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NAD^+ metabolism and cardiometabolic health: the human evidence

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Commentary on 'Nicotinamide mononucleotide increases muscle insulin sensitivity in prediabetic women' by M. Yoshino *et al.*, *Science*, 2021.

The metabolic cofactor nicotinamide adenine dinucleotide (NAD^+) has long been recognized as an essential molecule for cellular energy production and homeostasis due to its key role in cellular reduction–oxidation reactions. More than a century after this activity was first described, our understanding of NAD^+ has evolved well beyond redox and energy metabolism. In fact, NAD^+ serves as a co-substrate for enzymes contributing to vital cellular functions, including epigenetic and transcriptional regulation, post-translational protein modifications and cell signalling.[1] Accordingly, recent years have witnessed soaring interest in different aspects of NAD^+ metabolism, with the ultimate goal of harnessing its potential to treat chronic age-related diseases, which are typically multifactorial and lack efficient pharmacotherapies. Amongst these, cardiometabolic disorders represent an urgent and unmet medical need with an ever-growing prevalence due to the current demographic shift towards older populations, modern-day sedentary lifestyles and hypercaloric diets. Interestingly, intracellular NAD^+ levels progressively decline with ageing and obesity, while the salutary effects of exercise and caloric restriction coincide with increases in NAD^+.[1] Accordingly, NAD^+ has been posited as a potential actionable target to recapitulate the benefits of these healthy lifestyle modifications.[2] In fact, a growing body of preclinical evidence suggests that oral supplementation of NAD^+ precursors—otherwise known as vitamin B3 derivatives—improves metabolic health in rodent models of ageing, obesity, and diabetes.[1,2] However, the clinical evidence backing such health benefits in humans is still lagging behind.

In this regard, Yoshino *et al.*[3] recently reported in *Science* the results of a randomized, placebo-controlled, double-blind trial testing the short-term effects of the NAD^+ precursor nicotinamide mononucleotide (NMN) on body composition and insulin sensitivity in prediabetic women. In this small trial of 25 obese or overweight participants, NMN (250 mg/kg) did not affect body weight or composition as compared to the placebo group, yet improved measures of insulin sensitivity. Neither placebo nor NMN changed body mass index, fat-to-lean mass ratio, intra-abdominal fat levels, or hepatic triglycerides following the 10-week supplementation period (with the caveat that the NMN group began with lower hepatic triglycerides). Similarly, NMN did not affect plasma insulin, glucose, haemoglobin A1c, or cholesterol levels. However, hyperinsulinaemic-euglycaemic clamp testing revealed an improvement in insulin sensitivity over the course of NMN treatment, as determined by the rate of insulin-induced glucose clearance (normalized to fat-free body mass). While the placebo group showed no detectable change in insulin sensitivity, NMN-treated patients exhibited an average 25% increase in glucose disposal from baseline. At the same time, skeletal muscle insulin signalling was improved after NMNs supplementation, as denoted by the higher expression and phosphorylation levels of AKT—a major downstream effector of insulin action—in skeletal muscle biopsies of NMN-treated patients after insulin infusion. It is important to note that the AKT immunoblot analysis focused on within-groups (before vs. after treatment) changes and so, in absence of a direct comparison between the two groups on the same gel, no conclusion can be made on between-groups (NMN vs. placebo) differences. Regardless, the positive NMN effect on insulin sensitivity appeared to be skeletal muscle-specific, as both hepatic and adipose tissue insulin sensitivity indices were almost identical before and after NMN administration. Intriguingly, NMN did not increase NAD^+ in skeletal muscle, despite doing so in peripheral blood mononuclear cells. Instead, the skeletal muscle content of nicotinamide metabolites, such as *N*-methyl-nicotinamide, *N*-methyl-2-pyridone-5-carboxamide, and *N*-methyl-4-pyridone-5-carboxamide, showed a multi-fold increase, consistent with prior studies using the alternative precursor nicotinamide riboside.[4,5] Finally, NMN did not enhance skeletal muscle physical capacity, as evaluated by handgrip strength, nor did it improve mitochondrial respiratory function, as evaluated by high-resolution respirometery of quadriceps muscle biopsies.

Although larger trials are warranted, this pilot study represents a significant step towards human translation of the health-promoting effects of NAD^+ precursors. Like any other important study, this work raises several research questions worthy of future investigation. For instance, the authors performed an unbiased RNA-sequencing analysis of muscle biopsies obtained before and after NMN treatment, which revealed transcriptional up-regulation of platelet-derived growth factor (PDGF) signalling as well as collagen and extracellular matrix processing, at least upon insulin administration. These findings might be of relevance for

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Table 1 Ongoing clinical trials testing NAD$^+$ precursors for cardiometabolic outcomes

Precursor (daily dose)[a]	Disease/condition	Primary outcomes	Study design (enrolment estimate, *n*)	Duration or follow-up	Completion date	Trial identifier[b]
NR (up titrated to a final dose of 1000 mg)	Obesity	Mitochondrial biogenesis, histology, and mRNA expression in skeletal muscle and adipose tissue	Non-randomized, open label trial ($n = 56$)	5 months	May 2019	NCT03951285
NR (up titrated to a final dose of 2 × 1000 mg)	Systolic heart failure	Incidence of treatment-emergent adverse events (safety and tolerability)	Randomized, placebo-controlled, double-blind, phase 1 trial ($n = 30$)	12 weeks	June 2019	NCT03423342
NR (2 × 250 mg)	Ageing/lipaemia	NAD$^+$ content in blood and peripheral blood mononuclear cells	Randomized, placebo-controlled, double-blind trial with cross-over design ($n = 16$)	7 days	December 2019	NCT03501433
Niacin (2000 mg)	Dyslipidaemia	Plasma triglycerides as a measure of how niacin affects fatty acids	Randomized, placebo-controlled, double-blind, phase 1 trial with crossover design ($n = 20$)	12 h	December 2019	NCT01984073
NAM (2500 mg in three divided doses)	Preeclampsia	Mean arterial blood pressure (MAP)	Single group (open label) phase 2 trial ($n = 25$)	7 days	July 2020	NCT03419364
Niacin ER (up titrated to a final dose of 2000 mg)	Cardiovascular disease	Lipoprotein composition and vascular compliance	Single group (open label) phase 2 trial ($n = 24$)	16 weeks	July 2020	NCT02322203
NR (1000 mg)	Hypertension	Systolic blood pressure	Randomized, placebo-controlled, double-blind, phase 1 trial ($n = 74$)	6 weeks	June 2021	NCT04112043
Niacin (not specified)	Type 2 diabetes mellitus/obesity	Insulin-stimulated glucose disposal and insulin signalling in fat, muscle, and blood samples.	Non-randomized, open label, phase 1 trial ($n = 20$)	18 h	December 2021	NCT03867500
NR (1000 mg)	Peripheral artery disease	6 min walk distance	Randomized, placebo-controlled, double-blind, phase 3 trial ($n = 90$)	6 months	April 2022	NCT03743636
Niacin (0.01 mg/kg/min IV infusion)	Type 2 diabetes mellitus	Rate of endogenous glucose production	Randomized, single (participant)-blind, phase 2 trial with crossover design ($n = 45$)	7–7.5 h	April 2022	NCT03540758
NR (2 × 500 mg)	Ageing	Maximum oxygen uptake (VO_2 max), physical performance and skeletal muscle molecular phenotyping, as well as measures of bone turnover	Randomized, placebo-controlled, double-blind trial ($n = 48$)	6 months	December 2022	NCT03818802
NR (2 × 500 mg)	Hypertension/ageing	Systolic blood pressure	Randomized, placebo-controlled, double-blind phase 2 trial ($n = 118$)	3 months	December 2023	NCT03821623
NR (2 × 500 mg)	Vascular diseases/kidney disease	Aortic stiffness	Randomized, placebo-controlled, double-blind, phase 2a trial ($n = 118$)	3 months	September 2024	NCT04040959
NR (2 × 500 mg)	Peripheral diabetic neuropathy	Thigh intra-epidermal nerve fibre density (neuropathy)	Randomized, placebo-controlled, double-blind, phase 1-2 trial ($n = 54$)	6 months	October 2024	NCT03685253

ER, extended release; IV, intravenous; NAM, nicotinamide; NMN, nicotinamide mononucleotide; NR, nicotinamide riboside.
[a]Orally administered, unless indicated otherwise.
[b]*Source*: USA ClinicalTrials.gov (https://www.clinicaltrials.gov/).

future therapeutic testing of NAD^+ precursors particularly in cardiovascular medicine. For example, two recent studies have reported that NAD^+-replacement therapy by nicotinamide or nicotinamide riboside improves hypertrophy and myocardial stiffness in animal models of ageing, obesity, and heart failure with preserved ejection fraction; however, it remains to be determined whether altered extracellular matrix or PDGF signalling contribute to these effects.[6,7] Another important question is why the results of this study differ from prior work using higher doses of nicotinamide riboside, which was found to modestly increase fat-free mass with no effect on insulin sensitivity.[5,8] Further studies will be required to distinguish whether this reflects differences in the precursor molecule selected, the dose, the study population, or other more subtle aspects of the experimental designs.

Although human evidence for the cardiovascular benefits of NAD^+ supplementation using nicotinamide or nicotinamide riboside is only beginning to emerge,[9,10] the NAD^+ precursor nicotinic acid (niacin), which acts through ostensibly NAD^+-independent pathways to lower lipids, has historically been extensively tested for cardiovascular outcomes in patients. Whether the accompanying increase in NAD^+ is further contributing to the beneficial effects of niacin is an interesting question, particularly in light of the observations that prior niacin use in the Coronary Drug Project was associated with a significant reduction in mortality 9 years after stopping the drug,[11] and that dietary intake of NAD^+ precursors was inversely correlated with cardiac mortality during the 20-year follow-up of the Bruneck Study.[6] In fact, both niacin and the alternative NAD^+ precursor nicotinamide have recently been shown to protect against abdominal aortic aneurysms, whereas only niacin possesses lipid-lowering ability.[12] Since numerous human disorders, including those affecting the cardiovascular system are associated with NAD^+ deficiency, NAD^+ precursors are currently being tested in various trials with the aim to reinstate NAD^+ homeostasis (*Table 1*). A common limitation, including in the trial by Yoshino *et al.*, is the use of much lower doses than those tested in the animals that provided the basis for human testing. Even after adjusting for the difference in metabolic rate or body surface area between mice and humans, which may not be appropriate since NAD^+ turnover appears similar in human and mouse cells,[13] the dose used in this trial remains a fraction of that given to mice in preclinical studies. Using the human-equivalent dosage, NAD^+ precursors might exert a more favourable and consistent impact on body composition and other reported metabolic parameters that were not affected in the participants of this trial,[3] but strikingly improved in animals.[1,6] However, dosing at this level also has the potential for hepatotoxicity based on prior studies of niacin and nicotinamide, emphasizing the need to strike a delicate balance in future studies.

In conclusion, NAD^+ metabolism represents an exciting avenue to improve cardiometabolic health and, perhaps even, extend healthy lifespan in humans. The findings by Yoshino *et al.* support this potential, however, further clinical studies are still needed before NAD^+ precursors can be routinely recommended to patients, let alone to the general population.

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Conflict of interest: J.A.B. declares that he is an inventor on a patent for using NAD^+ precursors in liver injury and has received research support as noted Below. M.A. is involved in a patent application related to the cardiometabolic effects of nicotinamide.

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Biography: Dr Mahmoud Abdellatif is a post-doctoral research fellow at the Department of Cardiology, Medical University of Graz (Austria) and the Kroemer Lab (Institute Gustave Roussy and Centre de Recherche des Cordeliers, France). In his research, he studies the molecular mechanisms of cardiovascular ageing to exploit them in the development of effective interventions that extend lifespan and delay late-life chronic diseases, including heart failure. His contributions to the field include discovering the cardioprotective and longevity-promoting effects of spermidine, as a natural autophagy inducer and caloric restriction mimetic. More recently, he found that the NAD+ precursor nicotinamide—yet another caloric restriction mimetic—might be effective against a predominant and intractable form of heart failure in the elderly, known as heart failure with preserved ejection fraction. Mahmoud received various awards for his work including, the Sanofi Aventis Prize (2017), the Guido Tarone award of the ESC Heart Failure Association (2020), and the German Society of Cardiology AG23 Heart and Diabetes research prize (2021). As a board member of the European Society of Cardiology committee for young cardiovascular professionals and the Scientists of Tomorrow nucleus, he is actively involved in supporting and disseminating basic science amongst young scientists and clinicians.

Biography: Prof. Joseph A. Baur is an Associate Professor in the Department of Physiology and the Institute for Diabetes, Obesity, and Metabolism at the Perelman School of Medicine of the University of Pennsylvania. He has made key contributions to the understanding of how metabolism and dietary factors influence longevity. He led studies showing that a sirtuin activator, resveratrol, is able to improve insulin sensitivity and ameliorate premature mortality in obese mice and that rapamycin, a drug that extends life in mice, has off-target effects that impair glucose homeostasis. His laboratory at Penn is currently focused on the use of small molecules to understand and mimic the health-promoting effects of caloric restriction in rodents, with a particular focus on nicotinamide adenine dinucleotide (NAD+) metabolism. He has co-authored more than one hundred peer-reviewed publications, as well as several book chapters and numerous invited commentaries and reviews.