DEEP RESEARCH BRIEF / METABOLIC SCIENCE

The NAD+ Paradox

Why a molecule that reverses aging in mice does almost nothing in humans.

THE MYSTERY

Preclinical rodent data show NR and NMN reverse age-related metabolic decline, restore mitochondrial biogenesis, and rejuvenate stem-cell function. Human trials repeatedly confirm a two-fold rise in circulating NAD+ — and almost no functional benefit. This brief dissects the four structural barriers between the pill and the mitochondrion.

~2×
BLOOD NAD+ RISE

0
MITOCHONDRIAL CHANGE

4
STRUCTURAL BARRIERS

SUBJECT AREA
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EXECUTIVE SUMMARY

The Bottom Line Up Front

NAD^+ supplementation does not continually fail. It repeatedly succeeds at the wrong endpoint. Oral nicotinamide riboside (NR) and nicotinamide mononucleotide (NMN) reliably double whole-blood NAD^+ in human volunteers, but the field promised tissue-level mitochondrial rejuvenation based on rodent data, and that promise has not been kept. The translational gap is real, but it is structural, not mystical.

Four barriers stand between the swallowed pill and the intracellular NAD^+ pool that actually drives sirtuins, PARPs, and mitochondrial respiration. **(1) Presystemic clearance** — gut microbiota deamidate NR and NMN to nicotinic acid, purine nucleoside phosphorylase cleaves residual NR to nicotinamide within minutes, and the liver methylates and excretes the rest. **(2) Transport bottlenecks** — NR relies on ENT1/2, NMN requires CD73-mediated dephosphorylation or the intestine-dominant Slc12a8 transporter, so peripheral tissues see a different metabolite mix than blood does. **(3) The CD38 inflammatory sink** — inflammaging upregulates CD38 in macrophages and endothelium, hydrolyzing newly synthesized NAD^+ faster than precursors can replenish it. **(4) Trial-design flaws** — PBMC NAD^+ is a compliance marker, not an efficacy marker; healthy cohorts hit a ceiling effect; 4-to-12-week trials are too short for mitochondrial remodeling; and monotherapy neglects synergistic signals like exercise and CD38 inhibition.

The fix is not louder marketing. It is stratified cohorts with documented baseline NAD^+ deficiency, reduced precursors (NRH/NMNH) that bypass PNP cleavage and cross membranes by passive diffusion, combination protocols pairing a precursor with a CD38 inhibitor and structured exercise, and isotope-tracer flux endpoints in target-tissue biopsies rather than static blood biomarkers.

~2x	0	4
Whole-blood NAD^+ rise after 14 d NR or NMN	Significant change in skeletal-muscle mitochondrial respiration	Structural barriers between pill and mitochondrion

VERDICT

NR and NMN are not useless. They are misprescribed, mismeasured, and monotherapied. The preclinical-to-clinical gap is a pharmacokinetic, enzymatic, and methodological problem with a known solution set — not a failure of the NAD^+ hypothesis itself.

CHAPTER 1

The Paradox: Strong Preclinical Signal, Null Human Outcomes

Modern NAD^+ biology rests on a haystack of compelling rodent data. In aged mice, oral NR or NMN restores NAD^+ to near-youthful levels across liver, skeletal muscle, brain, and pancreas. Cantó-Bello and colleagues, and independently the Imai and Sinclair groups, demonstrated that precursor supplementation reverses age-related mitochondrial decline, restores muscle stem-cell regenerative capacity, rescues -cell function in diabetic BESTO mice, and extends lifespan in animals subjected to genotoxic stress [1,2]. The Imai & Guarente (2014) synthesis crystallized the field: *"the combination of sirtuin activation and NAD^+ intermediate supplementation may be an effective anti-aging intervention, providing hope to aging societies worldwide"* [1].

On the strength of those preclinical signals, NR and NMN moved into human trials. They have not delivered. Across more than 25 published randomized controlled trials, oral NR at 1,000–2,000 mg/day and NMN at 250–1,000 mg/day consistently elevate whole-blood or PBMC NAD^+ by 1.5- to 3-fold within two weeks [3,4]. Yet secondary endpoints — skeletal-muscle mitochondrial respiration ex vivo, insulin sensitivity by hyperinsulinemic-euglycemic clamp, body composition, aerobic capacity, blood pressure, lipid profile — overwhelmingly come back null [5,6,7]. Systematic reviews and meta-analyses now confirm the pattern: NMN modestly improves insulin sensitivity in some prediabetic subgroups but produces no broad metabolic or functional benefit in healthy adults [8,9].

This pattern is not what a null drug produces. A truly inert compound would not double NAD^+ in circulation. The pattern is what you see when a drug reaches the wrong compartment at the wrong dose for the wrong duration in the wrong population, and is measured with the wrong biomarker. The mystery dissolves once we stop asking "does NAD^+ augmentation work?" and start asking "where, when, for whom, and how would we know?"

Table 1.1 — Preclinical vs. human outcomes across organ systems

Organ System	Rodent preclinical finding	Human RCT outcome	Trial reference
Skeletal muscle	Restored mitochondrial biogenesis; reversed fiber-type shift; improved insulin signaling.	NAD^+ metabolites rise in biopsy, but ex vivo oxidative capacity and insulin sensitivity unchanged.	Remie 2020; Dollerup 2020
Liver	Reversed hepatosteatosis; restored SIRT1 / SIRT3 activity.	No significant change in hepatic triglyceride, insulin sensitivity, or mitochondrial gene expression.	Dollerup 2018
Adipose tissue	Adipocyte browning; improved glucose tolerance in HFD mice.	No measurable change in adipose insulin sensitivity or adipokine profile.	Yoshino 2021
Pancreatic cell	Restored glucose-stimulated insulin secretion in aged BESTO mice.	No change in fasting insulin, HOMA-IR, or glucose-stimulated C-peptide in prediabetic adults.	Yoshino 2021

Organ System	Rodent preclinical finding	Human RCT outcome	Trial reference
Cardiovascular	Reduced systolic BP in aged mice via SIRT3 endothelial effects.	Mixed results; modest 3-5 mmHg systolic reduction in some older cohorts, null in healthy adults.	Martens 2018
Brain / cognition	NR partially rescued memory in Alzheimer models; NMN attenuated neuroinflammation.	No published human cognitive RCT with positive primary endpoint; trials ongoing in Parkinson and mitochondrial disease.	NCT03965514 ; NCT04394936

Sources: cited trial registry numbers; primary trial publications (Remie 2020 [5]; Dollerup 2018, 2020 [6,7]; Yoshino 2021 [10]; Martens 2018 [11]).

CHAPTER 2 · BARRIER 1

Presystemic Clearance and Microbial Reprogramming

The first barrier is the simplest one: most of what you swallow never reaches your tissues intact. The assumption that oral NR or NMN enters the bloodstream as NR or NMN is biochemically nave. Three sequential gauntlets — the gut lumen, the intestinal mucosa, and the liver — transform these molecules into a different metabolite cocktail before peripheral tissues ever see them.

2.1 Gut microbiota convert NR and NMN to nicotinic acid

The 2026 head-to-head trial by Christen and colleagues, published in *Nature Metabolism*, is the cleanest demonstration of this principle in humans [3]. Sixty-five healthy adults received 14 days of NR (1 g/day), NMN (1 g/day), nicotinamide (0.5 g/day), or placebo. Whole-blood NAD^+ rose approximately 2-fold in the NR and NMN arms but did not rise significantly with nicotinamide. The counterintuitive finding was that intact NR and NMN were essentially undetectable in circulation; instead, the Preiss–Handler marker NAAD rose, and urinary excretion of nicotinic-acid riboside (NAR) and nicotinuric acid climbed. Ex vivo fermentation of NR and NMN with human fecal microbiota from multiple donors showed rapid conversion: NR → NAM → NA within 8–24 hours, and NMN → NAMN → NA through the same deamidation route.

In other words, the NAD^+ boost from oral NR and NMN is largely a nicotinic-acid boost mediated by gut bacteria. The same study showed that nicotinamide itself, which cannot be deamidated by the microbiome to NA, produced only a transient 1-hour NAD^+ spike with no chronic elevation. This is the opposite of what the field assumed for a decade — the amidated salvage precursors were supposed to be superior to NA because they avoided the flush. They avoid the flush, yes, but they also avoid direct tissue delivery, because the gut strips them of their amidation en route.

2.2 PNP cleaves residual NR within minutes

For the fraction of NR that escapes microbial deamidation, a second enzymatic wall awaits. Purine nucleoside phosphorylase (PNP) hydrolyzes the N-ribosidic bond of NR, splitting it into free nicotinamide and ribose-1-phosphate. This is not a slow process: in human whole blood, intact NR has a half-life of approximately three minutes [12]. The same enzyme does not cleave NMN, but extracellular NMN is rapidly dephosphorylated to NR by the cell-surface ectoenzyme CD73, after which PNP finishes the job. The net effect is that any NR or NMN reaching the systemic circulation arrives mostly as nicotinamide or its methylated downstream products, not as the parent nucleoside.

2.3 Hepatic first-pass clearance and the NNMT sink

Portal blood carries the surviving metabolites — predominantly NAM and NA — directly to the liver. Hepatic first-pass extraction clears the majority. The liver uses what it needs for its own considerable NAD^+ pool and excretes the rest through two routes: NA is conjugated and either flushed (the classic niacin flush) or routed into the Preiss–Handler pathway; NAM is methylated by nicotinamide N-methyltransferase (NNMT) to N1-methylnicotinamide (MeNAM), then oxidized to N1-methyl-2-pyridone-5-carboxamide (2PY) and

N1-methyl-4-pyridone-5-carboxamide (4PY), and renally eliminated [4]. These methylated pyridones are the dominant urinary NAD^+-related metabolites after chronic supplementation.

Two consequences follow. First, peripheral tissues — skeletal muscle, heart, brain — are exposed to systemic NAM, MeNAM, 2PY, and 4PY rather than to intact NR or NMN. They must rebuild NAD^+ from NAM via the salvage pathway, which is rate-limited by NAMPT. Second, sustained high NAM concentrations exert product inhibition on sirtuins and PARPs, the very enzymes the supplementation was supposed to activate. The Yusri et al. (2025) review in *npj Metabolic Health and Disease* describes this as a feedback loop that can *"paradoxically dampen downstream NAD^+-dependent functional responses"* [4].

Table 2.1 — What happens to a 1,000 mg oral NR dose

Compartment	Primary fate	Major metabolites detected	Time scale
Gut lumen	Bacterial deamidation; partial cleavage to NAM.	NR, NAM, NA, NAR, NAMN	0–6 h
Enterocyte	NR phosphorylation via NRK1; partial salvage to NAD^+.	NMN, NAD^+ (locally)	0–4 h
Portal vein	PNP cleavage of residual NR; NAM predominates.	NAM (peak), NA (low)	~30 min
Liver (first pass)	NAM methylation by NNMT; NAD^+ salvage; biliary excretion.	MeNAM, 2PY, 4PY, NAD^+ (hepatic)	1–6 h
Systemic circulation	NAM as dominant precursor; intact NR/NMN < 2 M.	NAM, MeNAM, 2PY, 4PY	Steady state
Peripheral tissue	NAMPT-dependent salvage; rate-limited by tissue NAMPT abundance.	NAD^+ (intracellular), NAM (recycled)	Hours
Renal excretion	Filtration of MeNAM, 2PY, 4PY, NAR.	Urinary MeNAM, 2PY, 4PY	4–24 h

Time-course values synthesized from Trammell 2016 [12], Christen 2026 [3], and the Yusri 2025 review [4].

PRACTICAL IMPLICATION

If you dose NR or NMN on an empty stomach hoping to flood muscle with intact nucleoside, you are biochemically out of luck. The bacteria and the liver will reach it first. Strategies that bypass the gut — sublingual delivery, IV, or reduced precursors like NRH that are PNP-resistant — are not quackery; they are the only plausible way to deliver intact parent molecule to systemic circulation.

CHAPTER 3 · BARRIER 2

Cellular Transport Bottlenecks and Tissue-Specific Disconnects

Even if intact precursor reached the systemic circulation, getting it across the cell membrane and into the intracellular NAD^+ pool is a separate problem. Each precursor has its own transport mechanism, and these mechanisms are not equally distributed across tissues. This is the single most underappreciated reason that blood NAD^+ rises while muscle NAD^+ barely moves: the cell types that dominate the blood NAD^+ signal (erythrocytes and PBMCs) are not the cell types whose mitochondria we are trying to rescue.

3.1 NR relies on equilibrative nucleoside transporters

NR crosses the plasma membrane via ENT1 and ENT2 (SLC29A1, SLC29A2), the same equilibrative nucleoside transporters that move adenosine, guanosine, and other purine and pyrimidine nucleosides [4]. Once inside the cytoplasm, NR is phosphorylated by NRK1 (ubiquitous) or NRK2 (skeletal muscle, heart, adipose) to NMN, which is then adenylated by NMNAT1-3 to NAD^+. The tissue-specific expression of NRK1/2 is critical: skeletal muscle expresses both isoforms and is therefore competent to use NR; white adipose tissue lacks NRK1 entirely and relies on NRK2, which is induced only under stress. The spleen lacks both. So even if NR reaches a tissue, that tissue must express the right kinase to convert it.

3.2 NMN requires CD73 dephosphorylation or Slc12a8

NMN is a charged nucleotide and cannot cross cell membranes by passive diffusion. Two routes have been proposed. The first is dephosphorylation to NR by the cell-surface ectoenzyme CD73 (5-prime-nucleotidase), followed by ENT-mediated NR uptake [4]. This is essentially a detour: NMN → NR → NMN → NAD^+, with two ATP-consuming phosphorylation steps. The second route is the sodium-dependent NMN transporter Slc12a8, identified by the Imai group in the small intestine [13]. Slc12a8 expression is predominantly intestinal, with variable and debated expression elsewhere. Tracer experiments in mice show that low-dose NMN can enter kidney and adipose intact, but at doses used in human trials the bulk of NMN is handled via the CD73→NR→ENT route, which is functionally equivalent to dosing NR.

3.3 Why this produces the trial results we see

These transport constraints explain the otherwise-puzzling disconnect in the Remie and Dollerup trials. Remie et al. (2020) gave 1,000 mg/day NR for six weeks to overweight/obese adults and paired the trial with skeletal-muscle biopsies [5]. Muscle NAD^+ metabolites (NAAD, MeNAM) rose, sleeping metabolic rate ticked up modestly, and fat-free mass increased slightly — but ex vivo mitochondrial respiration was unchanged, intramyocellular lipid was unchanged, and insulin sensitivity was unchanged. The biopsy data show that *something* reached the muscle, but it reached as NAM-derived metabolites rather than as intact NR, and the intracellular NAD^+ pool that drives SIRT3-mediated mitochondrial deacetylation was not meaningfully expanded.

Dollerup et al. (2018, 2020) escalated the dose to 2,000 mg/day for 12 weeks in obese, insulin-resistant men [6,7]. The result was the same null on mitochondrial respiration, mitochondrial content, and

oxidative-phosphorylation gene expression — and they found something more troubling: skeletal-muscle NAMPT protein abundance *decreased* after treatment. Exogenous precursor loading had induced negative feedback suppression on the endogenous salvage machinery. The body interpreted the precursor surplus as a signal to downscale its own NAD^+ production capacity.

Yoshino et al. (2021) provide the positive counterexample that proves the rule [10]. Postmenopausal women with prediabetes given 250 mg/day NMN for 10 weeks showed a significant improvement in skeletal-muscle insulin sensitivity, with upregulation of muscle remodeling pathways mediated by PDGF signaling. But the benefit was strictly localized: hepatic and adipose insulin sensitivity, blood pressure, and plasma lipids were unchanged. The positive signal appeared only in a tissue that (a) expresses NRK1/2 abundantly, (b) had a documented baseline deficit (prediabetic insulin resistance), and (c) was given a precursor dose matched to its uptake capacity.

Table 3.1 — Precursor-by-precursor transport and clinical outcome

Precursor	Primary uptake mechanism	Rate-limiting step / barrier	Tissue reach in humans	Meta-analytic outcome
Nicotinic acid (NA)	SLC5A8, SLC22A13 transporters; Preiss–Handler.	Cutaneous flushing (GPR109A); GI symptoms at high dose.	Liver, vascular endothelium.	Significant TG, TC, LDL reduction; HDL increase; risk of hyperglycemia.
Nicotinamide (NAM)	Passive diffusion / SLC29A1-2; salvage pathway.	Hepatic NNMT methylation; sirtuin/PARP product inhibition at high dose.	Systemic; liver clearance dominant.	Minimal lipid effect; transient 1-h NAD+ spike; no chronic elevation.
Nicotinamide riboside (NR)	ENT1/2; phosphorylation by NRK1/2.	PNP cleavage (~3 min $t_{1/2}$ in blood); microbial deamidation.	Whole blood, PBMCs, liver; limited muscle delivery.	Elevates blood NAD+/NAAD; null on muscle mitochondrial respiration and insulin sensitivity in obese/elderly cohorts.
Nicotinamide mononucleotide (NMN)	CD73 dephosphorylation to NR; Slc12a8 (intestine).	Dephosphorylation; gut bacterial deamidation to NAMN.	Skeletal muscle, intestinal epithelium, PBMCs.	Elevates muscle NAD+ turnover; selective muscle insulin-sensitivity gain in prediabetic women; null in general aging.
Dihydronicotinamide riboside (NRH)	Unassisted passive diffusion (neutral molecule).	ADK-dependent phosphorylation; toxicity signals at high dose in preclinical.	Liver, kidney, brain, pancreas (preclinical).	2- to 5-fold NAD+ elevation vs NR/NMN in head-to-head preclinical; human trials in progress.

Synthesized from Refs [3,4,5,6,7,10,12,14,15]. Transport mechanism data from [4]; NRH preclinical data from [14,15].

CHAPTER 4 · BARRIER 3

The CD38 Inflammatory Sink in Aging Tissues

The third barrier is the one most directly tied to aging itself. Even if you solve the pharmacokinetics and the transport and you successfully elevate intracellular NAD^+, you are now dumping fuel into a leaky tank. In aged tissue, that tank is the ectoenzyme CD38.

4.1 CD38 is the master NADase of inflammaging

CD38 (ADP-ribosyl cyclase 1) is a type-II transmembrane ectoenzyme expressed on the surface of immune cells, particularly tissue-resident macrophages and endothelial cells. It catalyzes the cyclization of NAD^+ to cyclic ADP-ribose (cADPR), a calcium-mobilizing second messenger, releasing free nicotinamide as a byproduct. The kinetic number is brutal: a single CD38 molecule can hydrolyze up to 100 molecules of NAD^+ per second to generate one cADPR molecule [16,17]. CD38 has a lower K_m for NAD^+ than most other NAD^+-consuming enzymes, meaning it is constitutively active at physiological NAD^+ concentrations and is the first to consume any surplus.

4.2 Aging upregulates CD38 via the senescence secretome

In young, homeostatic tissue, NAD^+ consumption is shared roughly evenly between sirtuins, PARPs, and CD38. In aging tissue, the balance shifts dramatically. Senescent cells accumulate with age and secrete a senescence-associated secretory phenotype (SASP) rich in TNF-, IL-6, and other pro-inflammatory cytokines. These cytokines drive massive transcriptional upregulation of CD38 in tissue-resident macrophages and endothelial cells [16,17]. Camacho-Pereira and colleagues showed that CD38 protein abundance in liver, spleen, and muscle increases 2- to 5-fold with age in mice, and that this increase accounts for the majority of age-related NAD^+ decline. CD38-knockout mice preserve youthful tissue NAD^+ concentrations into advanced age and resist high-fat-diet-induced metabolic dysfunction.

4.3 Why this makes precursor monotherapy futile in older adults

The arithmetic is unforgiving. If an older adult ingests enough NR or NMN to elevate intracellular NAD^+ by 30%, but their tissue CD38 abundance is 4-fold higher than a young adult's, the expanded NAD^+ pool is hydrolyzed to NAM and cADPR within minutes — faster than mitochondrial sirtuins can use it for protein deacetylation. The net effect is that the precursor dose produces more NAM (which inhibits sirtuins) and more cADPR (which mobilizes calcium and can paradoxically activate inflammatory pathways) without producing the SIRT3-mediated mitochondrial benefit that was the point of the exercise.

This is the structural reason why CD38 inhibition is now considered a necessary co-therapy, not an optional add-on. Synthetic CD38 inhibitors such as 78c restore tissue NAD^+ and SIRT3 activity in aged mice without requiring precursor supplementation [18]. The dietary flavonoids apigenin (parsley, celery, chamomile) and quercetin (onions, capers, apples) inhibit CD38 catalytic activity at micromolar concentrations achievable through diet or supplementation [19]. Co-administration of a CD38 inhibitor with an NAD^+ precursor prevents accelerated metabolite clearance, restores the intracellular NAD^+/NADH ratio, and rescues SIRT3-mediated mitochondrial protein deacetylation in aged animal models more effectively than precursor monotherapy.

Table 4.1 — CD38 intervention strategies

Strategy	Mechanism	Stage of evidence	Practical notes
78c (synthetic)	Potent competitive CD38 inhibitor; reduces NAD^+ hydrolysis.	Preclinical (mouse); restores aged-tissue NAD^+ without precursor.	Investigational; no human pharmacokinetic data yet.
Apigenin	Dietary flavonoid; inhibits CD38 at low-M concentrations.	Preclinical + early human PK; safe dietary intake.	Parsley, celery, chamomile; supplements widely available.
Quercetin	Dietary flavonoid; CD38 inhibition + senolytic effect.	Human trials as senolytic (with dasatinib); CD38 effect inferred.	Onions, capers, apples; FASEB-tier evidence for NAD^+ preservation.
Senolytic cocktails	Eliminate senescent cells → reduce SASP → reduce CD38 induction.	Early human trials in diabetic kidney disease and IPF.	Targets upstream cause of CD38 overexpression rather than enzyme itself.
Luteolin & other flavonoids	Multi-target CD38 / NADase inhibition.	Preclinical.	Less potent than 78c but broad dietary availability.

Sources: Camacho-Pereira 2016 [16]; Chini 2024 review [17]; Tarragó 2018 [18]; Rajman 2018 [19].

WHY THIS CHANGES THE TRIAL-DESIGN CONVERSATION

If you run a 12-week NR trial in 60-year-olds without controlling for CD38 activity, you have built a study that is biochemically biased toward null. The CD38 sink will erase the precursor signal before it reaches mitochondria. Future trials should either stratify by baseline CD38 activity (measurable in PBMCs by flow cytometry) or co-administer a CD38 inhibitor. Anything else is testing whether you can fill a bathtub with the drain open.

CHAPTER 5 · BARRIER 4

Methodological Trial-Design Flaws

The fourth barrier is not in the body — it is in the trials. Even if you had a perfect precursor that reached the right tissue at the right dose, most published RCTs would still fail to detect benefit because of four design choices that systematically bias toward null results. These are not minor quibbles; they explain why the same molecule produces "miracle in mice, dud in humans" headlines over and over.

5.1 Surrogate marker misalignment

The dominant endpoint in NAD^+ trials is steady-state NAD^+ concentration in whole blood or PBMCs. This is a compliance marker: it tells you the participant took the pill. It tells you almost nothing about whether the intervention changed intracellular NAD^+ flux in skeletal muscle, heart, or brain — the tissues whose mitochondrial function we actually care about. Blood NAD^+ and tissue NAD^+ are weakly correlated at best; the cell types that dominate the blood signal (erythrocytes, lymphocytes) have different NAD^+ kinetics, different transporter expression, and different consumption patterns than post-mitotic muscle fibers. Stable isotope tracer studies (13C- or 2H-labeled NR/NMN) measure real-time intracellular flux and organ distribution, and they are the only way to know whether the molecule is actually being used [20,21]. The ongoing NCT06882096 trial is among the first to apply isotope-labeled NMN and NAM in humans, mapping flux into blood, muscle, urine, and stool.

5.2 Cohort ceiling effects

Most published trials enrolled healthy, non-depleted young or middle-aged participants whose endogenous NAMPT-mediated salvage pathway is fully functional. In bioenergetically intact individuals, homeostatic feedback maintains tight physiological control over intracellular NAD^+ — the system actively resists surplus. This is why a 2-fold blood NAD^+ rise produces zero tissue-level functional change: the tissues did not need the surplus and down-regulated uptake or up-regulated consumption to absorb it. The therapeutic ceiling effect is real. Significant clinical benefits emerge predominantly in cohorts with documented baseline bioenergetic deficits: prediabetic postmenopausal women (Yoshino 2021 [10]), patients with dyslipidemia, heart failure, primary mitochondrial DNA disorders (Kerr 2025 [22]), or inflammaging-affected older adults. The field is slowly pivoting toward stratified enrichment designs.

5.3 Trial duration too short for mitochondrial remodeling

Mitochondrial biogenesis — the synthesis of new mitochondria, the expansion of the mitochondrial network, the upregulation of oxidative-phosphorylation complexes — is a structural process measured in months, not weeks. PGC-1 activation, mitochondrial DNA replication, electron-transport-chain assembly, and mitophagic turnover operate on timescales of 8–16 weeks in human skeletal muscle. The standard 4-to-12-week NR/NMN trial is timed to detect changes in blood metabolites, not mitochondrial structure. Asking whether NR improves muscle mitochondrial function in 6 weeks is like asking whether strength training adds muscle mass in 6 weeks. The answer is no, not because the intervention is ineffective, but because the window is too short to measure the structural endpoint.

5.4 Monotherapy neglects synergistic signals

The final design flaw is the assumption that NAD^+ supplementation is a monotherapy. In reality, the salvage pathway is upregulated by exercise (which induces NAMPT expression in skeletal muscle), the consumption side is gated by CD38 (which is suppressed by apigenin, quercetin, and senolytic interventions), and the downstream signaling requires functional sirtuins (which themselves require NAD^+ but are also regulated by circadian rhythm and substrate availability). Evaluating NR or NMN strictly as monotherapies in sedentary, inflammaging, sirtuin-attenuated older adults is testing the molecule under conditions designed to make it fail. Combination protocols — precursor + structured exercise + CD38 inhibitor + adequate sleep/circadian alignment — are what the biology predicts will work.

Table 5.1 — Trial-design flaws and their fixes

Design flaw	What it does to results	Fix
PBMC / whole-blood NAD^+ as primary endpoint	Measures compliance, not efficacy. Tissue NAD^+ flux invisible.	Stable isotope tracer (13C / 2H-labeled precursor) + target-tissue biopsy; fluxomics over static concentration.
Healthy young/middle-aged cohorts	Ceiling effect: homeostatic feedback absorbs surplus; no headroom for benefit.	Stratified enrichment: prediabetes, dyslipidemia, heart failure, mitochondrial DNA disease, inflammaging cohorts.
4–12-week duration	Sufficient for blood metabolites; insufficient for mitochondrial biogenesis or functional remodeling.	24–52-week trials with serial biopsies; structural co-endpoints (mitochondrial content, fiber-type shift).
Monotherapy design	Ignores NAMPT induction by exercise, CD38 suppression by flavonoids, circadian sirtuin regulation.	Combination protocols: precursor + CD38 inhibitor + exercise + circadian alignment.
Unstratified analyses	Positive subgroup signals (e.g., prediabetic women) get buried in null overall mean.	Pre-specified subgroup analyses by baseline NAD^+, CD38 activity, NAMPT level, age.
No measurement of CD38 / NAMPT baseline	Cannot distinguish non-responders from biochemically unsuitable participants.	Baseline PBMC CD38 flow cytometry; muscle NAMPT immunoblot; baseline isotope flux as enrollment criterion.

Synthesis of design recommendations from Refs [4,5,6,7,10,20,21].

CHAPTER 6

Trial Post-Mortem: What Four Landmark Studies Actually Showed

The fastest way to confirm the four-barrier diagnosis is to re-read the most-cited NAD^+ trials through that lens. Each headline-null trial contains positive mechanistic signals that were either underpowered, under-stratified, or measured at the wrong time horizon. None of them refute the NAD^+ hypothesis; all of them illustrate one or more of the barriers above.

6.1 Remie 2020 — NR in overweight adults

Six-week double-blind RCT of NR 1,000 mg/day versus placebo in overweight and obese adults, with paired skeletal-muscle biopsies [5]. Blood NAD^+ rose; muscle NAD^+ metabolites (NAAD, MeNAM) rose. Sleeping metabolic rate increased modestly; fat-free mass ticked up. But ex vivo mitochondrial respiration, intramyocellular lipid, and insulin sensitivity were unchanged. Re-reading through the four-barrier lens: the dose was likely too low to overcome PNP cleavage and CD38 consumption (Barriers 1 and 3); six weeks was too short for mitochondrial biogenesis (Barrier 4); and the cohort was bioenergetically intact enough to hit a ceiling (Barrier 4). The trial is not a refutation of NR; it is a refutation of low-dose, short-duration NR monotherapy in metabolically borderline adults.

6.2 Dollerup 2018, 2020 — high-dose NR in obese diabetic men

Twelve-week RCT of NR 2,000 mg/day in obese, insulin-resistant men [6,7]. Despite doubling the Remie dose and doubling the duration, skeletal-muscle mitochondrial respiration, mitochondrial content, and oxidative-phosphorylation gene expression were unchanged. The most important finding: skeletal-muscle NAMPT protein abundance *decreased* after NR treatment. The endogenous salvage machinery was down-regulated in response to exogenous precursor surplus — a homeostatic feedback effect that directly illustrates the ceiling mechanism (Barrier 4) and confirms that blood NAD^+ rise does not equal muscle NAD^+ rise (Barrier 2). The Dollerup trial is the strongest single piece of evidence that monotherapy in metabolically dysfunctional but bioenergetically salvageable adults is the wrong experimental design.

6.3 Yoshino 2021 — NMN in prediabetic postmenopausal women

Ten-week RCT of NMN 250 mg/day in postmenopausal women with prediabetes [10]. This is the trial most often cited as evidence that NAD^+ supplementation "works," and it is the one that most clearly demonstrates what right looks like. Primary endpoint — skeletal-muscle insulin sensitivity by hyperinsulinemic-euglycemic clamp — improved significantly in the NMN arm. Muscle remodeling pathways mediated by PDGF signaling were upregulated. But the benefit was strictly localized: hepatic and adipose insulin sensitivity, blood pressure, and plasma lipids were unchanged. The trial succeeded because it stratified into a tissue-specific deficit (prediabetic muscle), chose a precursor whose transport profile favors muscle (NMN via CD73/NRK1), used a low dose matched to uptake capacity rather than a high dose that would have triggered NAMPT suppression, and ran long enough for muscle signaling to shift. It is a proof-of-concept that barrier-aware design produces barrier-crossing results.

6.4 Christen 2026 — head-to-head NR vs. NMN vs. NAM

Fourteen-day open-label RCT in 65 healthy adults comparing NR 1 g/day, NMN 1 g/day, NAM 0.5 g/day, and placebo [3]. NR and NMN each approximately doubled whole-blood NAD^+; nicotinamide did not. Ex vivo fermentation with human fecal microbiota confirmed that the NAD^+ rise was mediated largely by microbially produced NA via the Preiss–Handler pathway, not by direct tissue uptake of intact NR or NMN. This is the trial that retroactively explains why every prior NR/NMN blood-NAD^+-positive trial produced tissue-function-negative results: the wrong metabolite was being measured in the wrong compartment. The Christen trial is the empirical foundation of Barrier 1.

Table 6.1 — Four-trial post-mortem matrix

Trial	Design	What rose	What did not	Barrier illustrated
Remie 2020	NR 1,000 mg/d x 6 wk; overweight/obese adults; paired muscle biopsy.	Blood NAD^+; muscle NAAD/MeNAM; sleeping metabolic rate; fat-free mass.	Ex vivo mitochondrial respiration; insulin sensitivity; intramyocellular lipid.	Dose too low (B1); duration too short (B4); ceiling effect (B4).
Dollerup 2018/20	NR 2,000 mg/d x 12 wk; obese insulin-resistant men; muscle biopsy.	Blood NAD^+; muscle NAD^+ metabolites.	Mitochondrial respiration /content/OXPHOS gene expression; NAMPT (decreased).	NAMPT feedback suppression (B2+B4); confirms blood/tissue disconnect.
Yoshino 2021	NMN 250 mg/d x 10 wk; prediabetic postmenopausal women; clamp.	Skeletal-muscle insulin sensitivity; PDGF muscle-remodeling pathway.	Hepatic/adipose insulin sensitivity; blood pressure; plasma lipids.	Demonstrates that barrier-aware design works; benefit localized to NRK1/2-rich tissue.
Christen 2026	NR vs NMN vs NAM, 14 d, 65 healthy adults; ex vivo microbiome fermentation.	Blood NAD^+ 2x with NR/NMN; NAAD; SCFAs; urinary NAR/NUR.	Chronic NAD^+ rise with NAM (acute only); intact NR/NMN barely detectable in blood.	Direct empirical foundation of Barrier 1 (microbial deamidation).

Full citations in References section. Trials selected for landmark status and paired-biopsy or mechanistic depth.

CHAPTER 7

Next-Generation Strategies: NRH, CD38 Inhibition, and Tracer-Driven Trials

If the four barriers explain why current NAD^+ supplementation underperforms, the obvious next question is what would actually perform. The translational pipeline now contains four answer categories: reduced precursors that bypass PNP and ENT bottlenecks, CD38 inhibitors that close the degradation sink, combination protocols that stack synergistic signals, and isotope-tracer trial designs that measure the right endpoint in the right compartment.

7.1 Reduced precursors: NRH and NMNH

Dihydronicotinamide riboside (NRH) and dihydronicotinamide mononucleotide (NMNH) are the reduced forms of NR and NMN. Their structural chemistry solves several problems at once. NRH is a neutral, uncharged molecule and crosses plasma membranes by unassisted passive diffusion, bypassing dependency on ENT1/2. It is completely resistant to hydrolysis by PNP, so the three-minute half-life that plagues NR does not apply. Once inside the cytoplasm, NRH bypasses the canonical NRK1/NRK2 pathway and is phosphorylated by adenosine kinase (ADK) to NMNH, then converted to NADH by NMNAT enzymes and oxidized to NAD^+. The ADK route was confirmed by pharmacological inhibition: ABT702 abolishes NRH phosphorylation and downstream NAD^+ synthesis [14]. In head-to-head animal and cell-culture studies, NRH achieves 2- to 5-fold higher intracellular NAD^+ concentrations than equivalent doses of NR or NMN across liver, kidney, muscle, and brain.

The caveat is toxicity. A 2026 chronic-supplementation study by Rumpler and colleagues in *Nature Communications* showed that high-dose NRH produces signs of toxicity not seen with NR or NMN [15], and a separate 2026 study (Venkateshvaran) showed biphasic effects on human skeletal-muscle differentiation. NRH is not a drop-in replacement for NR; it is a more potent molecule that needs dose-response characterization and human safety data. Multiple human trials are now in progress, but at the time of writing no published human efficacy data exist.

7.2 CD38 inhibitors: 78c, apigenin, quercetin

The case for pairing a CD38 inhibitor with a precursor is now overwhelming. The synthetic small molecule 78c is the most-studied CD38 inhibitor in preclinical models; it restores aged-tissue NAD^+ and SIRT3 activity without requiring precursor supplementation, and acts synergistically when combined with a precursor [18]. The dietary flavonoids apigenin and quercetin are weaker but achievable through diet or over-the-counter supplementation [19]. Quercetin has the additional advantage of being a senolytic when combined with dasatinib, hitting CD38 from the upstream side by eliminating the senescent cells whose SASP drives CD38 overexpression. A practical co-therapy protocol might combine NR or NMN with apigenin (50–100 mg/day) or quercetin (500–1,000 mg/day), with the flavonoid dosed separately from the precursor to avoid absorption competition.

7.3 Combination protocols with exercise

Exercise is the cheapest, most reliable NAMPT inducer known. Skeletal-muscle NAMPT expression rises 2- to 3-fold after a structured endurance-training block. This is not a confounder to control for; it is a synergistic signal to exploit. Trial designs that randomize participants to NR/NMN alone, exercise alone, or NR/NMN + exercise are the cleanest way to test whether precursor loading amplifies the bioenergetic remodeling that exercise initiates. The combination hypothesis is biologically well-motivated: exercise upregulates the salvage machinery (NAMPT) that the precursor requires, and the precursor provides the substrate that the upregulated machinery needs.

7.4 Isotope-tracer trial design

The single most important methodological shift is replacing static blood NAD^+ with isotope-tracer flux. NCT06882096 (ongoing) uses 13C- or 2H-labeled NMN and NAM to map flux from oral precursors into blood, muscle, urine, and stool in young versus older adults [21]. This is the trial design that can actually answer the question "did the precursor reach the tissue and contribute to the intracellular NAD^+ pool?" Once isotope-tracer flux becomes the standard endpoint, the field will be able to distinguish pharmacokinetic failures (precursor never reached tissue) from pharmacodynamic failures (precursor reached tissue but did not translate to functional benefit) — a distinction the current literature collapses.

Table 7.1 — Next-generation NAD^+ intervention pipeline

Strategy	Mechanism / rationale	Current status	Limitations
NRH / NMNH (reduced precursors)	Neutral, PNP-resistant; passive diffusion; ADK pathway; 2-5x NAD^+ rise preclinical.	Human PK/safety trials in progress; no published efficacy data.	Toxicity signals at high dose; biphasic muscle differentiation effects; long-term safety unknown.
78c + precursor combination	Synthetic CD38 inhibitor; prevents NAD^+ hydrolysis in aged tissue.	Preclinical only; restores aged-tissue NAD^+ and SIRT3 activity.	No human pharmacokinetic or safety data; drug-development pathway required.
Apigenin / quercetin + precursor	Dietary flavonoid CD38 inhibition; senolytic co-benefit (quercetin + dasatinib).	Apigenin: early human PK. Quercetin: human senolytic trials completed.	Lower potency than 78c; absorption and bioavailability variable; multi-target effects.
Exercise + precursor	Exercise induces NAMPT 2-3x in skeletal muscle; precursor provides substrate.	Combination trials in design; biological rationale strong.	Adherence burden; exercise effect size dwarfs precursor effect, complicating attribution.
Isotope-tracer flux endpoints	13C / 2H-labeled precursor measures real-time tissue flux rather than static blood concentration.	NCT06882096 ongoing; first human flux data expected 2026-2027.	Requires biopsy; expensive; small cohorts; not yet widely available.
Stratified enrichment cohorts	Enroll only participants with documented baseline NAD^+ deficit or CD38 elevation.	Increasingly adopted; prediabetes and mitochondrial-disease cohorts leading.	Recruitment slower; generalizability narrower; requires baseline biomarker screening.

Sources: Refs [14,15,18,19,21,22]. Trial numbers verifiable at ClinicalTrials.gov.

CHAPTER 8

The Verdict: What Works, What Doesn't, What to Do

So is NAD^+ supplementation useless? No. Is it the anti-aging miracle the marketing implied? Also no. The honest verdict sits in between, and it depends on who is asking.

8.1 What the evidence actually supports

For healthy adults under 50 with normal glucose tolerance, normal lipid profile, and regular exercise habits: NR and NMN supplementation reliably elevate blood NAD^+ and produce essentially no measurable functional benefit. The bioenergetic ceiling is real; the homeostatic system resists surplus; the CD38 sink is not yet overactive; the mitochondria are not yet dysfunctional. Save your money, or spend it on exercise and sleep.

For adults with documented baseline deficits — prediabetes, dyslipidemia, heart failure, primary mitochondrial DNA disorders, inflammaging-affected older adults — the evidence is more encouraging. Yoshino's prediabetic postmenopausal women gained muscle insulin sensitivity. The Kerr 2025 mitochondrial-disease cohort showed functional improvement in genetically confirmed mito disease [22]. The Martens 2018 cohort of older adults with hypertension showed a modest systolic reduction [11]. These are not null results; they are stratified signals buried in cohorts that, on aggregate, looked null.

8.2 Practical protocol considerations

For the biohacker or clinician who decides to supplement despite the mixed evidence, the four-barrier framework translates into concrete protocol choices:

Dose. Most published trials used 250–1,000 mg/day NR or NMN. Higher doses (2,000 mg/day) do not produce proportionally larger tissue NAD^+ rises — they trigger NAMPT feedback suppression (Dollerup). The sweet spot for cost-benefit appears to be 250–500 mg/day.

Timing. The NAD^+ salvage pathway is under circadian control via BMAL1/CLOCK-mediated NAMPT transcription. Dosing in the morning aligns with the physiological NAD^+ peak; dosing at night may disrupt circadian rhythm. There is no strong trial evidence for this, but the biology is clear.

Co-factors. A CD38 inhibitor (apigenin 50 mg or quercetin 500 mg, taken separately from the precursor) addresses Barrier 3 directly. Quercetin's senolytic co-benefit is a bonus. The combination is the most defensible real-world protocol given current evidence.

Exercise. The single most reliable way to upregulate NAMPT in skeletal muscle is endurance exercise. A precursor plus exercise is biologically more rational than a precursor alone. This is also free.

What to track. Whole-blood NAD^+ confirms you absorbed the pill. It does not confirm efficacy. If you want to know whether the protocol is working at the tissue level, the only currently accessible proxies are functional (insulin sensitivity via CGM, aerobic capacity, body composition) rather than biochemical. Push for isotope-tracer endpoints in any clinical decision-making — demand better biomarkers, not just more pills.

8.3 What we got wrong, what to do next

The NAD^+ field over-promised based on mouse data and under-delivered because it ignored pharmacokinetics, transport biology, enzymatic consumption, and trial design. The translational gap is real, but it is a gap of execution, not of concept. The hypothesis — that declining NAD^+ drives age-related mitochondrial dysfunction and that restoring it should help — remains the most coherent bioenergetic theory of aging we have. The four barriers identified here are all addressable. Reduced precursors solve the transport and PNP problems. CD38 inhibitors solve the consumption sink. Stratified cohorts and isotope-tracer endpoints solve the trial-design problem. Combination protocols with exercise solve the synergy problem.

The next decade of NAD^+ research will be defined by whether the field adopts these fixes or continues to run low-dose, short-duration, monotherapy trials in healthy cohorts and report null results as if they were informative. The mystery is solved. The question is whether anyone will act on the solution.

FINAL VERDICT

NAD^+ supplementation is not a scam, but the way it has been tested and sold has been scientifically inadequate. NR and NMN reliably elevate blood NAD^+ and produce real but tissue-specific, population-specific, dose-specific, and duration-specific benefits. The next generation of interventions — NRH, CD38 inhibitors, combination protocols, isotope-tracer trials — will tell us whether the NAD^+ hypothesis matures into a clinical tool or remains a biohacker enthusiasm. The biology says it should work. The execution is what has been missing.

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