De novo NAD\(^+\) biosynthetic impairment in acute kidney injury in humans

Ali Poyan Mehr\(^{1,12}\), Mei T. Tran\(^{1,12}\), Kenneth M. Ralto\(^{1,2,3,12}\), David E. Leaf\(^4\), Vaughan Washco\(^1\), Joseph Messmer\(^1\), Adam Lerner\(^5\), Ajay Kher\(^1\), Steven H. Kim\(^1\), Charbel C. Khoury\(^6\), Shoshana J. Herzig\(^7\), Mary E. Trovato\(^8\), Noemie Simon-Tillaux\(^8\), Matthew R. Lynch\(^1\), Ravi I. Thadhani\(^8\), Clary B. Clish\(^9\), Kamal R. Khabbaz\(^8,13\), Eugene P. Rhee\(^6,9,10\), Sushrut S. Waikar\(^4\), Anders H. Berg\(^{11,13}\) and Samir M. Parikh\(^{11,13}\)*

Nicotinamide adenine dinucleotide (NAD\(^+\)) extends longevity in experimental organisms, raising interest in its impact on human health. De novo NAD\(^+\) biosynthesis from tryptophan is evolutionarily conserved yet considered supplanted among higher species by biosynthesis from nicotinamide (NAM). Here we show that a bottleneck enzyme in de novo biosynthesis, quinolinate phosphoribosyltransferase (QPRT), defends renal NAD\(^+\) and mediates resistance to acute kidney injury (AKI). Following murine AKI, renal NAD\(^+\) fell, quinolinate rose, and QPRT declined. QPRT\(^+/−\) mice exhibited higher quinolinate, lower NAD\(^+\), and higher AKI susceptibility. Metabolomics suggested an elevated urinary quinolinate/tryptophan ratio (uQ/T) as an indicator of reduced QPRT. Elevated uQ/T predicted AKI and other adverse outcomes in critically ill patients. A phase 1 placebo-controlled study of oral NAM demonstrated a dose-related increase in circulating NAD\(^+\) metabolites. NAM was well tolerated and was associated with less AKI. Therefore, impaired NAD\(^+\) biosynthesis may be a feature of high-risk hospitalizations for which NAD\(^+\) augmentation could be beneficial.

Nicotinamide adenine dinucleotide (NAD\(^+\)) is a universal electron acceptor from glycolysis and the Krebs cycle. It is also a substrate for non-redox enzymes that consume NAD\(^+\), such as poly (adenosine diphosphate-ribose) polymerases, sirtuins, and eutonucleotidases\(^{1−5}\). Chronic deficiency of nicotinamide (NAM) or nicotinic acid, vitamin B\(_3\) analogs that are NAD\(^+\) precursors, affects several metabolically active organs. Deficiency of a third NAD\(^+\) precursor, tryptophan, can develop from inherited defects in a neutral amino acid transporter and impairs metabolically active organs. Although the biosynthesis of NAD\(^+\) proceeds through distinct routes depending on the dietary precursor, evidence from dietary NAM deficiency or tryptophan transporter defects demonstrates that supplementation with one precursor can effectively treat deficiency of the other\(^{6,4}\). More recently, NAD\(^+\) metabolism (Fig. 1a) has been suggested as a therapeutic target for diverse diseases ranging from diet-induced obesity to neuronal degeneration and glaucoma, conditions that share substantial metabolic stress\(^{6−9}\). NAD\(^+\) preservation may also prevent age-related decline in health and even extend life span\(^{10−12}\). Critically ill patients are often subjected to severe metabolic stress—arising from systemic inflammation and ischemia—and are susceptible to an array of age-associated complications. One such complication, acute kidney injury (AKI), affects 3–10% of all hospitalized adults, can be deadly, and lacks a specific treatment\(^{13}\).

Results
Renal and urinary quinolinate elevation in ischemic AKI. To study AKI, we conducted an unbiased metabolomics screen on the urine of mice with AKI induced by transient renal ischemia (Fig. 1b). Of 204 metabolites measured (Supplementary Table 1), 27 were more than twofold increased in postischemic urines compared to controls including several sugars and amino acids, a pattern consistent with tubular impairment (Supplementary Table 2). Among these metabolites was quinolinate, an intermediate in the de novo NAD\(^+\) biosynthetic pathway from tryptophan (Fig. 1c). After normalizing for tryptophan, urinary quinolinate elevation persisted (Fig. 1d). To assess whether excess urinary quinolinate reflected intrarenal processes rather than filtration into urine from extrarenal sources, we then measured kidney tissue levels. Both renal quinolinate and the ratio of renal quinolinate to renal tryptophan were strongly related to renal function and postischemic injury (Fig. 1e–h). This suggested a reduction in AKI of renal quinolinate phosphoribosyltransferase (QPRT), an enzyme that connects the initial steps of opening tryptophan’s pyrrole ring to the final steps of NAD\(^+\) biosynthesis (Fig. 1a). Whereas quinolinate can only be

\(^{1}\)Division of Nephrology and Department of Medicine, Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, MA, USA. \(^{2}\)Division of Pulmonary and Critical Care and Department of Medicine, Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, MA, USA. \(^{3}\)Division of Pulmonary and Critical Care, Massachusetts General Hospital and Harvard Medical School, Boston, MA, USA. \(^{4}\)Division of Renal Medicine and Department of Medicine, Brigham and Women’s Hospital and Harvard Medical School, Boston, MA, USA. \(^{5}\)Department of Anesthesia and Critical Care, Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, MA, USA. \(^{6}\)Division of Nephrology and Department of Medicine, Massachusetts General Hospital and Harvard Medical School, Boston, MA, USA. \(^{7}\)Division of General Medicine and Primary Care Medicine and Department of Medicine, Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, MA, USA. \(^{8}\)Cardiovascular Institute and Department of Surgery, Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, MA, USA. \(^{9}\)Broad Institute of Harvard and MIT, Cambridge, MA, USA. \(^{10}\)Endocrine Unit and Department of Medicine, Massachusetts General Hospital and Harvard Medical School, Boston, MA, USA. \(^{11}\)Division of Clinical Chemistry and Department of Pathology, Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, MA, USA. \(^{12}\)These authors contributed equally: Ali Poyan Mehr, Mei T. Tran, Kenneth M. Ralto. \(^{13}\)These authors jointly supervised: Kamal R. Khabbaz, Anders H. Berg, Samir M. Parikh. *e-mail: sparikh1@bidmc.harvard.edu
transformed to penultimate NAD\(^+\) precursors, tryptophan, and other upstream metabolites have multiple metabolic fates, thus making quinolinate the first fully committed NAD\(^+\) precursor in de novo biosynthesis (Kyoto Encyclopedia of Genes and Genomes; see URLs). Renal NAD\(^+\) and renal NAD\(^+\) phosphate (NADP\(^+\)) were also significantly reduced by AKI (Fig. 1i,j).

**QPRT mediates resistance to AKI.** Data from public repositories demonstrated selective enrichment of human and mouse QPRT in the kidney and liver among the body’s major organs (Supplementary Fig. 1a,b). In the kidney, QPRT protein was abundant in the epithelium of the proximal tubule, its most metabolically active cellular compartment (Supplementary Fig. 1c). Renal QPRT expression was attenuated by transient renal ischemia (Fig. 2a), attenuated in mice hypersensitive to renal ischemia and induced in mice resistant to renal ischemia (Supplementary Fig. 1d)

The involvement of QPRT in AKI has not been previously described. Therefore, we created QPRT\(^{+/−}\) mice by CRISPR–Cas9 gene editing and studied several founder lines (Supplementary Fig. 2). Loss of one allele recapitulated the extent of QPRT reduction associated with AKI (Fig. 2b). Urinary quinolinate and the urinary quinolinate/tryptophan ratio (uQ/T) were elevated in QPRT\(^{+/−}\) mice even in the absence of renal injury. This corroborated the specificity of the AKI metabolite profile for reduced QPRT that was originally suggested by the biochemistry, namely that quinolinate is only known to be made and utilized in this single pathway of de novo NAD\(^+\) biosynthesis (Fig. 1c–e and Supplementary Table 3). Among several metabolically active organs, QPRT reduction lowered NAD\(^+\) content. Based on renal NAD\(^+\) deficiency, we hypothesized that QPRT\(^{+/−}\) mice would be more vulnerable to acute ischemic stress. Renal function and injury to tubular cells (Fig. 2g, h) 24 h after transient renal ischemia was indeed worse in QPRT\(^{+/−}\) mice. We then tested the role of QPRT-independent augmentation of NAD\(^+\) metabolism via salvage biosynthesis by administering NAM in this model. NAM overcame the sensitivity of QPRT\(^{+/−}\) mice to transient renal ischemia (Fig. 2g). Together, these data showed that impairment of
QPRT mediates resistance to experimental AKI. a, Mouse renal QPRT mRNA 24 h after transient ischemia (n = 6 animals per group). b, QPRT mRNA in littermate controls (wild-type (WT)) versus QPRT−/− mice (n = 5 animals per group). c, Volcano plot comparing urinary metabolites (n = 204) in littermate controls (WT = 4 animals) versus QPRT−/− mice (n = 5 animals). The vertical dashed lines indicate the threshold for the twofold abundance difference. The horizontal dashed line indicates the P = 0.05 threshold. X-axis, log₂[fold change for right condition/ left condition]. Red dot, quinoline; red dot with black border, quinoline/tryptophan (Q/T) ratio. Y-axis, −log₁₀[P value]. P value computed by using a two-sided unpaired t-test without adjustment for multiple comparisons. d,e, Urinary quinoline (uQUIN) and urinary Q/T (uQ/T) ratio from c, a.u., arbitrary unit. f, Tissue NAD⁺ content in littermate controls (WT) versus QPRT−/− mice (n = 5 animals per group). g, Renal function 24 h after transient renal ischemia-reperfusion injury (IRI) in littermate controls (WT) versus QPRT−/− mice receiving vehicle (Veh) or NAM (400 mg kg⁻¹ intraperitoneally) −24, −1, and +4–6 h relative to surgery (n = 10, 10, 8, and 9 animals per group, respectively). h, Representative examples from 3 independent animals per group of intratubular cast (arrowhead) and tubular necrosis (arrows) 24 h after transient renal ischemia in littermate controls (WT) versus QPRT−/− mice. Scale bar, 20 μm. Data in a, b, d-f, and g displayed as mean ± s.e.m.; pairwise comparisons by Mann–Whitney U test with two-sided *P < 0.05 and **P < 0.01.
reported to be well tolerated in humans at high doses administered chronically. We first tested a 3-day regimen of 3 g per day of oral NAM among healthy volunteers to confirm that the pharmacological supply of NAM led to measurable changes in circulating NAM (Supplementary Table 8). We detected elevation of circulating NAM, consistent with a previous report of oral NAM obtained from different manufacturers (Supplementary Fig. 6a). Serum creatinine concentration among adults undergoing cardiac surgery (trial number NCT02701127, see URLs; Fig. 4a,b). The primary end point compared blood NAM levels between blinded participants randomized to receive placebo, 1 g per day NAM, or 3 g per day NAM once daily relative to cardiopulmonary bypass.

**Fig. 3 | Elevation of the uQ/T ratio in human AKI.** a, De novo biosynthesis of NAD⁺ from tryptophan. IDO, indole dioxygenase; TDO, tryptophan dioxygenase; AFMID, arylformamidase; KMO, kynurenine monooxygenase; KYNU, kynureninase; HAAO, 3-hydroxyanthranilate 3,4-dioxygenase. Quinolinate is then ribosylated by quinolinate phosphoribosyltransferase. b–f, Urinary metabolites in cardiac surgery patients. Measurements were compared by time relative to cardiopulmonary bypass and whether patients developed AKI (n = 6 per group). CPB, during cardiopulmonary bypass. ICU, immediate postoperative period during which patients were still intubated for mechanical ventilation. +6 h, 6 h after surgery completion. Otherwise, times are day relative to surgery. For b–f, significance was assessed by two-way analysis of variance with the two-sided P value indicating treatment effect and data displayed as mean ± s.e.m. g–i, Urinary metabolites in a prospective cohort study of ICU patients (n = 215 patients, 51 of whom subsequently developed AKI and 164 without AKI). g–i, Urinary metabolite and uQ/T ratio in those who did or did not develop AKI. Data displayed as median ± interquartile range. Model 1 is adjusted for the following demographics and comorbidities: age; sex; race; baseline estimated glomerular filtration rate; hypertension; and diabetes mellitus. Model 3 is further adjusted for the following severity of illness covariates: ICU type; need for mechanical ventilation; and APACHE II score. Quartile I (Q1) was the reference in all models. Error bars for 95% confidence interval (CI). j, Forest plot for incident AKI and other outcomes as listed per s.d. of log-transformed uQ/T. Error bars for 95% CI. Two-sided ***P < 0.01 and ****P < 0.001.

We then designed a phase I pilot study of oral NAM administration among adults undergoing cardiac surgery (trial number NCT02701127, see URLs; Fig. 4a,b). The primary end point compared blood NAM levels between blinded participants randomized to receive placebo, 1 g per day NAM, or 3 g per day NAM once daily by mouth or orogastric tube on days −1, 0, and +1 relative to surgery. The treatment groups were well balanced across 37 baseline demographic, clinical, and laboratory characteristics (Table 1 and Supplementary Tables 9 and 10). NAM administration significantly increased blood and urine NAM (Fig. 4c,d). NAM can either contribute to de novo NAD⁺ biosynthesis through the intermediate NAM mononucleotide (NMN) or undergo methylation to N°-methylnicotinamide (MNA) followed by irreversible oxidation to waste products. NAM administration at the 3 g per day regimen increased NMN whereas either NAM regimen increased MNA relative to placebo (Fig. 4e,f). NAM administration was not associated with increased adverse events compared to placebo. Serious adverse events were uncommon, were distributed evenly across the study arms, and were independently adjudicated to be unrelated to study participation (Table 2).

Given that either NAM treatment arm yielded significant higher exposure to NAM than the placebo arm, the comparable safety of either NAM dose to placebo, and the comparable effect on sCr of either NAM dose, we also combined the two NAM arms into one treatment group. One of the safety assessments evaluated whether NAM increased perioperative cardiac injury. NAM treatment was associated with lower blood levels of the cardiac injury marker troponin T compared to placebo (Fig. 4g,h). In a second safety assessment, we examined renal function because cardiac surgery increases AKI risk. NAM was associated with better estimated renal function compared to placebo (Fig. 4i,j). AKI events were significantly lower with NAM treatment than placebo (Supplementary Table 11 and Supplementary Fig. 7). Taken together, short-term NAM administration increased NAM levels and appeared to be well tolerated by
cardiac surgery patients. Safety assessments linked NAM administration to lower AKI risk.

Discussion

The present studies investigated the metabolic changes associated with AKI by applying a localized injury model in mice and unbiased metabolomic screening. A highly specific indicator of reduced QPRT emerged. Mimicking this acquired defect by gene editing corroborated the quantitative contribution of de novo biosynthesis to QPRT, or both. Elevating NAD+ metabolism may have several beneficial mechanisms of action, such as postischemic augmentation of fatty acid oxidation—for which NAD+ levels are rate-limiting15—to the provision of substrate for cytoprotective sirtuin enzymes25. NADP+ may also be critical in AKI because its reduced form, NADPH, promotes detoxification of reactive oxygen species26. Parsing a singular kidney-protective mechanism downstream of NAD+ augmentation may be challenging given the ~400 redox reactions involving NAD+/NADH, ~50 reactions involving NAD+ consumption, and ~30 redox reactions involving NADP+/NADPH throughout the cell27.

The de novo NAD+ biosynthetic pathway has been considered a minor contributor to intracellular NAD+ levels in mammals. However, a recent study identified rare loss-of-function mutations HAAO and KYN that reduced NAD+ in humans. These genes encode two other de novo enzymes, 3-hydroxyanthranilate 3,4-dioxogenase and kynureninase. Not only did this study independently corroborate the quantitative contribution of de novo biosynthesis to NAD+ metabolism, but affected individuals exhibited major renal anomalies28. This report identified a pivotal role for de novo NAD+ biosynthesis during human development and proposed the therapeutic potential of “orthogonal” NAD+ augmentation during gestation, i.e., independent of the de novo pathway. In the present context of acquired de novo NAD+ impairment related to reduced QPRT, there is a striking parallel in that kidney involvement is one of the most common complications suffered by critically ill patients and may also respond to orthogonal NAD+ augmentation. The present data propose both a mechanism of locally diminished NAD+ biosynthesis triggered by metabolic stressors such as ischemia and an inexpensive strategy for its therapeutic replenishment. Further, acquired impairments in the de novo pathway associated with aging or chronic kidney disease—two major AKI risk factors—may reduce renal NAD+ and thereby enhance susceptibility to AKI.
The transcriptional coactivator peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC1α) coordinates expression of QPRT and other de novo enzymes in the kidney both at baseline (Supplementary Fig. 1d) and during ischemic AKI; however, other QPRT regulators may also be important. For example, enrichment of QPRT expression in the kidney and liver may relate to their shared blood detoxification function. Shortage of NAD+ may also be a deleterious side effect of an otherwise adaptive response to injury. Since reduced QPRT would favor the accumulation of earlier injury. Since reduced QPRT would favor the accumulation of earlier

Table 1 | Baseline characteristics for oral NAM phase 1 pilot study in cardiac surgery patients

| Patient characteristics | Treatment group | Placebo group |
|-------------------------|-----------------|--------------|
| Age, yr, median (IQR)   | 52 (48.5–70.5)  | 64.5 (56–72.5)| 58 (52–72) |
| BMI, kg m⁻², mean (s.d.)| 31 (8)          | 29 (5.3)     | 30 (6.7)   |
| Female sex, n (%)       | 4 (31)          | 1 (7)        | 5 (19)     |
| African American, n (%) | 1 (8)           | 1 (7)        | 2 (7)      |
| CKD, eGFR ≤ 45 ml min⁻¹ or proteinuria, n (%) | 1 (8) | 3 (21) | 4 (15) |
| Cleveland Clinic score ≥ 5, n (%) | 3 (23) | 0 | 3 (7) |
| Ejection fraction < 35%, n (%) | 1 (8) | 1 (7) | 2 (7) |
| Hypertension, n (%)     | 8 (62)          | 10 (71)      | 18 (67)    |
| Heart failure, n (%)    | 4 (31)          | 4 (29)       | 8 (30)     |
| Diabetes, n (%)         | 5 (38)          | 2 (14)       | 7 (26)     |
| Tobacco use, n (%)      | 9 (69)          | 8 (57)       | 17 (63)    |

Surgical characteristics

| Aortic cross-clamp time, min, mean (s.d.) | Treatment group | Placebo group |
|------------------------------------------|-----------------|--------------|
| 60 ± 27                                   | 78 ± 37         | 69 ± 33      |
| CABG, n (%)                               | 6 (46)          | 5 (36)       |
| CABG + valve, n (%)                       | 3 (23)          | 1 (7)        |
| Valve, n (%)                              | 4 (31)          | 8 (57)       |
| Previous heart surgery, n (%)             | 0               | 2 (14)       |

Laboratory characteristics

| Sodium, mEq l⁻¹, mean (s.d.)             | Treatment group | Placebo group |
|-----------------------------------------|-----------------|--------------|
| 137 ± 2                                  | 138 ± 2         | 138 ± 2      |
| Potassium, mEq l⁻¹, mean (s.d.)         | 41 ± 0.4        | 4.0 ± 0.4    |
| Bicarbonate, mEq l⁻¹, mean (s.d.)       | 25 ± 3          | 25 ± 3       |
| BUN, mg dl⁻¹, mean (s.d.)               | 22 ± 4          | 19 ± 6       |
| Creatinine, mg dl⁻¹, median (IQR)       | 1 (0.7–1.1)     | 0.9 (0.7–1.2)|
| eGFR, ml min⁻¹, median (IQR)            | 83 (69–103)     | 86 (67–98)  |
| Hematocrit, %, mean (s.d.)              | 40 ± 5          | 40 ± 4       |
| Hemoglobin, g dl⁻¹, mean (s.d.)         | 13 ± 1.9        | 13.3 ± 1.7   |
| Platelets, k µl⁻¹, mean (s.d.)          | 208 ± 42        | 188 ± 67     |
| WBC, k µl⁻¹, mean (s.d.)                | 7.3 ± 1.7       | 7.5 ± 2.5    |
| AST, IU l⁻¹, mean (s.d.)                | 27 ± 18         | 31 ± 28      |
| ALT, IU l⁻¹, mean (s.d.)                | 28 ± 21         | 29 ± 22      |
| Total bilirubin, mg dl⁻¹, mean (s.d.)   | 0.5 ± 0.2       | 0.7 ± 0.2    |
| LDH, IU l⁻¹, mean (s.d.)                | 249 ± 86        | 244 ± 105    |
| CRP, mg l⁻¹, mean (s.d.)                | 18 ± 25         | 12 ± 25      |
| Troponin T, ng ml⁻¹, median (IQR)       | 0.02 (0.01–0.03)| 0.01 (0.01–0.01)|
| CK-MB, ng ml⁻¹, median (IQR)            | 2 (2–3)         | 2 (2–5)     |
| CK, IU l⁻¹, median (IQR)                | 140 (64–194)    | 113 (52–210)|
| PT, s, mean (s.d.)                      | 12 ± 2          | 12 ± 1      |
| PTT, s, mean (s.d.)                     | 51 ± 25         | 45 ± 22     |
| INR, mean (s.d.)                        | 1.1 ± 0.2       | 1.1 ± 0.1   |

Baseline demographic and clinical parameters (trial number NCT02701127; see URLs). There were no statistically significant between-group differences in any of the listed variables. IQR, interquartile range; BMI, body mass index; CKD, chronic kidney disease; eGFR, estimated glomerular filtration rate; CABG, coronary artery bypass graft; BUN, blood urea nitrogen; WBC, white blood cell count; AST, aspartate aminotransferase; ALT, alanine aminotransferase; LDH, lactate dehydrogenase; CRP, C-reactive protein; CK-MB, creatine kinase-muscle brain fraction; CK, creatine kinase; PT, prothrombin time; PTT, partial thromboplastin time; INR, international normalized ratio.
Table 2 | Perioperative assessments and adverse events for oral NAM phase 1 pilot study in cardiac surgery patients

| Safety monitoring | Treatment group | Placebo group (n = 14) | P value † |
|-------------------|-----------------|------------------------|----------|
|                   | NAM 1 g per day (n = 13) | NAM 3 g per day (n = 14) | Combined (n = 27) |
| Perioperative assessment | | | |
| Intraoperative urine output, ml kg h⁻¹, median (IQR) | 5.4 (2.2–6.9) | 1.7 (0.5–5.1) | 3.6 (1.6–6.8) | 2.4 (1.4–4.3) | 0.581 |
| Intraoperative total volume administration, ml, median (IQR) | 3,520 (2,988–3,704) | 3,475 (3,088–4,419) | 3,520 (3,100–4,050) | 3,690 (3,330–4,060) | 0.518 |
| Hospital stay, d, median (IQR) | 8 (7-10) | 9 (8-10) | 8 (7-10) | 10 (5-11) | 0.831 |
| Postoperative hospital stay, d, median (IQR) | 5 (4-7) | 6 (4-8) | 5 (4-8) | 5 (4-7) | 0.720 |
| ICU stay, d, median (IQR) | 2 (1-5) | 2 (1-3) | 2 (1-4) | 2 (1-3) | 0.826 |
| ALT, IU l⁻¹, mean (s.d.) | | | |
| Day 0 | 32 ± 21 | 40 ± 15 | 36 ± 18 | 35 ± 13 | 0.848 |
| Day 1 | 42 ± 23 | 46 ± 20 | 44 ± 21 | 35 ± 13 | 0.208 |
| Day 2 | 37 ± 19 | 40 ± 22 | 38 ± 20 | 37 ± 23 | 0.838 |
| Day 3 | 27 ± 12 | 35 ± 20 | 32 ± 17 | 33 ± 20 | 0.877 |
| AST, IU l⁻¹, mean (s.d.) | | | |
| Day 0 | 31 ± 26 | 29 ± 14 | 30 ± 20 | 28 ± 20 | 0.793 |
| Day 1 | 23 ± 18 | 20 ± 10 | 21 ± 14 | 23 ± 17 | 0.747 |
| Day 2 | 17 ± 11 | 17 ± 7 | 17 ± 9 | 18 ± 12 | 0.684 |
| Day 3 | 14 ± 7 | 22 ± 19 | 18 ± 15 | 19 ± 9 | 0.814 |
| LDH, IU l⁻¹, mean (s.d.) | | | |
| Day 0 | 222 ± 81 | 267 ± 57 | 244 ± 72 | 271 ± 104 | 0.409 |
| Day 1 | 260 ± 89 | 300 ± 96 | 280 ± 92 | 287 ± 79 | 0.843 |
| Day 2 | 325 ± 165 | 349 ± 220 | 339 ± 194 | 298 ± 87 | 0.502 |
| Day 3 | 279 ± 110 | 284 ± 89 | 282 ± 96 | 278 ± 67 | 0.916 |
| CK-MB, ng ml⁻¹, median (IQR) | | | |
| Day 0 | 14 (4-17) | 16 (5-26) | 15 (4-19) | 20 (12-41) | 0.020 |
| Day 1 | 13 (9-28) | 16 (5-26) | 16 (11-28) | 18 (9-27) | 0.490 |
| Day 2 | 5 (3-11) | 5 (3-10) | 5 (3-10) | 7 (3-17) | 0.242 |
| Day 3 | 3 (1-6) | 3 (2-5) | 3 (1-5) | 2 (1-5) | 0.294 |
| CK, IU l⁻¹, median (IQR) | | | |
| Day 0 | 144 (132-215) | 186 (124-486) | 172 (132-417) | 247 (177-445) | 0.538 |
| Day 1 | 507 (266-740) | 577 (279-737) | 542 (276-738) | 414 (371-569) | 0.268 |
| Day 2 | 359 (119-959) | 484 (202-1614) | 427 (176-958) | 392 (331-588) | 0.354 |
| Day 3 | 328 (114-627) | 243 (92-535) | 279 (111-595) | 187 (131-198) | 0.218 |
| Adverse events | | | |
| Nausea, n (%) | 3 (23) | 2 (14) | 5 (19) | 3 (21) | 0.673 |
| Poor appetite, n (%) | 3 (23) | 2 (14) | 5 (19) | 4 (29) | 0.692 |
| Rash, n (%) | 0 | 0 | 0 | 0 | |
| Delirium, n (%) | 1 (8) | 1 (7) | 2 (7) | 1 (7) | > 0.999 |
| Heart failure, n (%) | 2 (15) | 1 (7) | 3 (11) | 1 (7) | > 0.999 |
| Fever, n (%) | 1 (8) | 1 (7) | 2 (7) | 1 (7) | > 0.999 |
| Serious adverse events | | | |
| 30-day rehospitalization (unrelated to study participation) | 54 | 0 | 5 | 1 | – |
| Dialysis | 0 | 0 | 0 | 0 | – |
| Death | 0 | 0 | 0 | 0 | – |
| Complete heart block | 0 | 1 | 1 | 0 | – |
| Hemothorax | 0 | 0 | 0 | 1 | – |

IQR, interquartile range; ALT, alanine aminotransferase; AST, aspartate aminotransferase; LDH, lactate dehydrogenase; CK-MB, creatine kinase-muscle brain fraction; CK, creatine kinase. †P values were computed between combined treatment group and placebo group by Mann–Whitney U test (continuous variables) or chi-squared test (categorical variables). To enhance the detection of between-group differences, no adjustments were made for multiple comparisons. a Gastrointestinal symptoms, including nausea, were the most common adverse drug effects of NAM in past clinical trials; therefore, patients were specifically asked on each study visit through to day 30. b Rash (flush), a common symptom of niacin, and infrequently reported to occur with NAM was not reported or identified during study visits. c Heart failure beyond general postoperative fluid overload, requiring diuresis after discharge from the ICU occurred in all three treatment arms and was adjudicated as unrelated to study participation. d Left flank pain, likely musculoskeletal. e Sternal wound infection. f Diabetes and heart failure exacerbation, and diabetic foot infection. g Heart failure exacerbation. h Pulmonary embolus two weeks postoperatively in the setting of morbid obesity and prolonged immobilization. i Pneumonia. j Complete heart block on postoperative day 2, and resolved on postoperative day 4. k Postoperative day 1 return to operating room for intrathoracic bleed. Full recovery and discharge by postoperative day 9. Unrelated to study participation.
metabolites in the de novo pathway such as kynurenine, reduction of QPRT may offer the injured kidney a chemical mechanism to modulate local signaling responses in a manner that affects subsequent tissue repair\textsuperscript{26–28}.

Several areas require further investigation. First, upstream regulators of QPRT need to be identified. We found that PGC1α induces QPRT expression, but the relevant transcriptional partner(s) remain elusive. Second, the present results suggest that renal and urinary quinoline deriv generate impaired renal QPRT action, but this could also reflect renal accumulation from extrarenal sources that respond to renal ischemia. For example, QPRT’s expression is highest in the liver, where it should be studied further. Third, if QPRT is an important link between AKI and risk factors such as aging and chronic kidney disease (CKD), then one or more mechanisms implicated in aging and/or CKD may be important. Proposed mechanisms and effectors of aging include autophagy, redox balance, telomere shortening, epigenetic changes, and stem cell function\textsuperscript{29}. QPRT may be affected by or may impact several of these processes. Finally, the urinary metabolomic profiles of AKI and QPRT deficiency share 24 differentially regulated metabolites—approximately 40% of each profile. The nonoverlapping metabolites may illuminate novel QPRT-independent metabolic stressors in AKI and/or actions of QPRT outside the scope of postischemic AKI.

The clinical studies propose both a new noninvasive indicator of NAD\textsuperscript{+} metabolism in the context of AKI and a novel set of targets for future therapeutic studies. Several important limitations should be noted. Because quinoline is unique to the de novo NAD\textsuperscript{+} biosynthesis pathway, the evidence implicating uQ/T in clinical AKI strongly suggests that NAD\textsuperscript{+} metabolism is important in the human AKI context. Although verification of this signal in a larger, prospectively collected ICU cohort (Fig. 3g–j) helped to answer questions of confounding and overfitting in the discovery study, future studies will need to address the timing and amplitude of uQ/T deflection in different AKI populations before considering this metabolite signature an AKI biomarker. Routine safety assessments of renal and cardiac function in the high-risk cardiac surgery population yielded signals suggesting benefit. However, larger studies powered for these and related end points are clearly needed to address safety and efficacy. In this light, the present results provide valuable guidance for future trials. For example, since the 3 g per day dose was well tolerated and led to higher levels of circulating NMN than the 1 g per day dose, this regimen may be more effective for increasing NAD\textsuperscript{+} metabolism. The severity of AKI was modest in our phase 1 pilot study. Severe AKI after cardiac surgery is uncommon and would require much larger interventional studies to accumulate a sufficient number of severe events for analysis\textsuperscript{30}. Nonetheless, the incidence of AKI and the average postoperative rise in creatinine we observed in the placebo arm were comparable to large phase 3 trials\textsuperscript{31,32}.

The optimal method to boost NAD\textsuperscript{+} remains to be determined. Although our choice of NAM was based on its extensive human safety record, we also considered NAM’s ability to inhibit stress-activated poly (adenosine diphosphate-ribose) polymerases as a possible adjunctive benefit\textsuperscript{33}. However, NAM can also inhibit the cytoprotective enzyme NAD-dependent protein deacetylase sir-tuin-1. Both NAM and another natural NAD\textsuperscript{+} precursor, nicotinamide riboside, have been shown to increase intracellular NAD\textsuperscript{+}; however, nicotinamide riboside may also augment sirtuin activity more effectively than NAM\textsuperscript{34}. Complementary to orthogonal NAD\textsuperscript{+} supplementation, an add-on therapeutic strategy could also involve increasing flux through the de novo pathway, e.g., by administering de novo precursors or inhibiting enzyme(s) that deplete this pathway. Finally, compared to other metabolically active organs such as the brain or heart, which are also affected by aging or metabolic stressors, kidney function can be assessed quantitatively, reproducibly, and simply. Therefore, renal end points may become useful for future trials related to aging. More broadly, recognizing that age-dependent loss of resistance to acute metabolic stressors may be an aging phenotype suggests that exploratory interventional studies on aging could be conducted in a cost-efficient, acute context. In short, the biology of NAD\textsuperscript{+} should be examined in multiple clinical contexts.

To summarize, the present results demonstrate that impairment in the de novo biosynthesis of NAD\textsuperscript{+} characterizes patients at risk for AKI, a common and morbid complication of critical illness for which no specific treatment exists. Urinary measurement of de novo precursors in at-risk humans implicates impairment in this pathway and, furthermore, predicts adverse outcomes. The orthogonal NAD\textsuperscript{+} precursor NAM may be safe to administer to high-risk patients. Novel treatments to restore NAD\textsuperscript{+} could constitute an important advance for patients at risk of AKI. Further studies are needed to verify these findings.

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**Methods**

Methods, including statements of data availability and any associated accession codes and references, are available at https://doi.org/10.1038/s41591-018-0138-z.

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**URLs**

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Author contributions
A.P.M. and K.R.K. were co-principal investigators on the phase 1 pilot study of oral NAM in cardiac surgery patients for which V.W., J.M., A.L., and M.E.T. were coinvestigators. S.J.H. served as the independent medical monitor for this trial. K.M.R., N.S.-T., and A.H.B. analyzed samples and data from all human studies. A.H.B. developed targeted metabolic assays and conducted all related measurements. M.T.T. conducted the mouse studies and analyzed the results with assistance from N.S.-T. and M.R.L. D.E.L. and S.S.W. enrolled the ICU cohort, created that repository, and performed statistical analyses of the uQ/T results in the ICU cohort. A.K. and S.H.K. enrolled the discovery cohort of cardiac surgery patients and created that repository with guidance from S.M.P. C.C.K., E.P.R., and R.I.T. developed and conducted the trial of oral NAM in healthy volunteers. E.P.R. also conducted metabolic screening of mouse samples on the platform developed in C.B.C’s laboratory with input from C.B.C. A.P.M. and K.R.K. K.M.R., V.W., A.K., and M.R.L. were supported by award K23AG042459; N.S.-T. by a grant from Assistance Publique—Hôpitaux de Paris; and S.M.P. assumed primary responsibility for writing the manuscript. All authors reviewed, provided substantive input, and approved of the final manuscript.

Competing interests
S.M.P. is listed as an inventor on disclosures filed by BIDMC pertaining to NAD+.}

Additional Information
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Methods

Materials. All chemicals, except when noted, were purchased from Sigma-Aldrich.

Mouse studies. Ischemia-reperfusion injury (IRI) model. All studies involving mice were approved by the Beth Israel Deaconess Medical Center (BIDMC) Institutional Animal Care and Use Committee. Experiments were performed using littermate controls by an operator blinded to genotype and randomized within each cage to sham versus AKI model. Creatinine from mouse serum was measured using liquid chromatography–tandem mass spectrometry at the University of Alabama Birmingham O’Brian Core Center for Acute Kidney Injury Research in a blinded fashion (NH1 P30 DK079337). Bilateral renal IRI was performed as previously described.1 NAM or saline vehicle was administered as 400 mg kg⁻¹ intraperitoneal injections given 24 h before IRI surgery, 1 h before surgery, and 4–6 h after surgery. sCr was measured in all IRI experiments at 24 h after surgery.

CRISPR–Cas9 QPR™ mouse. Single-guide RNAs (sgRNAs) were designed using the CRISPR guide-design application from MIT (see URLs). All CRISPR reagents (Cas9 nickase and sgRNAs) were purchased from PNA Bio. Pronuclear stage zygotes were injected with CRISPR reagents and sgRNAs by the BIDMC Transgenic Core to generate founder mice on the C57BL6/J background. Founder mice were verified by genotyping with the primers shown in Supplementary Fig. 2 and Supplementary Table 1. Male mice of 8–12 weeks of age were used in the experiments.

Quantitative PCR. Total RNA extraction and cDNA synthesis were performed as previously described.2 PCR reactions were performed in duplicate using the QuantStudio 7 Flex Real-Time PCR System (Thermo Fisher Scientific). SYBR primers were designed using the PrimerQuest Tool (Integrated DNA Technologies) and the sequences are shown in Supplementary Table 12. Relative expression levels were determined using the comparative threshold method.

Metabolomics measurements. Mouse urine and renal samples were analyzed in a blinded fashion with two distinct liquid chromatography–mass spectrometry (LC–MS)-based methods. For positively charged polar analytes, 10 μl of urine were extracted with 90 μl of 74.9:24.9:0.2 (vol/vol/vol) acetonitrile/methanol/formic acid containing L-Valine-d₈ (Sigma-Aldrich). After centrifugation, 10 μl of supernatant underwent chromatography on a 150 × 2.1 mm Atlantis hydrophilic interaction chromatography column (Waters), and mass spectrometry data were acquired on an Exactive Plus Orbitrap Mass Spectrometer (Thermo Fisher Scientific) using electrospray ionization in the positive ion mode. For negatively charged polar analytes, 20 μl of urine was extracted with the addition of 70 μl of 80:20 (vol/vol) methanol/water containing isotope-labeled insulin (15N₄, 95%); 10 μl of supernatant underwent chromatography on a 150 × 2.0 mm Luna NH2 column (Phenomenex), and mass spectrometry data were acquired on an Exactive Plus Orbitrap Mass Spectrometer (Thermo Fisher Scientific) using electrospray ionization in the negative ion mode. Data were processed with the TraceFinder 3.0 for Windows (Thermo Fisher Scientific) software, matching retention times and mass-to-charge ratio to synthetic mixtures of reference compounds and characterized pooled plasma reference samples included in each sample queue.

NAD⁺ measurement. A 20–30 mg quantity of mouse kidney was homogenized with a Bullet Blender (Next Advance). Homogenization was performed in the NAD⁺/NADH extraction buffer supplied with the NAD⁺/NADH Quantification Colorimetric Assay (Biovision); 25 μl of homogenate was assayed according to the manufacturer’s instructions and the plate was read at 450 nm using a 96-well microplate reader (Bio-Rad).

Histopathology. Formalin-fixed, paraffin-embedded blocks were sectioned and stained with hematoxylin and eosin and photographed with Zen 2012 on Zeiss Axiocam 503.

Statistical considerations. Sample size for metabolomics experiments was based on previous experience.4 For the IRI experiments focused on renal function (i.e., sCr) outcome, sample size was based on previous experience and consideration of the following estimation: sCr of 1.4 ± 0.5 mg d⁻¹⁺⁻¹ among controls versus 2.1 ± 0.5 mg d⁻¹⁺⁻¹ among IRI mice requires n = 6 mice per group for 80% power and 5% α-level. For all mouse studies, nonparametric tests were used to compare continuous variables (Mann–Whitney U test or Kruskal–Wallis H test if more than two groups) unless otherwise noted. Data are presented as mean ± s.e.m. unless otherwise specified. Results were prepared using GraphPad Prism version 7 (GraphPad Software). Two-tailed P values < 0.05 were considered significant (*P < 0.05, **P < 0.01, ***P < 0.001, and ****P < 0.0001, unless otherwise indicated).

Human studies. uOT/ICU discovery nested case-control study. We examined the uOT/ICU ratio as an indicator of risk for AKI in a prospective cohort of patients undergoing nonurgent, on-pump cardiac surgery through a nested case-control analysis. The prospective cohort for this nested case-control study was enrolled over a period of six months. The primary inclusion criterion was planned, on-pump cardiac surgery. Patients were excluded for any of the following reasons: unresolved AKI 1 week before surgery; a history of kidney transplant; end-stage renal disease; pregnancy: younger than 18 years of age; unable to consent; off-pump cardiac surgery; and individuals held in an institution by legal or official order. The study was in accordance with the Declaration of Helsinki 2013, and approved by the Institutional Review Board (IRB) of the BIDMC (IRB 2010-P-0000052). All patients provided written informed consent before enrolling on the study. Study participants were patients undergoing cardiac surgery at the BIDMC. From January 2011 to June 2011, a total of 29 patients were enrolled (Supplementary Table 4), of which 6 had AKI. Using risk-set sampling,5 we randomly selected controls from the subgroup who had no AKI.

AKI was defined by the Kidney Disease Improving Global Outcomes (KDIGO) criteria for sCr during any of the postoperative days 1 through to 3.6 The first sCr was measured in each 24 h calendar day was used as the daily value. Patients with AKI (n = 6) were matched with controls at a 1:1 ratio for age (±11 years), ejection fraction (±12%) based on echocardiogram results, aortic cross-clamp time (±12 min) obtained from anesthesia records, and baseline creatinine (±0.5 mg d⁻¹⁺⁻¹) obtained from the patient’s medical record and defined as the most recent creatinine value before cardiac surgery. Assessment of other factors, including body mass index, sex, race, smoking history, clinical diagnosis of heart failure, hypertension, cardiopulmonary bypass time, valve surgery, and use of an intra-aortic balloon pump, was performed through review of the medical records. All patients had complete clinical information.

Samples were collected from patients in standard polyethylene collection cups. Samples were collected at the following time points: preoperative; intraoperative; arrival at the ICU; 6 h postoperative; and postoperative days 1 through to 5. All samples were stored at −80°C and labeled with a unique identifier. Laboratory analysis for the uOT/ICU ratio was performed by investigators blinded to the exposure (K.M.R. and A.H.B.). Routine laboratory testing of human samples (creatinine, blood gases, electrolytes, troponin, liver enzymes, inflammatory function tests, C-reactive protein, and complete blood count) was performed in Clinical Laboratory Improvements Amendments (CLIA)-certified clinical laboratory tests from the site at which the study was conducted.

Based on mouse data showing a uOT/ICU ratio of 1.1 ± 0.6 in controls versus 2.8 ± 0.3 with AKI (Fig. 5), and assuming a comparable difference in humans, examining 6 controls versus 6 AKI-affected patients was estimated to provide 85% power to detect a between-group difference with an α-level of 0.05. To further analyze for temporal trends between cases and controls in uOT/ICU, we used a two-factor analysis of variance. No imputation was performed. All P values were two-tailed; P values < 0.05 were considered to indicate statistical significance. All analyses were performed using Prism 7 (GraphPad Software).

uOT/ICU validation cohort. The ICU validation cohort included patients admitted to the medical or surgical ICU at Brigham and Women’s Hospital between September 2008 and January 2013. Further details of this cohort have been published.2 The study was performed in accordance with the Declaration of Helsinki 2013 and was approved by the Partners Human Research Committee (IRB protocol #2007P000894). All patients provided their written and informed consent.

The primary end point was incident AKI, as defined by changes in sCr (urine output data were not available) in accordance with the KDIGO criteria.7 The highest sCr value from each 24 h calendar day was recorded and used to assess AKI. The cut point of the study was set at the value at which the study was defined as new AKI occurring within 7 days after enrollment. Accordingly, patients who already had AKI at the time of enrollment were excluded from these analyses (Supplementary Fig. 5). Secondary end points included a composite of incident AKI or in-hospital mortality (AKI/death), severe AKI, rapid response team (RRT) or in-hospital mortality (RRT/death), and death (assessed while in hospital and at one year). Severe AKI was defined as the doubling of sCr or the need for RRT, corresponding to stages 2 and 3 of the KDIGO criteria.

Urinary samples were collected from patients in standard polyethylene collection cups. Samples were collected within 48 h of arrival at the ICU, and then daily thereafter for 5 days. All samples were stored at −80°C and labeled with a unique identifier. Laboratory analysis for the uOT/ICU ratio was performed by investigators blinded to the exposure (K.M.R. and A.H.B.). sCr was measured for routine clinical purposes by the central clinical laboratory at Brigham and Women's Hospital.

A post hoc sample size calculation for the ICU cohort study demonstrated that 215 patients with an incident AKI event rate of 24% (n = 51) provided > 80% power to detect an odds ratio of 1.7 per s.d. of uOT/ICU. All P values were two-tailed; P values < 0.05 were considered to be statistically significant. All analyses were performed using SAS version 9.4 (SAS Institute). Covariates included in multivariable models consisted of both comorbidities (e.g., hypertension and diabetes mellitus) and severity of illness factors. Comorbidities were ascertained by manual chart review. At the first day of analysis for the uOT/ICU ratio was performed by investigators blinded to the exposure (K.M.R. and A.H.B.). sCr was measured for routine clinical purposes by the central clinical laboratory at Brigham and Women's Hospital.
system ranging from 0 to 71, with a higher score indicating more severe disease. Comparison of metabolite levels between those with AKI and those without AKI was performed by Wilcoxon rank-sum test. Multivariate logistic regression was used to assess confounding relationships between each metabolite concentration and assessment for adverse events. Per specific guidance from the protocol, we performed an on-pump cardiac surgery. Secondary end points were changes in urinary NAM concentration and assessment for adverse events. Per specific guidance from the protocol amendment due to lack of sufficient patients meeting this criterion. The study was conducted according to the principles of the Declaration of Helsinki 2013. The inclusion criterion for this study was planned on-pump cardiac surgery; and individuals held in an institution by legal or official order. Patients were stratified according to a history of CKD, defined as an estimated glomerular filtration rate (eGFR) < 60 ml min⁻¹ 1.73 m² or an estimated glomerular filtration rate < 45 ml min⁻¹ before enrollment. Screening and enrollment took place between July 2016 and January 2017. Randomization was concealed and carried out by the research pharmacy at the BIDMC. Patients, attending surgeons, and other members of the health-care team, as well as the investigators analyzing the molecular markers, were blinded to treatment assignment. Placebo and NAM (Rugby Laboratories) were supplied by the BIDMC research pharmacy.

The primary end point in this phase 1 pilot trial was the change of serum NAM in the setting of high-dose oral NAM administration in patients undergoing on-pump cardiac surgery. Secondary end points were changes in urinary NAM concentration and assessment for adverse events. Per specific guidance from the IRB before study approval regarding the periprocedural risks related to cardiac surgery, laboratory safety assessments included cardiac enzymes, renal function tests, and blood gas analyses. We also evaluated length of ICU stay, the length of hospital stay, 30-day rehospitalization, and all-cause mortality. A detailed schedule of assessments is included (Supplementary Table 10). Blood and urine samples for NAD⁺ metabolite measurements were obtained as part of routine clinical care. Blood samples to determine NAD⁺ metabolites were successfully collected for 82% (placebo), 71% (1 g per day arm), and 72% (3 g per day arm) of the prespecified schedule of assessments. More than 95% of collected specimens were evaluated successfully for NAD⁺ metabolites. Unanticipated adverse events were managed through participation of the multidisciplinary care team, and through manual chart review. Safety data were monitored on an ongoing basis. The principal investigator (A.P.M.) and co-investigator (V.W.) monitored all patients matriculating through the trial for signs of toxicity and other adverse events. Adverse events including, but not limited to, cardiovascular events and abnormal laboratory findings were reviewed, adjudicated, and shared with an independent medical monitor (S.J.H.) for review. All serious adverse events were reported to the IRB. For primary and secondary end points, all patients had complete clinical data. No imputation of the data was performed. Study data were collected and managed using the REDCap electronic data capture tools hosted at the BIDMC. REDCap (Research Electronic Data Capture) is a secure, Web-based application designed to support data capture for research studies. It provides: (1) an intuitive interface for validated data entry; (2) audit trails for tracking data manipulation and export procedures; (3) automated export procedures for seamless data downloads to common statistical packages; and (4) procedures for importing data from external sources.

Baseline blood and urine samples were drawn at the preoperative evaluation visit if the study participant was an outpatient, or the day before surgery if the participant was already hospitalized. Subsequent follow-up assessments were performed immediately after surgery and on postoperative days 1 through to 3. All samples were stored at −80°C and labeled with a unique identifier. Routine laboratory tests for safety were performed using an API 5000 Triple Quadrupole mass spectrometer (AB Sciex) coupled to a Prominence UFLC liquid chromatography system with autosampler (Shimadzu Scientific Instruments).

Within-patient sCr levels over time were compared to baseline using paired t-tests. All P values were two-tailed; P values < 0.05 were considered to indicate statistical significance. All analyses were performed using Prism 7 (GraphPad Software).

Phase 1 pilot study of oral NAM in cardiac surgery patients. In this phase 1 pilot randomized, single-blind, placebo-controlled clinical trial, patients were identified and screened through surgical appointment logs and electronic medical records. Patients were approached at their outpatient predonation evaluation or on inpatient hospital wards, at which time the study was explained and written, and signed informed consent was obtained before enrollment. The trial was approved by the IRB of the BIDMC (IRB 2016P0000028) and registered at ClinicalTrials.gov (trial number NCT02701127; see URLs). The study was conducted according to the principles of the Declaration of Helsinki 2013. The inclusion criterion for this study was planned on-pump cardiac surgery. An additional inclusion criterion was a Cleveland Clinic score ≥ 6 (Supplementary Table 9) was removed through IRB protocol amendment due to lack of sufficient patients meeting this criterion. Patients were excluded for any of the following reasons: any new AKI within 1 week before surgery; kidney transplant status; end-stage renal disease; pregnancy; young age of < 18 years of age; and those who gave consent in context of on-pump cardiac surgery; and individuals held in an institution by legal or official order. No patients with urgent cases were enrolled. Blocked randomization schedules were electronically generated with a block size of six and maintained by the BIDMC research pharmacy, which was not involved in patient care or data analysis. Patients were randomly assigned on a 1:1 basis into the three study arms (1 g per day NAM, 2 g per day NAM, or placebo). Patients were stratified according to a history of CKD, defined as a history of proteinuria (diastolic reading ≥ 1+), or an estimated glomerular filtration rate < 45 ml min⁻¹ 1.73 m² before enrollment. Screening and enrollment took place between July 2016 and January 2017. Randomization was concealed and carried out by the research pharmacy at the BIDMC. Patients, attending surgeons, and other members of the health-care team, as well as the investigators analyzing the molecular markers (K.M.R. and A.H.B.), were blinded to treatment assignment. Placebo and NAM (Rugby Laboratories) were supplied by the BIDMC research pharmacy.

The primary endpoint in this phase 1 pilot trial was the change of serum NAM in the setting of high-dose oral NAM administration in patients undergoing on-pump cardiac surgery. Secondary end points were changes in urinary NAM concentration and assessment for adverse events. Per specific guidance from the IRB before study approval regarding the periprocedural risks related to cardiac surgery, laboratory safety assessments included cardiac enzymes, renal function tests, and blood gas analyses. We also evaluated length of ICU stay, the length of hospital stay, 30-day rehospitalization, and all-cause mortality. A detailed schedule of assessments is included (Supplementary Table 10). Blood and urine samples for NAD⁺ metabolite measurements were obtained as part of routine clinical care. Blood samples to determine NAD⁺ metabolites were successfully collected for 82% (placebo), 71% (1 g per day arm), and 72% (3 g per day arm) of the prespecified schedule of assessments. More than 95% of collected specimens were evaluated successfully for NAD⁺ metabolites. Unanticipated adverse events were managed through participation of the multidisciplinary care team, and through manual chart review. Safety data were monitored on an ongoing basis. The principal investigator (A.P.M.) and co-investigator (V.W.) monitored all patients matriculating through the trial for signs of toxicity and other adverse events. Adverse events including, but not limited to, cardiovascular events and abnormal laboratory findings were reviewed, adjudicated, and shared with an independent medical monitor (S.J.H.) for review. All serious adverse events were reported to the IRB. For primary and secondary end points, all patients had complete clinical data. No imputation of the data was performed. Study data were collected and managed using the REDCap electronic data capture tools hosted at the BIDMC. REDCap (Research Electronic Data Capture) is a secure, Web-based application designed to support data capture for research studies. It provides: (1) an intuitive interface for validated data entry; (2) audit trails for tracking data manipulation and export procedures; (3) automated export procedures for seamless data downloads to common statistical packages; and (4) procedures for importing data from external sources. Baseline blood and urine samples were drawn at the preoperative evaluation visit if the study participant was an outpatient, or the day before surgery if the participant was already hospitalized. Subsequent follow-up assessments were performed immediately after surgery and on postoperative days 1 through to 3. All samples were stored at −80°C and labeled with a unique identifier. Routine laboratory tests for safety were performed using an API 5000 Triple Quadrupole mass spectrometer (AB Sciex) coupled to a Prominence UFLC liquid chromatography system with autosampler (Shimadzu Scientific Instruments).

Within-patient sCr levels over time were compared to baseline using paired t-tests. All P values were two-tailed; P values < 0.05 were considered to indicate statistical significance. All analyses were performed using Prism 7 (GraphPad Software).

Human metabolite measurements. Isotopic standards. Isotopic standards (1-13C) areodan-d5 (indole-d5), nicotinamide-d4 (C-creatine) were purchased from Toronto Research Chemicals, North York, Ontario, Canada. Isotopic standards were mixed at a concentration of 50 μM and mixed 1:1 with urine and serum samples before protein precipitation with acetonitrile (80% v/v final concentration).

Instrumentation. All LC–MS analyses were performed using an API 5000 Triple Quadrupole mass spectrometer (AB Sciex) coupled to a Prominence UFLC liquid chromatography system with autosampler (Shimadzu Scientific Instruments). High-performance chromatography of both peptides and amino acids was performed using hydrophilic interaction chromatography on Luna HILIC HPLC columns (100 × 3.0 mm, 2.6 μm bead diameter, 100 A pore size; Phenomenex). Instrument control, data acquisition, and quantification were performed using the Analyst version 1.6.2 for Windows software (Sciex).

Serum and urine small molecule analyses. A quantity of 10 μl of serum or urine were mixed with 10 μl isotopic standards (50 μmol l⁻¹ each) diluted in water. Serum/urine proteins were then precipitated by mixing with 80% acetonitrile (80% final concentration), and precipitates were cleared by centrifuging at 14,000 rpm for 10 min. The supernatant was transferred into a 96-well microplate for analysis. Analysis was performed by hydrophilic interaction chromatography on a Luna HILIC HPLC column using a gradient elution protocol. Chromatograms of the
extracted ion multiple reaction monitoring metabolite peaks from a representative urine sample are shown in Supplementary Fig. 4. Calibration standards for each metabolite were purchased from Sigma-Aldrich, weighed, and diluted into phosphate buffered saline at 100 mM, and calibrator mixtures were made from stock solutions starting at 100 μM. The coefficient of variation for individual metabolites was 4–7%.

Data availability. The data that support the findings of this study are available from the corresponding author upon reasonable request. For any patient-related information, data requests will be addressed in consultation with the IRB overseeing the study.

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Reporting Summary

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Statistical parameters

When statistical analyses are reported, confirm that the following items are present in the relevant location (e.g. figure legend, table legend, main text, or Methods section).

| Item | Confirmed |
|------|-----------|
| n/a | Confirmed |
| □ | □ The exact sample size ($n$) for each experimental group/condition, given as a discrete number and unit of measurement |
| □ | □ An indication of whether measurements were taken from distinct samples or whether the same sample was measured repeatedly |
| □ | □ The statistical test(s) used AND whether they are one- or two-sided |
| Only common tests should be described solely by name; describe more complex techniques in the Methods section. |
| □ | □ A description of all covariates tested |
| □ | □ A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons |
| □ | □ A full description of the statistics including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals) |
| □ | □ For null hypothesis testing, the test statistic (e.g. $F$, $t$, $r$) with confidence intervals, effect sizes, degrees of freedom and $P$ value noted |
| Give $P$ values as exact values whenever suitable. |
| □ | □ For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings |
| □ | □ For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes |
| □ | □ Estimates of effect sizes (e.g. Cohen's $d$, Pearson's $r$), indicating how they were calculated |
| □ | □ Clearly defined error bars |
| □ | □ State explicitly what error bars represent (e.g. SD, SE, CI) |

Our web collection on statistics for biologists may be useful.

Software and code

Policy information about availability of computer code

Data collection

Zen 2012 and Zeiss Axiocam (Thornwood, NY) for PC for image analysis; Graphpad Prism Version 7 (La Jolla, CA) and SAS version 9.4 (Cary, NC) for statistical analysis; TraceFinder (v 3.0, Thermo Scientific, Waltham, MA) for metabolite peak analysis

Data analysis

Data were analyzed and results were prepared using GraphPad Prism 7 or SAS version 9.4 as indicated in the Methods document.

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors/reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.

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☐ Life sciences  ☐ Behavioural & social sciences  ☐ Ecological, evolutionary & environmental sciences

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Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size

MOUSE STUDIES
Sample size for metabolomics experiments was based on previous experience. 17 For IRI experiments focused on renal function (i.e., serum creatinine) outcome, sample size was based on previous experience and consideration of the following estimation: serum creatinine of 1.4 ± 0.5 mg/dl among controls vs. 2.1 ± 0.5 mg/dl among IRI mice requires n = 6 mice per group for 80% power and 5% alpha-error.

HUMAN STUDIES
(1) Sample size was calculated for the uQ:T nested case control study based on uQ:T of 1.1 ± 0.6 in controls vs. 2.8 ± 0.2 in mice with AKI (Fig 1d) and assuming a comparable difference in humans, examining 6 controls vs. 6 AKI-affected subjects was estimated to provide 85% power to detect a between-group difference with α-error 0.05. (2) A post hoc sample size calculation for the ICU cohort study demonstrated that 215 patients with an incident AKI event rate of 24% (n = 51) provided > 80% power to detect an odds ratio of 1.7 per standard deviation of uQ:T. (3) Based upon a comparably-sized previous study of oral Nam pharmacokinetics in healthy volunteers (Ref 6) nine participants were consented for the healthy subject Nam PK study. (4) For the Phase 1 pilot study of oral Nam in cardiac surgery patients, We estimated that 5 subjects per group would provide >95% power to detect differences in serum Nam concentration between the 3 gm/d Nam and placebo groups with α-error 1%. However, based on recent literature describing an AKI event rate of ~40%,8 we estimated a sample size of 10-15 patients per group for this Phase 1 study (each yielding approximately 5 patients with AKI) would be sufficient to assess pharmacokinetic effects of oral Nam in on-pump cardiac surgery patients with and without AKI. To account for screen failures and loss to follow-up, we set the target enrollment for 20 patients per group.

Data exclusions
None

Replication
Serum creatinine measurements in mice were measured in duplicate by an independent core facility. Metabolite measurements were performed in duplicate by investigators blinded to experimental setting. All attempts at replication were successful.

Randomization
MOUSE: All studies involving mice were approved by the Beth Israel Deaconess Medical Center Institutional Animal Care and Use Committee (IACUC). Experiments were performed using littermate controls by an operator blinded to genotype and randomized within each cage to sham vs. AKI model. Creatinine from mouse serum was measured using LC/MS-MS at the University of Alabama Birmingham O’Brien Core Center for Acute Kidney Injury Research in a blinded fashion (NIH P30 DK079337).

HUMAN: The Phase 1 pilot study of oral Nam in cardiac surgery patients included blocked randomization schedules that were electronically generated with a block size of 6, and maintained independently by the Beth Israel Deaconess Medical Center research pharmacy, which was not involved in patient care or data analysis. Patients were randomly assigned on a 1:1:1 basis into the three study arms (1 gm/d Nam, 2 gm/d Nam, or placebo).

Blinding
For mouse studies, measurements of serum creatinine were performed by an independent core facility at University of Alabama, Birmingham, that was blinded to experimental setting. For the clinical studies, metabolite measurements were performed by an investigator (AHB) blinded to experimental setting. Furthermore, patients participating in the Phase 1 pilot study of oral Nam in cardiac surgery patients were blinded to study group assignment. For investigators participating in this interventional trial, participants were designated as Groups A, B, and C by the Beth Israel Deaconess research pharmacy.

Reporting for specific materials, systems and methods

Materials & experimental systems

| n/a | Involved in the study |
|-----|-----------------------|
| ☒   | Unique biological materials |
| ☒   | Antibodies |
| ☒   | Eukaryotic cell lines |
| ☒   | Palaeontology |
| ☒   | Animals and other organisms |
| ☒   | Human research participants |

Methods

| n/a | Involved in the study |
|-----|-----------------------|
| ☒   | ChIP-seq |
| ☒   | Flow cytometry |
| ☒   | MRI-based neuroimaging |
Animals and other organisms

Policy information about studies involving animals: ARRIVE guidelines recommended for reporting animal research

| Laboratory animals | Mus musculus C57BL6/J male mice of 8-12 week age for both general AKI experiments and for QPRT+/- experiments. Note that this mouse model was developed in the C57BL6J background by CRISPR gene editing as described in the Methods. |
| Wild animals | N/A |
| Field-collected samples | N/A |

Human research participants

Policy information about studies involving human research participants

Population characteristics

Covariates and clinical characteristics of the human research participants are described in the Methods and summarized as below:

1. uQ:T Discovery Nested Case-Control Study
   Study Population: We examined the uQ:T ratio as an indicator of risk for AKI in a prospective cohort of patients undergoing nonurgent, on-pump cardiac surgery through a nested case-control analysis. The prospective cohort for this nested case-control study was enrolled over a period of six months. The primary inclusion criterion was planned, on-pump cardiac surgery. Subjects were excluded for any of the following reasons: unresolved AKI 1 week prior to operation, history of kidney transplant, end stage renal disease, pregnancy, younger than 18 years of age, unable to consent, off-pump cardiac surgery, and individuals held in an institution by legal or official order.

2. uQ:T ICU Validation Cohort
   Study Population: The ICU validation cohort included subjects admitted to the medical or surgical intensive care unit (ICU) at Brigham and Women's Hospital (BWH; Boston, MA) between September 2008 and January 2013. Further details of this cohort have been published (Ref 4 of methods).

3. Healthy Subject PK Study
   Study Population: Based upon a comparably-sized previous study of oral Nam pharmacokinetics in healthy volunteers, 6 nine participants were consented. One subject withdrew from the study following a new diagnosis of a chronic medical condition. The remaining eight, who were free of chronic illness, were given oral Nam 3 gm once daily supplied by the Massachusetts General Hospital research pharmacy (Rugby Laboratories) at 0, 24 and 48 hours between January 2016 to June 2016.

4. Phase 1 Pilot study of Oral Nam in Cardiac Surgery Patients
   Study Population: In this phase 1 pilot randomized, single blind, placebo-controlled clinical trial, patients were identified and screened through surgical appointment logs and electronic medical records. Patients were approached at their outpatient preadmission evaluation or on inpatient hospital wards, at which time the study explained and written and signed informed consent was obtained prior to enrollment. The trial was approved by the Institutional Review Board of Beth Israel Deaconess Medical Center (IRB 2016P0000028) and registered at clinicaltrials.gov (NCT02701127). The study was conducted according to the principles of the Declaration of Helsinki. The inclusion criterion for this study was planned on-pump cardiopulmonary bypass surgery. An additional inclusion criterion of Cleveland Clinic Score \( \geq 6 \) (Extended Data Table 9) was removed through IRB-protocol amendment due to lack of sufficient patients meeting this criterion. Subjects were excluded for any of the following reasons: any new AKI within 1 week prior to operation, kidney transplant status, end stage renal disease, pregnancy, younger than 18 years of age, inability to consent, off-pump cardiac surgery, and individuals held in an institution.

Recruitment

Patients were approached at their outpatient preadmission evaluation or on inpatient hospital wards, at which time the study explained and written and signed informed consent was obtained prior to enrollment. For the healthy subject oral Nam PK study, eligible subjects were identified through flyers posted throughout the Massachusetts General Hospital campus, and online on the Partners Healthcare Clinical Trials in need of Volunteers Website (https://clinicaltrials.partners.org/). Subjects who completed the study were remunerated with a check for $120.