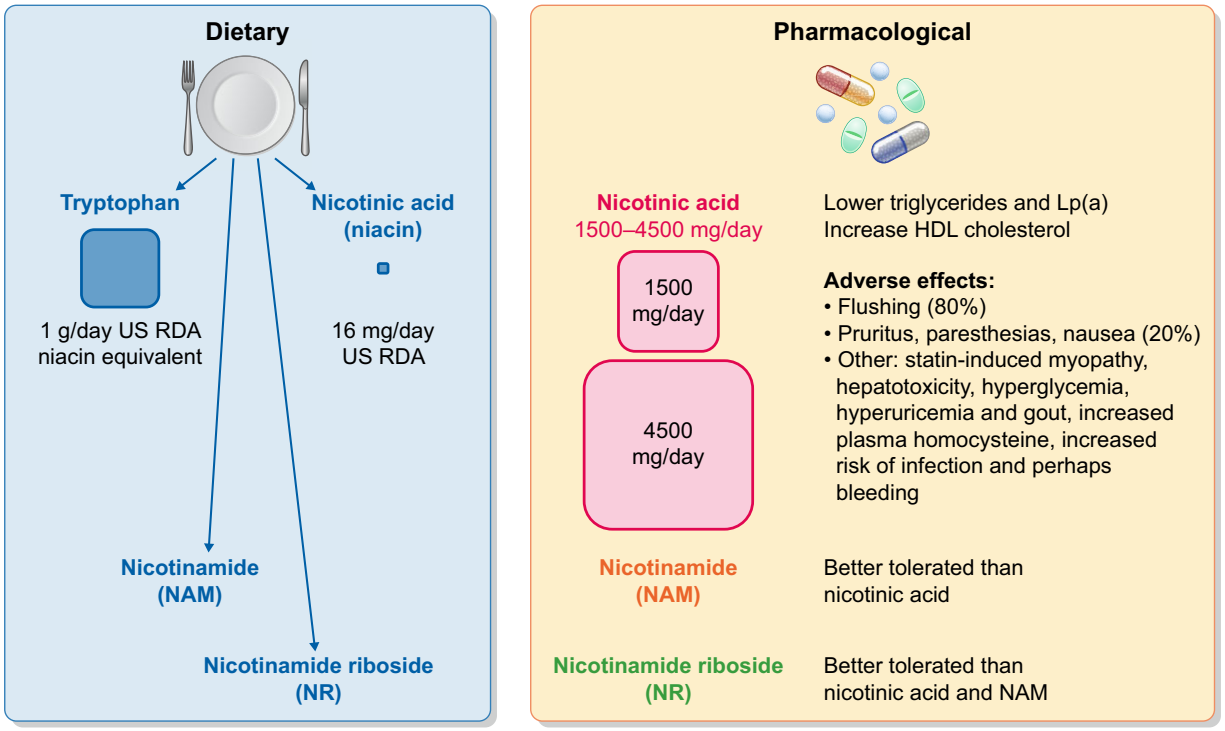

FIGURE 2: Dietary sources of vitamin B3 vitamers and pharmacological dosing of niacin. Pharmacological doses of niacin are over 100-fold higher that the US recommended dietary allowance. The size of each square is proportional to the magnitude of the dose.

from the kynurenine pathway (kynurenine, kynurenic acid, anthranilic acid, 3-hydroxykynurenine, 3-hydroxyanthranilic acid and quinolinic acid), the circulating levels of which are increased while those of tryptophan are decreased in persons with chronic kidney disease (CKD), or toxins derived from the gut microbiota metabolism of tryptophan to indole [e.g. indoxyl sulphate (IS), indole acetic acid (IAA) and indoxyl-β-D-glucuronide (IDG)] [6, 7] (Figure 1).

NAD^+ has three key functions: it is an essential coenzyme in electron-transfer reactions, a substrate for NAD^+-consuming enzymes and an endogenous agonist of P2Y1 and P2Y11 cell surface purinergic receptors.

The role of NAD^+ in electron-transfer reactions is essential, because >400 enzymes require NAD^+ and $NADP^+$, mainly to accept or donate electrons for redox reactions. NAD^+ is involved mainly in energy-producing reactions in the mitochondria, meaning catabolism, while $NADP^+$ commonly functions in biosynthetic reactions, meaning anabolism. $NADP^+$ is also crucial for the regeneration of components of detoxification and antioxidant systems. To accomplish these functions, cellular NAD^+ is kept in the oxidized state (NAD^+), whereas cellular $NADP^+$ is maintained in a reduced state (NADPH) [2, 8].

NAD^+-consuming enzymes include ADP-ribosyltransferases (ARTs), poly (ADP-ribose) polymerases (PARPs), sirtuins and NAD glycohydrolases/ADP-ribosylcyclases like CD38 and CD157. ARTs and PARPs catalyse ADP-ribosyl transfer reactions [9], while sirtuins catalyse the removal of acetyl groups from acetylated proteins, using ADP-ribose from NAD^+ as an acceptor for acetyl groups. They play a key role in histone post-translational modifications [10].

NAD^+ binding to P2Y1 receptors functions as an inhibitory neurotransmitter at neuromuscular junctions in visceral smooth muscles [11], while binding to P2Y11 receptors in neutrophils activates a signalling cascade involving cyclic ADP-ribose and increased intracellular calcium, stimulating superoxide generation and chemotaxis [12].

CLINICAL USE OF VITAMIN B3

Vitamin B3 vitamers niacin and NAM may be prescribed to treat pellagra. Clinical trials of higher dose vitamin B3 for other indications date back to the 20th century. In 1998, Guyton stated that 'the use of niacin to prevent or treat atherosclerotic cardiovascular disease is based on strong and consistent evidence from clinical trials' [13]. The ClinicalTrials.gov web page lists 140 completed clinical trials testing niacin, NAM or NR (Figure 3A). Despite this, current clinical indications are limited. Niacin has been used to treat dyslipidaemia, especially hypertriglyceridaemia with low high-density lipoprotein cholesterol levels, but NAM does not have the lipid-lowering effect [14]. Niacin also lowers lipoprotein(a) (Lp(a)) levels by around 25%, but there is no evidence that this reduces clinical events. However, niacin is no longer recommended for dyslipidaemia, except in specific clinical situations (e.g. severe hypertriglyceridaemia resistant or intolerant to other therapeutic approaches) [15]. Niacin extended-release still lists an indication to reduce the risk of recurrent non-fatal myocardial infarction in patients with a history of myocardial infarction and hyperlipidaemia [16]. Oral NAM was also safe and effective in reducing the rates of new non-melanoma skin cancers and actinic keratoses in high-risk patients [17]. However, pharmacological doses of niacin or NAM are poorly tolerated. The dose-limiting adverse effect of niacin is skin flushing, caused by an increase in the vasodilator prostacyclin. Additional adverse effects of niacin and NAM include pruritus, skin rash, gastrointestinal disturbances and thrombocytopaenia [18, 19]. Liver cell damage has been observed at intakes of niacin as little as 750 mg/day [20]. In addition, high niacin doses impair glucose tolerance, presumably by

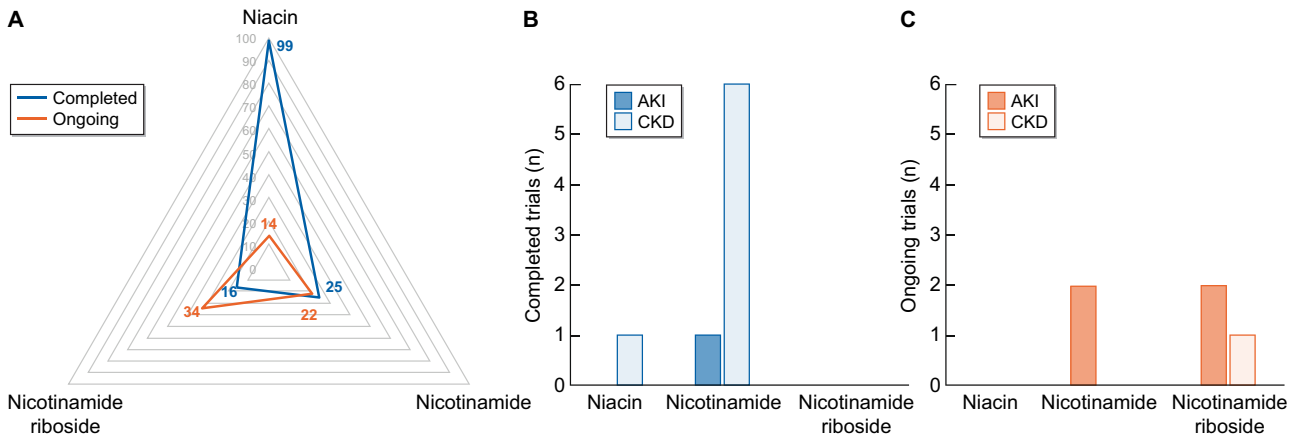

FIGURE 3: Clinical trials of vitamin B3 vitamers, according to ClinicalTrials.gov (accessed on 10 July 2021). (**A**) Completed and ongoing clinical trials involving the vitamin B3 vitamers, NAM, niacin and NR. Note that most completed trials tested niacin, followed by NAM and NR. In contrast, most ongoing trials are testing NR. (**B**) Completed clinical trials involving persons with CKD or prevention or treatment of AKI. (**C**) Ongoing clinical trials involving persons with CKD or prevention or treatment of AKI. As is the case for other trials, a shift can be observed in kidney disease to testing of NR as well as an increasing interest in AKI.

decreasing insulin sensitivity, and long-term niacin therapy was associated with a modest increase in risk of new onset Type 2 diabetes mellitus [21]. NAM is generally better tolerated than niacin, although gastrointestinal disturbances and signs of liver toxicity have been reported at doses >10 g/day [22].

NR was beneficial in pre-clinical and early clinical studies on diabetes, neurodegenerative disorders and cardiovascular diseases without the serious adverse effects observed with similar doses of niacin or NAM [23, 24]. In healthy volunteers, peak doses of up to 1000 mg twice daily were tolerated in a short-term (up to 9 days) open-label trial, and resulted in doubling of circulating NAD^+ levels [25]. Single doses of 100, 300 and 1000 mg of NR led to dose-dependent increases in the blood NAD^+ metabolome [23]. The rise in peripheral blood mononuclear cells (PBMCs) niacin adenine dinucleotide was a sensitive biomarker of effective NAD^+ repletion, despite not being in the NR to NAD^+ pathway (Figure 1).

VITAMIN B3 IN CKD

Vitamin B3 vitamers have been tested in clinical trials for the treatment of hyperphosphataemia in CKD (Figure 3B and Table 1). In CKD, niacin [26] or NAM [18, 19, 30], either alone or in combination with other phosphate binders, reduced serum phosphate levels in most trials through inhibition of the expression of the intestinal phosphate transporter NaPi2b by poorly characterized mechanisms. The main metabolite of NAM, N-methyl-2-pyridone-5-carboxamide, may accumulate in haemodialysis patients [19]. Overall, tolerance was suboptimal and regulatory authorities did not approve this indication. Additional studies unsuccessfully explored the impact of niacin on flow-mediated dilation and of NAM in sirtuin deacetylase activity [27, 28] (Table 1). An ongoing trial is testing NR to improve aerobic capacity in persons with CKD, while four other trials are testing NAM (1–3 g/day) or NR (1 g/day) for acute kidney injury (AKI), as discussed below (Figure 3C and Table 1).

VITAMIN B3 AS A KIDNEY PROTECTIVE STRATEGY IN EXPERIMENTAL CKD

Administration of vitamin B3 vitamers NAM or NR, as well as downstream metabolites such as NAM mononucleotide (NMN), has been tested in experimental CKD with the aim of preserving kidney function.

NAM supplementation [200, 400, 800 mg/kg, intraperitoneal (i.p.) 1 h before unilateral ureteral obstruction (UUO) and daily until Day 13] reduced renal interstitial fibrosis, tubular injury and inflammation at Day 14 in mice [31]. NAM (250 mg/kg i.p. 3 days before UUO and continuing to Day 7) also reduced fibrosis and Neutrophil gelatinase-associated lipocalin (NGAL) levels in mice at Day 7 [32]. Moreover, in murine aristolochic acid nephropathy NAM (500 mg/kg, i.p. 1 day before and continuously up to Day 14) reduced tubular injury and cytokine expression on Day 15, but this effect was weaker than in the UUO model [32]. Drinking NAM (0.3, 0.6 or 1.2% NAM) from Day 0 until the end of study (6 weeks) improved kidney function and decreased tubular injury and inflammation in murine adenine-induced CKD. However, in advanced stages of adenine-CKD, drinking 0.3 or 0.6% NAM from Week 6 of adenine diet until sacrifice at 10 weeks did not improve kidney fibrosis and function [33].

Dietary NR supplementation (800 mg/kg/day) 1 week prior to CKD induction until sacrifice did not prevent CKD progression, assessed as kidney function [glomerular filtration rate (GFR), blood urea nitrogen (BUN) and serum creatinine (sCr)] and fibrosis, in two murine models of CKD induced by UUO or proteinuria, despite increased kidney NAD^+ levels [34].

NMN administration (500 mg/kg, i.p.) 20 min prior to unilateral ischaemia–reperfusion injury (IRI) and daily for 3 days after IRI prevented tubular damage and cell death at Day 3 and attenuated tubular senescence and fibrosis at Day 21 [35]. Additionally, NMN in the recovery phase (500 mg/kg i.p. on Days 3 and 14 after surgery) reduced DNA damage, inflammation and fibrosis at Day 21 after IRI [35].

Overall, published experience supports a preventive but not therapeutic role of NAM or NMN in pre-clinical CKD. However, the lack of benefit observed for NR, despite increasing kidney NAD^+ levels, casts doubt on the mechanisms of protection and raises the spectrum of publication bias.

VITAMIN B3 IN AKI

Vitamin B3 vitamers are up taken by proximal tubule brush borders through the sodium monocarboxylate transporter for niacin and through unidentified transporters for NAM [36, 37]. Additionally, kidneys may also synthetize NAD^+ from

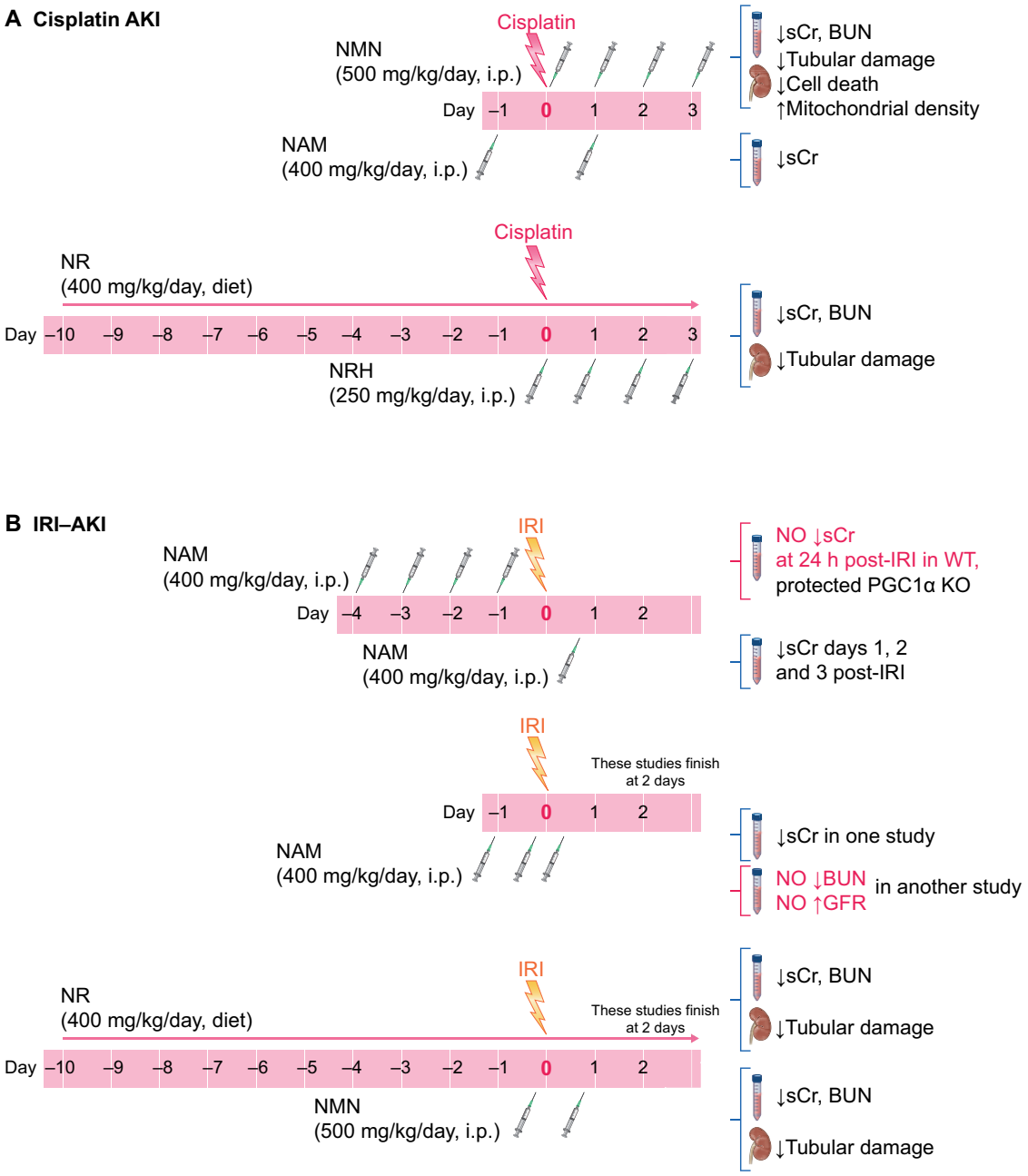

FIGURE 4: Dosing schedules and outcomes of pre-clinical studies in AKI. (**A**) Cisplatin AKI. (**B**) IRI AKI.

tryptophan, as do hepatocytes [38]. During AKI, mitochondrial respiration and function are impaired [39], leading to a substantial decrease in NAD^+ levels, which may compromise energy production and ultimately the core kidney function of selective solute transport [1, 40]. Peroxisome proliferator-activated receptor gamma co-activator 1 alpha (PGC-1α) is a master regulator of mitochondrial biogenesis. Upon deacetylation by Sirtuin 1, PGC-1α translocates to the nucleus where it is a transcriptional coactivator of genes encoding mitochondrial proteins [39]. Indeed, kidney tubule PGC-1α coordinates NAD^+ *de novo* biosynthesis [40], constituting a positive feedback loop where PGC-1α and NAD^+ upregulate each other, and both are downregulated in AKI [40–42]. Therefore, decreased NAD^+ levels may lead to reduced PGC-1α activity and mitochondrial biogenesis and vice versa. During AKI, PGC-1α is downregulated, and PGC-1α deficiency promotes local inflammation and increases the sensitivity to AKI [39, 42, 43]. The expression of quinolinate phosphoribosyltransferase (QPRT), which catalyses the final step of the tryptophan pathway, is also reduced in AKI, leading to increased quinolate levels and decreased NAD levels [27]. Indeed, as is the case for PGC-1α-deficient mice, $QPRT^{+/-}$ mice have increased susceptibility to IRI AKI [27]. The increased sensitivity of these genetically modified mice to AKI may be improved by vitamin B3 vitamers, as discussed below.

Based in part on these observations, AKI has been proposed to represent a state of NAD^+ impairment that can be therapeutically targeted by approaches that replete NAD^+ [44]. Therapeutic approaches aimed at increasing kidney NAD^+ levels in AKI include administration of NAM, NR, NRH or NMN (Figure 4) (Table 2).

Table 1. Clinical trials of vitamin B3 vitamers in kidney disease: niacin, NAM or NR

Trial	Population	*n*	Phase	Comparator	Primary endpoint	Key secondary endpoint	Status/results	Completion	Comments
Ongoing trials									
CoNR (NCT03579693)	Non-KRT CKD (eGFR <50)	30	2	Coenzyme Q10/NR/ placebo	Aerobic capacity	Inflammation	Recruiting	2021	–
NCT04342975	Elective open aortic arch replacement or repair	238	2	NR 1 g/day + pterostilbene (Basis™)/ placebo	AKI incidence (eGFR)		Recruiting	2024	–
NIRVANA (NCT04818216)	COVID-19 with persistent AKI	100	2	NR 1 g/dday/placebo	Whole blood NAD^+, safety	Kidney function, proteinuria	Recruiting	2023	–
VITAKI (NCT04589546)	Septic shock	310	3	NAM 1 g/day/placebo	MAKE30[a]		Not yet recruiting	2024	–
NACAM (NCT04750616)	Cardiac surgery-associated myocardial injury	304	2	NAM 3 g/day pre-surgery and post-surgery Days 1 and 2 a/ placebo	Troponin T	eGFR	Not yet recruiting	2024	–
Completed trials[b]									
NCT00852969	CKD	30	4	Niacin/placebo	Flow-mediated dilation by brachial artery reactivity	HDL-C	No differences in outcomes	2012	–
AIM-HIGH NCT00120289 [26]	$eGFR_{cys+cr}$ <60, >45 years old, established vascular disease and atherogenic dyslipidaemia, sCr <2.5 mg/L	352 of 3414	3	Niacin 1.5 or 2 g + simvastatin/niacin 50 mg + placebo + simvastatin	Composite CVD endpoint[c]	Other CVD endpoints Serum phosphate CKD-MBD parameters	Terminated/lack of efficacy for primary endpoint. *Post hoc* analysis: lower serum phosphate at 3 years, no change in other CKD-MBD parameters	2011	–
NCT02701127 [27]	Cardiac surgery at risk of AKI	55	1	NAM 1 or 3 g/placebo	Serum NAM metabolites	Urine NAM metabolites	Increased circulating NAD^+ metabolites, well tolerated, associated with less AKI	2017	–
NIAC-PKD1 (NCT02140814) [28]	Polycystic kidney disease	10	2	NAM/uncontrolled	Sirtuin deacetylase activity (12 months)	Sirtuin deacetylase activity (6 months)	No sustained inhibition of sirtuin activity	2016	–
NIAC-PKD2 (NCT02558595) [28]	ADPKD, eGFR >50	36	Pilot	NAM/placebo	Acetylated/total p53 ratio in PBMC	TKV, eGFR, urinary MCP-1	No differences in outcomes	2017	–
COMBINE/ NCT02258074	CKD	205	2	NAM/lanthanum/ placebo	Serum phosphate, FGF23	Markers of CKD-MBD, CVD, CKD progression	No differences in outcomes	2019	Up to 42% stopped study drug
NCT00508885 [29]	Peritoneal dialysis	17	1, 2	NAM/placebo plus phosphate binder	Plasma phosphate	CKD-MBD parameters	Lower plasma phosphate	2007	–
2013-000488-95 [19]	Haemodialysis patients	738	3	NAM/placebo plus phosphate binder	Serum phosphate	CKD-MBD parameters, lipids	Lower serum phosphate	2017	–

(continued)

Table 1. (continued)

Trial	Population	*n*	Phase	Comparator	Primary endpoint	Key secondary endpoint	Status/results	Completion	Comments
NICOREN [20]	Haemodialysis patients	176	3	NAM/sevelamer	Serum phosphate	LDL/HDL-C, CKD-MBD parameters, NAM metabolites	Lower serum phosphate	2013	NAM worse tolerated than sevelamer

ClinicalTrials.gov identifier and/or publication provided to identify trials.
[a]MAKE30: in-hospital mortality, receipt of new KRT, or persistent renal dysfunction defined as a final inpatient sCr value ≥2-fold over baseline sCr.
[b]Status is 'completed' unless otherwise specified.
[c]Endpoint of CHD death, non-fatal myocardial infarction, ischaemic stroke, hospitalization for non-ST segment elevation, acute coronary syndrome, or symptom-driven coronary or cerebral revascularization.
CKD-MBD, CKD-mineral and bone disorder; COVID-19, coronavirus disease 2019; KRT, kidney replacement therapy; ADPKD, autosomal dominant polycystic kidney disease; FGF23, fibroblast growth factor-23; CVD, cardiovascular disease; HDL-C, high-density lipoprotein cholesterol; TKV, total kidney volume; eGFR, estimated GFR in mL/min/1.73 m^2.

Intraperitoneal NAM improved kidney function as assessed by sCr in mice with bilateral IRI or cisplatin-induced AKI [27, 40] (Table 2). However, in one IRI AKI study, the therapeutic effect was only noted in PGC-1α knock-out (KO) mice, which have impaired mitochondrial function and lower spontaneous kidney NAM levels. Tran *et al*. explored two dosing schemes in IRI. Administration of four daily doses (400 mg/kg NAM, i.p.) before IRI protected PGC-1α KO mice, as assessed by sCr levels at 24 h (sCr ∼1.6 versus ∼0.6 mg/dL, $P < 0.01$), but not wild-type (WT) mice (sCr ∼0.75 versus ∼0.50 mg/dL, P = not significant) [40]. Control WT AKI mice had less severe AKI at this time-point than PGC-1α KO mice. A single dose of 400 mg/kg NAM administered i.p. 18 h after IRI protected WT mice as assessed by sCr levels at 24–72 h [40] (Figure 4). In cisplatin nephrotoxicity, two doses of 400 mg/kg NAM, administered i.p. 1 day before and at the time of cisplatin injection, reduced sCr levels at 72 h [40]. Poyan Mehr *et al*. administrated three doses of 400 mg/kg NAM i.p. 24 and 1 h prior to IRI and 4–6 h after IRI, and observed milder AKI in both WT (sCr ∼1.9 versus ∼1.1 mg/dL at 24 h, $P < 0.05$) and QPRT-deficient mice (sCr ∼2.6 versus ∼1.5 mg/dL at 24 h, $P < 0.01$), noting that AKI was more severe in control QPRT-deficient AKI mice than in control WT mice with AKI ($P < 0.05$) [27].

NR administered 10 days before AKI induction until sacrifice (400 mg/kg/day in diet) improved kidney function and decreased tubular injury at 48 h in IRI-induced AKI and at 72 h in cisplatin AKI [34]. In contrast, NR administration by oral gavage (500 mg/kg/day) for 2 weeks before IRI surgery and up to 24 h later did not protect rats at 24 h or 14 days after IRI, as assessed by sCr or kidney NGAL mRNA, despite repleting tissue NAD^+ [48].

A reduced form of NR, NRH, administered i.p. (250 mg/kg) at the time that cisplatin and at 24, 48 and 72 h thereafter, significantly reduced BUN levels, tubular injury and recovered kidney NAD levels at 72 h in mice [46]. NMNH is a reduced form of NMN that protects cultured kidney cells from hypoxia and *in vivo* increased NAD^+ levels in blood and several organs including the kidneys [49].

NMN administration (500 mg/kg i.p.) immediately after cisplatin injection and for three consecutive days until sacrifice significantly protected 3- and 20-month-old mice from cisplatin-induced AKI at 72 h as assessed by sCr, BUN, tubular damage and tubular cell death [45]. In the same report, NMN 500 mg/kg i.p. right before IRI and 24 h after reperfusion, significantly protected 3-month-old mice as assessed at 48 h by sCr, BUN and tubular damage [45].

Overall, as it was the case for CKD, published experience supports a preventive and even therapeutic role of NAM, NR, NRH or NMN in pre-clinical AKI. However, as for CKD, there were mixed results reported for NR, with one study failing to observe benefit in rat IRI AKI despite increasing kidney NAD^+ levels.

Regarding clinical translation, urine quinolinate levels were higher in cardiac surgery patients that develop AKI [27]. A Phase I pilot study addressed the safety and impact on NAM levels of administering NAM to prevent AKI in cardiac surgery [27]. Three daily doses of either 1 ($n = 13$) or 3 g ($n = 14$) oral NAM or placebo ($n = 14$) were administered on the day prior to on-pump cardiac surgery, on the day of surgery and on the first post-operative day. Circulating and urinary NAM, NMN and N1-methyl-NAM (MNA, a metabolite not in the NAD^+ pathway) increased without major safety concerns. Patients on the higher dose had persistently elevated serum NAM and NMN and urine NAM at 48 h. In a *post hoc* analysis, treatment with NAM resulted in lower frequency of AKI events in both AKI groups combined: 4/27 (15%)

Table 2. Therapeutic approaches aimed at increasing NAD^+ availability in preclinical AKI

Model	Drug	Dosing schedule	Mice	Time of readout	Result	Comments	Ref
Cisplatin	NAM	400 mg/kg i.p. 1 day before and at time of cisplatin	PGC-1α$^{-/-a}$	24, 48 and 72 h	↓sCr at 72 h ↑Kidney NAM at 24 h	Protection	[40]
	NMN	500 mg/kg i.p. immediately after cisplatin injection and at 24, 48 and 72 h	3- and 20-month-old WT	72 h	↓sCr, BUN ↓Tubular damage ↓Cell death ↑Mitochondrial density	Protection in 3- and 20-month old	[45]
	NR	400 mg/kg/day, in diet 10 days prior to cisplatin and up to sacrifice	WT	72 h	↓sCr, BUN ↓Tubular damage	Protection	[34]
	NRH	250 mg/kg i.p. at time of cisplatin and at 24, 48 and 72 h	WT	72 h	↓BUN ↓Tubular damage ↑ Kidney NAD	Protection	[46]
IRI	NAM	400 mg/kg i.p. 4 daily doses before IRI	WT, PGC-1α$^{-/-}$	24 h	↓sCr ↑Kidney NAM in PGC-1α$^{-/-}$ mice	No protection in WT mice	[40]
		400 mg/kg i.p. 18 h after IRI	PGC-1α$^{-/-}$	24, 48 and 72 h	↓sCr at all points	Protection	[40]
		400 mg/kg i.p. 24 and 1 h pre-IRI and 4–6 h after IRI	WT, QPRT$^{+/-}$	24 h	↓sCr	Protection QPRT$^{+/-}$ and WT	[27]
		400 mg/kg i.p. 24 and 1 h pre-IRI and 4–6 h after IRI	WT	BUN 48 h GFR –24 h, +24 h	BUN and measured GFR do not change	No protection	[47]
	NMN	500 mg/kg i.p. right before IRI and 24 h after reperfusion	WT	48 h	↓sCr, BUN ↓Tubular damage	Protection	[45]
	NR	400 mg/kg/day, in diet 10 days before IRI and up to sacrifice	WT	48 h	↓sCr, BUN ↓Tubular damage	Protection	[34]

NRH, 1-[(2R , 3R , 4S , 5R)-3,4-Dihydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl]-4H-pyridine-3-carboxamide, which is the NAD^+ precursor in mammalian cells. BUN, blood urea nitrogen.
[a]Not specified.

versus 7/14 (50%) in the placebo group ($P < 0.05$) [27]. However, it has been pointed out that sCr started to decrease in NAM-treated patients on day 0 and reached a nadir on Day 1, raising the spectrum of interference with sCr method and/or creatinine kinetics [47]. Four ongoing placebo-controlled clinical trials exploring NAM or NR to prevent or treat AKI are expected to be completed between 2023 and 2024 (Table 1). Of these, one Phase 2 (NR in aortic arch surgery) and one Phase 3 clinical trial (NAM in septic shock) have kidney function as primary outcome.

DIVERGENT EFFECTS OF NAM SUPPLEMENTATION ON MURINE IRI AKI

In a recent issue of CKJ, Piedrafita *et al.* [47] reported that urine tryptophan and kynurenine were reduced in cardiac bypass surgery patients who developed AKI, especially in those with more severe AKI. However, receiver operating characteristic (ROC) curves with both markers showed a poor discrimination for AKI severity or post-operative mortality. Based on the low levels of urine tryptophan and kynurenine, they hypothesized that reduced activity of the kynurenine pathway may contribute to AKI and explored the effect of NAM supplementation on murine IRI AKI. However, NAM did not improve kidney function as assessed by measured GFR at 36 h or BUN at 48 h in male or female mice, nor did it reduce histological injury or kidney Kim-1 expression, the latter being even higher in NAM-treated males. These findings are in contrast with the protection provided by NAM in murine IRI AKI in studies by Poyan Mehr *et al.*, but may be aligned with results by Tran *et al.* [27, 40]. What may explain the different results obtained?

The dosing schedule was similar for Piedrafita and Poyan Mehr. Surgical methods were also similar, but ischaemia time differed: 20 min for Tran and Poyan Mehr [27, 40] and 25 min for Piedrafita [47] . One possibility is that the longer ischeamia time decreased the efficacy of the intervention, but this hypothesis should be assessed experimentally. Furthermore, Tran and Poyan Mehr assessed sCr, while Piedrafita assessed BUN and measured GFR by sinistrin-FITC clearance. Therefore, further studies, using the same readouts of kidney injury, are necessary to clarify the effect of NAM over AKI. In this regard, an impact of NAM on creatinine generation, kinetics or assessment cannot be excluded, and Tran and Poyan Mehr did not report on further evidence of kidney injury such as kidney gene expression or histological injury.

Of interest, only Piedrafita *et al.* assessed the impact of NAM supplementation on kidney NAD^+ levels, and failed to observe improvement over AKI controls at the studied timepoint. This may explain the failure to observe kidney protection, but it would be necessary to compare these data with the impact of NAM over kidney NAD^+ levels in studies in which NAM was protective.

Finally, the origin of mice differed: Janvier supplied C57BL/6JRj mice to Piedrafita, and Jackson supplied C57BL6J for Tran and Poyan Mehr. A different origin for the mice may have impacted their gut microbiota on top of any potential strain

differences. The gut microbiota is a key modulator of dietary tryptophan metabolism, including the pathway leading to niacin [50].

GAPS IN KNOWLEDGE THAT NEED TO BE FILLED

In conclusion, administering vitamin B3 vitamers and other metabolites in the NAD^+ biosynthesis pathway is a hot topic in kidney protection. In addition to mostly positive pre-clinical data in models of both AKI and CKD, several randomized clinical trials are ongoing in AKI and at least two have a primary endpoint of kidney function. The clinical development stage generates a sense of urgency in the need to fully understand which are the optimal molecules, dose, timing and route of administration that protect the kidneys, as well as to understand the molecular mechanisms of protection. However, several studies could not reproduce the protection described by other authors in AKI or CKD, in some cases despite documentation of repletion of kidney NAD^+. This raises questions that so far remain answered. The recent concept of pre-clinical multicentre randomized controlled trials may help address some of the pending issues regarding the impact of NAM or other vitamin B3 vitamers or NAD^+ precursors on pre-clinical kidney injury [51]. Knowledge derived from such studies, which incorporate the different levels of heterogeneity that are expected in clinical care, may better guide the design of clinical trials.

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CONFLICT OF INTEREST STATEMENT

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