NAD^+ RESTORATION AND CD38 INHIBITION

A Comprehensive Analysis of Precursor Supplementation and NADase Pharmacology in the Context of Neurodegenerative Therapeutics

A Synthetic Monograph in the Organic Network Synthesis Methodology
Bioenergetic Pharmacology Series · Volume II

Ben Gustafsson

Prepared under the ONS Methodology

AdultCognitiveDisease.com

June 2026

First Edition

Second volume of the ONS Bioenergetic Pharmacology series. Companion to Volume I (Pharmacological Modulation of Mitochondrial ATP Synthase) and Volume III (Iron Chelation and GPX4 Stabilization). Integrates the Chini, Brenner, Sinclair, Auwerx, and Bohr research programs into a single analytical framework on the pharmacology of NAD^+ restoration.

“The supply of electrons that drives every cellular thought is older than thought itself. When the supply contracts, the thought contracts with it.”

— After Lautrup, Sinclair, Mattson & Fang, 2019

For the laboratories of the Mayo Clinic and the NIH, and for the patients whose substrate supply is contracting faster than their cells can compensate.

THE ONS BIOENERGETIC PHARMACOLOGY SERIES

I. Pharmacological Modulation of ATP Synthase

II. NAD^+ Restoration and CD38 Inhibition ☐ this volume

III. Iron Chelation and GPX4 Stabilization

Each volume of the Bioenergetic Pharmacology series examines a single rate-limiting catalytic node in the mitochondrial-autophagic axis and the medicinal-chemistry pipelines that engage it. The series is the translational arm of the Collapse Trilogy: where the trilogy mapped the circuit, cellular, and substrate failures, the pharmacology series examines the pharmacological wedges by which those failures can be prevented, slowed, or in principle reversed.

Abstract

The age-associated decline in nicotinamide adenine dinucleotide (NAD^+) — the universal electron carrier of intermediary metabolism, the obligate co-substrate of the sirtuin deacylases, and the principal substrate of the PARP and CD38 enzymes — is among the most rigorously documented metabolic changes in the aging brain. NAD^+ concentrations decline by approximately fifty percent between early adulthood and the eighth decade in cortex, hippocampus, and muscle tissue, with steeper declines in disease-affected regions of patients with Alzheimer's disease, Parkinson's disease, and ALS.

This monograph evaluates the two complementary pharmacological strategies that have emerged from this mechanistic understanding. The *substrate-restoration strategy* supplements the NAD^+ pool with the direct biosynthetic precursors nicotinamide riboside (NR) and nicotinamide mononucleotide (NMN). The *drain-arrest strategy* inhibits the CD38 NADase using small molecules — the flavonoid scaffold exemplified by apigenin and luteolin, the synthetic compound 78c developed by the Chini program, and the next-generation MK-class compounds. The two strategies are mechanistically complementary in the same hose-and-bucket sense that direct ATP synthase modulation (J-147) and upstream protection (AG18051-class NQO2 inhibition) are complementary in Volume I of this series.

The monograph develops the substrate-restoration and drain-arrest strategies through six analytical chapters, situates them within the Collapse Trilogy framework, examines the disappointing clinical translation of first-generation NAD^+ precursors and the lessons it carries for the field, and concludes with seven falsifiable experimental predictions. The argument advanced is that NAD^+ restoration in the aging brain requires *coordinated* intervention against both the synthetic deficit and the catabolic surplus — that precursor supplementation alone is insufficient because the restored substrate is consumed by CD38 before it can engage the downstream sirtuin and PARP machinery — and that the optimal therapeutic regimen will combine an NAD^+ precursor with a CD38 inhibitor as the foundational layer of bioenergetic intervention.

Keywords: NAD^+, CD38, nicotinamide riboside, nicotinamide mononucleotide, sirtuins, PARP, 78c, apigenin, mitochondrial biogenesis, mitophagy, geroneuroprotection, Alzheimer's disease

Note on Sources

This monograph is the second volume of the ONS Bioenergetic Pharmacology series. It integrates the Chini, Brenner, Sinclair, Auwerx, Bohr, and Bredesen research programs into a single analytical narrative on the pharmacology of NAD^+ restoration. Where the prevailing literature is unsettled — particularly around the comparative cellular bioavailability of nicotinamide riboside (NR) and nicotinamide mononucleotide (NMN), and around the clinical translation of CD38 inhibitors — sources are explicitly weighted. All claims trace to peer-reviewed sources listed in the References section. The monograph should be read as a synthetic review under the Organic Network Synthesis methodology, not a primary experimental report.

Contents

Executive Summary

The age-associated decline in nicotinamide adenine dinucleotide (NAD^+) — the universal electron carrier of intermediary metabolism, the obligate co-substrate of the sirtuin deacylases, and the principal substrate of the PARP and CD38 enzymes — is among the most rigorously documented metabolic changes in the aging brain. The decline reflects the integrated effect of three principal mechanisms: falling synthetic flux through the salvage pathway, falling cellular import of precursor molecules, and rising consumption by NAD^+-degrading enzymes — of which the cyclic-ADP-ribose hydrolase CD38 has emerged in the last decade as the dominant variable in aging tissue.

This monograph evaluates the two complementary pharmacological strategies that have emerged from this mechanistic understanding. The *substrate-restoration strategy* supplements the NAD^+ pool with the direct biosynthetic precursors nicotinamide riboside (NR) and nicotinamide mononucleotide (NMN), which are imported into cells, phosphorylated, and incorporated into the mature NAD^+ pool via the salvage pathway. The *drain-arrest strategy* inhibits the CD38 NADase using small molecules — the flavonoid scaffold exemplified by apigenin and luteolin, the synthetic compound 78c developed by the Chini program, and the next-generation MK-class compounds — which preserve the existing NAD^+ pool by suppressing its principal consumer.

The two strategies are mechanistically complementary in the same hose-and-bucket sense that direct ATP synthase modulation (J-147) and upstream protection (AG18051-class NQO2 inhibition) are complementary in Volume I of this series. The monograph develops them through six analytical chapters, situates them within the Collapse Trilogy framework, and concludes with seven falsifiable experimental predictions. The argument advanced is that the optimal therapeutic regimen will combine an NAD^+ precursor with a CD38 inhibitor as the foundational layer of bioenergetic intervention.

Chapter I

Introduction

The NAD^+ Decline as the Substrate-Supply Problem

1.1 The Research Problem

In the framework developed in Volume I of this series, mitochondrial ATP synthase emerged as the *catalytic* chokepoint of neuronal bioenergetics — the molecular machine whose preservation or restoration determines whether the cell can convert the proton-motive force into ATP. But every catalytic machine requires substrate. The substrate supply on which ATP synthase ultimately depends — through the reducing equivalents that drive proton pumping at Complexes I, III, and IV — is the NAD^+/NADH redox couple. When NAD^+ concentrations decline, the substrate supply to the electron transport chain declines in proportion; Complex I activity falls; the proton-motive force diminishes; ATP synthase output collapses; and the entire downstream edifice of bioenergetic, proteostatic, and redox homeostasis fails along with it.

This dependence makes the NAD^+ pool one of the most consequential single variables in neuronal aging. Its decline is observed across every major disease examined — Alzheimer's, Parkinson's, ALS, frontotemporal dementia, Huntington's, and a growing list of mitochondrial encephalomyopathies — and across every aged tissue measured. Restoring it is one of the most mechanistically tractable interventions in the entire geroneuroprotective pharmacopoeia. The reason NAD^+ restoration has not yet delivered the disease-modifying benefit that its mechanistic promise suggests is, this monograph will argue, that the field has pursued precursor supplementation in isolation rather than as part of a coordinated regimen against both the synthetic deficit and the catabolic surplus.

1.2 Why NAD^+ Matters: Three Roles in One Molecule

NAD^+ occupies a uniquely privileged position in cellular biochemistry because it serves three distinct functions simultaneously, none of which is fully substitutable by other molecules. *First*, NAD^+ is the principal electron carrier of intermediary metabolism: it accepts a hydride from glyceraldehyde-3-phosphate dehydrogenase in glycolysis, from pyruvate dehydrogenase at the gateway to the TCA cycle, from the three NAD^+-linked TCA dehydrogenases (isocitrate, α-ketoglutarate, malate), and from the β-oxidation cycle, becoming NADH in the process. NADH then delivers its electrons to Complex I of the electron transport chain, where they drive proton pumping and ultimately ATP synthesis. The NAD^+/NADH ratio in the mitochondrial matrix is therefore the principal determinant of the respiratory chain's capacity to function.

Second, NAD^+ is the obligate co-substrate of the sirtuin deacylases — SIRT1 through SIRT7 — which remove acetyl, succinyl, glutaryl, and other acyl modifications from lysine residues of histone tails, transcription factors, and metabolic enzymes. The sirtuins control approximately one hundred and fifty metabolic and transcriptional targets, including PGC-1α (the master regulator of mitochondrial biogenesis), FOXO transcription factors, p53, and the autophagy-initiating Atg proteins. When NAD^+ falls below the sirtuin K_m (approximately 150–250 μM in the matrix), sirtuin activity is rate-limited by substrate availability; below this threshold, the entire sirtuin-dependent regulatory program decompensates.

Third, NAD^+ is the substrate of the PARP family of poly(ADP-ribose) polymerases — particularly PARP1, the principal sensor of single-strand DNA breaks. PARP1 cleaves NAD^+ and transfers ADP-ribose to nuclear acceptor proteins, generating PAR chains that recruit repair machinery. Sustained DNA damage drives PARP1 hyperactivation, which can deplete cellular NAD^+ within minutes and trigger necrotic cell death. These three roles — electron carrier, sirtuin substrate, PARP substrate — compete for a single cellular pool. When the pool contracts, all three functions decompensate together.

1.3 The Age-Related Decline: A Cross-Tissue Invariant

The age-related decline in NAD^+ has been documented in every mammalian tissue examined to date. Massudi and colleagues (2012) reported a forty-eight percent decline in skin NAD^+ between ages 30 and 80; Zhu and colleagues (2015) documented similar declines in skeletal muscle using P-MRS; Camacho-Pereira and colleagues (2016) extended the observation to brain, liver, and adipose tissue in mice. The decline is larger in metabolically active tissues than in quiescent tissues and is reproducibly accelerated in disease-affected regions of patients with Alzheimer's disease, Parkinson's disease, and ALS.

The mechanistic dissection has been the major contribution of the Chini program at the Mayo Clinic. Through papers beginning in 2016, Eduardo Chini, Claudia Camacho-Pereira, and colleagues established that the decline is not driven primarily by failing synthesis — the salvage pathway enzymes remain abundant in aged tissue — but by rising consumption by CD38. CD38-null mice are protected from the age-related NAD^+ decline. CD38 expression rises three- to ten-fold across tissues between young and old animals. The implication is that *the NAD^+ decline is principally a consumption problem, not a synthesis problem* — a finding with major pharmacological consequences.

1.4 The Strategic Case for Two-Pronged Intervention

If the NAD^+ decline were purely a synthesis problem, precursor supplementation would be sufficient. If it were purely a consumption problem, CD38 inhibition would be sufficient. The reality is that both mechanisms operate simultaneously, and pharmacological intervention against only one arm leaves the other operating unmodified. The data from first-generation precursor trials suggest that this is precisely why those trials underwhelmed: NR and NMN administration reliably elevate plasma NAD^+ metabolite levels but produce only modest and inconsistent elevations in tissue NAD^+, because the supplemented substrate is consumed by the unchecked CD38 drain before it can be incorporated into the functional pool.

The strategic case advanced in this monograph is that NAD^+ restoration requires *coordinated* intervention against both arms simultaneously. The cardiovascular analog is the combination of a statin (reducing endogenous cholesterol synthesis) and a PCSK9 inhibitor (reducing LDL receptor degradation): each alone produces a modest benefit,

but the combination produces a substantial benefit because the two interventions compose multiplicatively. The NAD^+ analog is the combination of an NR or NMN precursor with a CD38 inhibitor.

1.5 Significance

The reframing of NAD^+ restoration as a coordinated supply-and-demand intervention carries four significant implications. *First*, it identifies a target combination — NAD^+ precursor plus CD38 inhibitor — that has not yet been tested at scale in clinical neurodegeneration trials. *Second*, it accounts for the disappointing results of first-generation NR and NMN trials in Parkinson's disease and mild cognitive impairment. *Third*, it links the NAD^+ axis to the microglial homeostatic collapse central to the ONS framework: CD38 is upregulated on activated microglia, and microglial CD38 expression is itself a principal contributor to tissue NAD^+ depletion in the inflamed brain. *Fourth*, it positions NAD^+ restoration as the second component of a multi-target geroneuroprotective regimen alongside ATP synthase modulation (Volume I) and the iron-GPX4 axis (Volume III).

1.6 Scope and Method

This monograph is a synthetic review under the ONS methodology, integrating eight research programs into a unified analytical framework. The work is biased toward Alzheimer's disease because the NAD^+ literature in AD is the deepest, but each chapter systematically addresses Parkinson's, ALS, and Huntington's where evidence permits. The monograph cannot resolve the open question of whether NR and NMN produce equivalent intracellular NAD^+ elevations or whether one is mechanistically superior; this is flagged as the principal open empirical issue in Chapter IV.

Chapter II

NAD^+ Biology

Synthesis, Salvage, Compartmentalization, Consumption

2.1 The Three Biosynthetic Pathways

Cellular NAD^+ is synthesized through three distinct biosynthetic routes that converge on the mature dinucleotide. *The de novo pathway* begins with tryptophan, which is converted through an eight-step sequence to nicotinic acid mononucleotide (NaMN); this NaMN is then adenylated by NMNAT enzymes to NaAD and amidated by NADS to NAD^+. The de novo pathway is the principal source of NAD^+ in liver but contributes only a small fraction in brain and most peripheral tissues. *The Preiss–Handler pathway* begins with nicotinic acid (niacin, vitamin B3) and proceeds through the same NaMN ☐ NaAD ☐ NAD^+ steps. *The salvage pathway* recycles nicotinamide (NAM) — the byproduct of every sirtuin, PARP, and CD38 reaction — back into NAD^+ via NAMPT (rate-limiting) and NMNAT. In most tissues, the salvage pathway provides the majority of NAD^+ flux.

The relative dominance of the salvage pathway is significant pharmacologically. The cellular NAD^+ pool is, in steady state, a balance between the rate of NAM regeneration and the rate of NAD^+ consumption. If consumption rises (as it does in aging through CD38 upregulation), the salvage pathway must accelerate to keep pace; if it cannot, the steady-state pool falls. This is the mechanistic foundation of the supply-and-demand reframing developed in Chapter I.

2.2 NAMPT: The Salvage Gatekeeper

NAMPT (nicotinamide phosphoribosyltransferase) catalyzes the conversion of nicotinamide and 5-phosphoribosyl-1-pyrophosphate to nicotinamide mononucleotide (NMN), the rate-limiting step of the salvage pathway. NAMPT exists in two forms: an intracellular form (iNAMPT) that catalyzes the salvage reaction in cytoplasm and nucleus, and an extracellular form (eNAMPT) that circulates in plasma. Circulating eNAMPT declines with age in both mice and humans, and eNAMPT supplementation in aged mice partially restores tissue NAD^+ and extends lifespan (Yoshida et al., 2019). This has motivated the development of NAMPT activators as a potential third arm of NAD^+ intervention.

2.3 NAD^+ Compartmentalization

The cellular NAD^+ pool is not a single homogeneous concentration but a set of distinct subcellular pools — cytosolic, mitochondrial, nuclear, and endoplasmic-reticulum-associated — that exchange through dedicated transport mechanisms. The mitochondrial pool is the largest by absolute content (~250 μM matrix NAD^+, two- to three-fold higher than cytosolic) and the most kinetically isolated, because the inner mitochondrial membrane is impermeable to NAD^+ itself. Mitochondrial NAD^+ is generated in situ from NMN imported through SLC25A51 (identified in 2020 by Luongo and colleagues). Cytosolic NMN is therefore the principal upstream determinant of mitochondrial NAD^+ availability.

The compartmentalization is pharmacologically important because the beneficial sirtuin and biogenesis programs are predominantly nuclear and mitochondrial, while the detrimental CD38 catabolism operates in distinct ER and plasma-membrane compartments. A pharmacological strategy that preferentially elevates nuclear and mitochondrial NAD^+ while leaving CD38-accessible pools unchanged would, in principle, decouple beneficial from detrimental functions.

2.4 The Sirtuin–PARP–CD38 Triangle

Three enzyme families consume NAD^+ in physiologically important quantities. *The sirtuins* (SIRT1–SIRT7) deacetylate lysine residues on histones, transcription factors, and metabolic enzymes, with one NAD^+ consumed per deacylation event. SIRT1 (nuclear) and SIRT3 (mitochondrial) are the two most relevant to geroneuroprotection. *The PARPs* cleave NAD^+ and transfer ADP-ribose to acceptor proteins; PARP1 is the dominant isoform and the dominant NAD^+ consumer under acute DNA damage. *CD38* is a membrane-bound NADase that hydrolyzes NAD^+ to ADP-ribose and nicotinamide; CD38 expression is strongly upregulated by inflammatory stimuli and rises three- to ten-fold across tissues with aging. The Chini work has established CD38 as the dominant NADase in aging tissue.

2.5 NADH/NAD^+ Redox Coupling and Mitochondrial Function

The NAD^+/NADH ratio in the mitochondrial matrix is approximately 8 under basal conditions and falls to approximately 0.5 under maximal respiratory load. This ratio is the principal thermodynamic driver of Complex I activity: at high NAD^+/NADH, electrons flow rapidly from NADH into the chain; at low ratios, the chain becomes substrate-limited. The mitochondrial NAD^+ pool size therefore sets the upper ceiling on respiratory capacity and, by extension, on ATP synthase output — linking Volume II directly to Volume I.

The age-related decline reduces both the absolute matrix NAD^+ pool and the NAD^+/NADH ratio at any given respiratory load. The consequence is a leftward shift in the substrate-limitation curve of Complex I: aged mitochondria become substrate-limited at lower respiratory demands, and the ATP-synthesis capacity falls in proportion. Restoring the mitochondrial NAD^+ pool through precursor supplementation or CD38 inhibition restores the substrate supply and, in mechanistically clean experimental systems, restores respiratory capacity to youthful levels.

Chapter III

CD38

The Dominant NADase of Aging

3.1 Discovery and Biology

CD38 was originally identified in 1980 as a lymphocyte surface antigen. The discovery that CD38 is enzymatically a multifunctional NAD^+-degrading enzyme came later: in 1992, Howard and colleagues showed that CD38 catalyzes the conversion of NAD^+ to cyclic ADP-ribose (cADPR), a calcium-mobilizing second messenger. Subsequent work established that CD38 catalyzes three reactions: NAD^+ ☐ cADPR + nicotinamide; NAD^+ ☐ ADP-ribose + nicotinamide; and cADPR ☐ ADP-ribose. The enzyme is a type II membrane protein with its catalytic domain oriented toward the extracellular space on the plasma membrane and toward the lumen on intracellular membranes.

CD38 is expressed at low levels in most resting cells and at high levels on immune cells, brain microglia, and several endocrine tissues. Expression is dramatically upregulated by inflammatory stimuli — LPS via TLR4-NF-κB signaling, IFN-γ via JAK-STAT signaling, and senescence-associated secretory phenotype cytokines. This inflammation-induced upregulation places CD38 at the intersection of the chronic low-grade inflammation characteristic of aging (“inflammaging”) and the bioenergetic decline that defines the aged cellular phenotype.

3.2 The Chini Program: CD38 as the Dominant Consumer

Eduardo Chini and colleagues at the Mayo Clinic have developed the most comprehensive characterization of CD38 in aging tissue. Their canonical demonstration rests on three observations (Camacho-Pereira et al., 2016). *First*, CD38 protein and enzymatic activity rise three- to ten-fold in liver, muscle, adipose tissue, and brain between young and aged mice. *Second*, CD38-null mice are protected from the age-related NAD^+ decline; their tissue NAD^+ remains at youthful levels into the twenty-fourth month. *Third*, pharmacological inhibition of CD38 in aged wild-type mice (using 78c) reverses the NAD^+ decline and restores mitochondrial function, glucose tolerance, and exercise capacity to levels indistinguishable from young controls.

The Chini program has also established the mechanism by which CD38 rises with age. The principal driver is the senescence-associated secretory phenotype: as cells accumulate senescent fates, they secrete inflammatory cytokines (IL-6, IL-1β, TNF-α, CXCL1, CXCL10) that act in a paracrine manner to upregulate CD38 on neighboring macrophages and microglia. Senescent-cell clearance with senolytic compounds reduces CD38 expression in aged tissue. The implications for therapeutic strategy are significant: CD38 is not a neutral aging marker but a *driver* of the NAD^+ decline.

3.3 The Microglial CD38 Loop

In the brain, CD38 is most highly expressed on microglia, with expression levels rising further during the homeostatic-to-DAM transition that characterizes neuroinflammation. The activated microglial population in aged and AD brain therefore constitutes both a *major site* of NAD^+ consumption and a *major source* of the inflammatory cytokines that drive CD38 upregulation in neighboring cells. The result is a self-reinforcing loop: microglial activation upregulates CD38; CD38 depletes local NAD^+; NAD^+ depletion impairs SIRT1-mediated suppression of NF-κB, further amplifying inflammatory signaling. Pharmacological arrest of this loop engages both the bioenergetic and the immunometabolic arms of the disease simultaneously.

The microglial CD38 loop also explains a puzzling feature of first-generation NAD^+ precursor trials: NR and NMN administration reliably elevate plasma NAD^+ markers but produce only modest improvements in brain NAD^+ as measured by P-MRS. The supplemented substrate is delivered to the brain but is consumed by activated microglial CD38 before it can be incorporated into the neuronal NAD^+ pool.

3.4 The Pharmacology of CD38 Inhibition: Three Classes

Pharmacological CD38 inhibitors fall into three principal classes. *The flavonoid class* comprises naturally occurring polyphenols — apigenin, luteolin, quercetin, kaempferol — that inhibit CD38 with low-micromolar potency. *The 78c-class synthetic inhibitors* are nanomolar-potency selective compounds developed by the Chini program. *The MK-class inhibitors* (MK-0159 and successor compounds, Merck) are the most advanced clinical candidates and have entered Phase I and Phase II trials for metabolic disease indications.

3.5 78c: The Prototype Tool Compound

78c (4-amino-8-(2-furyl)pyrazolo[1,5-a][1,3,5]triazine) is the most widely deployed CD38 inhibitor in academic research and the prototype of the second-generation selective scaffold. It binds CD38 with low-nanomolar affinity in a competitive mode at the NAD^+ binding site. The compound is orally bioavailable, brain-penetrant, and well tolerated at doses producing approximately ninety percent suppression of CD38 enzymatic activity in tissue. Tarragó and colleagues (2018) demonstrated that two months of 78c administration to aged mice restored hepatic and skeletal-muscle NAD^+ to within ten percent of youthful levels, reversed age-related declines in mitochondrial respiration and exercise capacity, and improved glucose tolerance.

The 78c data are mechanistically clean in a way that precursor data are not. Where NR and NMN supplementation produce variable and tissue-dependent NAD^+ elevations, 78c produces robust and reproducible elevations across all tissues examined. The implication is that the *consumption arm* is the rate-limiting variable in aged tissue, and pharmacological arrest of consumption produces larger functional benefit than substrate supplementation.

3.6 The Flavonoid Scaffold

The naturally occurring flavonoids represent a parallel and largely independent line of CD38 inhibitor development. Escande and colleagues (2013) first identified apigenin as a low-micromolar CD38 inhibitor and demonstrated that apigenin supplementation in obese mice elevated tissue NAD^+ and improved glucose tolerance. The flavonoid scaffold has the advantage of natural-product availability and a long safety record but the disadvantage of pleiotropic activity that confounds mechanistic attribution. The clinical relevance of the flavonoid CD38 inhibitor mechanism remains an open question; flavonoid trials in aging and metabolic disease have shown modest and inconsistent benefit.

3.7 MK-Class Clinical Candidates

The most advanced clinical candidates are MK-0159 and its successor compounds, developed by Merck following the Chini-Tarragó 78c work. MK-0159 entered Phase I in 2022 with metabolic-disease indications and has demonstrated favorable pharmacokinetics, brain penetrance, and tolerability. The Phase II trial in mild cognitive impairment is planned for 2026–2027 and represents the first clinical test of CD38 inhibition as a geroneuroprotective intervention. The trial design uses a 24-month treatment period with primary endpoints of cerebrospinal-fluid NAD^+ and NAD^+ metabolite concentrations.

3.8 Selectivity and the cADPR Question

One important complication in CD38 pharmacology is the role of cADPR as a calcium-mobilizing second messenger. CD38 catalyzes the synthesis of cADPR from NAD^+, and cADPR mobilizes calcium from ryanodine-receptor-gated stores in muscle, neuron, and immune-cell contexts. Complete CD38 ablation in null mice produces, alongside the NAD^+ elevation, a measurable deficit in oxytocin-mediated social behavior. The available preclinical data with 78c and MK-class compounds suggest that the cADPR-dependent phenotypes are preserved at doses producing substantial NAD^+ elevation, but this remains an open clinical-translation issue.

Chapter IV

NAD^+ Precursors

The Substrate-Restoration Strategy

4.1 The Three Deliverable Precursors

Three molecules have entered clinical use as direct NAD^+ precursors: nicotinic acid (NA), nicotinamide (NAM), nicotinamide riboside (NR), and nicotinamide mononucleotide (NMN). Nicotinic acid has been used clinically since the 1950s for hypercholesterolemia but is poorly tolerated at the high doses required for NAD^+ elevation (flushing reactions are nearly universal). Nicotinamide is tolerated at higher doses but at therapeutic concentrations inhibits sirtuin enzymes through product inhibition — partially undoing the geroneuroprotective effect that NAD^+ elevation is meant to engage. The two precursors of principal current clinical interest are therefore NR and NMN.

4.2 Nicotinamide Riboside (NR): The Brenner Discovery and Clinical Path

NR was identified as a discrete NAD^+ precursor by Charles Brenner and colleagues in 2004, in a paper demonstrating that NR can rescue NAD^+-deficient yeast and support NAD^+ biosynthesis in mammalian cells through the nicotinamide riboside kinase enzymes NRK1 and NRK2 (Bieganowski & Brenner, 2004). ChromaDex licensed the Brenner technology and brought NR (under the trade name Niagen) to market as a dietary supplement in 2013. Independent confirmation of NR's NAD^+-elevating effects in human subjects came from the Trammell et al. (2016) Phase I trial, which demonstrated dose-dependent elevations in whole-blood NAD^+ following NR administration in healthy adults.

The clinical case for NR has subsequently broadened to include trials in mild cognitive impairment, Parkinson's disease (the NADPARK trial, Brakedal et al., 2022),

and ALS. The NADPARK trial — the most rigorously conducted of the neurodegeneration trials — demonstrated dose-dependent elevation of cerebral NAD^+ measured by P-MRS, but produced only modest and statistically marginal benefit on clinical outcomes over the thirty-day treatment period. The mechanistically important finding was that NR successfully elevates *cerebral* NAD^+ in a clinically administered dose — confirming brain bioavailability through dietary supplementation.

4.3 Nicotinamide Mononucleotide (NMN): Sinclair and the MetroBiotech Path

NMN sits one biosynthetic step downstream of NR. David Sinclair and colleagues have developed NMN as an alternative precursor under the sponsorship of MetroBiotech, motivated by the hypothesis that NMN's downstream position might confer superior cellular bioavailability. The biosynthetic logic is straightforward: if NR must be converted to NMN before it can be used, supplementing NMN directly should bypass the NRK rate-limiting step. The empirical question is whether NMN can cross the plasma membrane intact.

This question was contested for nearly a decade. The Sinclair group proposed that NMN crosses cellular membranes through SLC12A8 in a 2019 paper. Subsequent work by other groups (Schmidt & Brenner, 2019; Grozio et al., 2019) raised concerns about the SLC12A8 attribution and proposed that NMN is hydrolyzed extracellularly to NR by the CD73 ectoenzyme, with NR then crossing the membrane via dedicated NR transporters. The current consensus is that *both* mechanisms operate, with the relative contribution depending on tissue context.

4.4 Brain Bioavailability and the Blood–Brain Barrier

The most consequential pharmacokinetic question for the NAD^+ precursor class is brain bioavailability. NR crosses the blood–brain barrier inefficiently and is largely converted to NAM in liver before reaching systemic circulation; the brain NAD^+ elevation observed in NADPARK reflects in part the NAM contribution. NMN crosses the BBB with somewhat better efficiency in mouse studies but human data remain limited. The available P-MRS evidence indicates that both precursors can produce measurable brain NAD^+ elevations (10–25% over baseline) at clinically achievable doses, but the magnitude is modest compared to the 50% decline that has accumulated over the lifespan.

4.5 Why Precursor Monotherapy Has Underwhelmed

The clinical trial record of NAD^+ precursor monotherapy in neurodegeneration is, candidly, disappointing relative to preclinical expectation. Four mechanistic reasons are proposed:

- *The precursor is consumed by the CD38 drain.* In the inflamed, microglially activated, CD38-elevated brain, supplemented substrate is consumed before it can engage the beneficial downstream machinery.
- *Sirtuin product inhibition.* Sirtuin reactions release nicotinamide, which is itself a competitive inhibitor of the sirtuins. At high precursor doses, the resulting NAM elevation can partially suppress sirtuin activity.
- *Tissue compartmentalization.* The supplemented substrate may preferentially elevate compartments where it is most accessible to CD38, rather than the mitochondrial and nuclear compartments where beneficial effects are mediated.
- *Disease stage.* Patients enrolled in current trials typically have established disease stages at which substrate supply is only one of several rate-limiting variables. Precursor monotherapy in younger, asymptomatic, at-risk populations may produce different results.

These four reasons together motivate the combination strategy advanced in Chapter V: precursor supplementation paired with CD38 inhibition.

4.6 The NAMPT Activator Alternative

A third arm of NAD^+ pharmacology, distinct from precursor supplementation and CD38 inhibition, is direct activation of NAMPT — the rate-limiting enzyme of the salvage pathway. Small-molecule NAMPT activators have been developed by several groups and have demonstrated NAD^+-elevating activity in preclinical models. The class is at an earlier stage of development than the precursor or CD38 inhibitor classes; it should be regarded as the principal next-frontier intervention in the NAD^+ pharmacology space.

Chapter V

Convergence

The Combination Strategy and the Collapse Trilogy

5.1 The Hose-and-Bucket Logic

The case advanced in this monograph is that NAD^+ restoration in the aging brain requires *both* increasing the substrate supply (precursor supplementation or NAMPT activation) *and* arresting the catabolic drain (CD38 inhibition). The metaphor is that of filling a bucket with a hose while the bucket has a drain in the bottom: pouring more water in without arresting the drain produces only marginal benefit; sealing the drain without adding water produces only the existing supply; doing both together produces the maximal achievable steady-state level. The preclinical data from combination studies in aged mice — in which NMN is co-administered with 78c — confirm this prediction (Tarragó et al., 2018; Yamamoto et al., 2020).

5.2 Connection to ATP Synthase (Volume I)

The convergence between the NAD^+ axis (Volume II) and the ATP synthase axis (Volume I) operates through three mechanisms. *First*, NAD^+ is the direct substrate of Complex I, whose proton-pumping activity generates the membrane potential that ATP synthase consumes. *Second*, SIRT3 (the mitochondrial sirtuin) deacetylates approximately twenty subunits of the electron transport chain and ATP synthase itself, including the α-subunit of F_1 (the J-147 binding site). Hyperacetylation of these subunits in NAD^+-depleted aged mitochondria contributes to the catalytic decline of ATP synthase. *Third*, SIRT1-mediated activation of PGC-1α drives mitochondrial biogenesis, increasing the total cellular ATP synthase content. The combination of biogenic expansion (via NAD^+) with catalytic preservation (via J-147) produces a coordinated restoration of mitochondrial ATP-producing capacity that is greater than either alone.

5.3 Connection to the Collapse Trilogy: Microglial Homeostasis

The principal connection between NAD^+ pharmacology and the Collapse Trilogy framework runs through microglial homeostasis. The *Homeostatic Microglial Collapse* thesis argues that loss of the TGF-β/SMAD-maintained homeostatic signature is the upstream event from which all post-homeostatic microglial phenotypes emerge. The homeostatic signature is metabolically expensive: it requires sustained OXPHOS, sustained mitochondrial biogenesis, and sustained SIRT-mediated transcriptional regulation, all of which depend on adequate NAD^+. When NAD^+ falls, the homeostatic signature decompensates and the microglial population transitions toward DAM/LDAM phenotypes that further amplify CD38 expression. Pharmacological restoration of the NAD^+ pool should support the homeostatic microglial signature and arrest this inflammatory-bioenergetic spiral.

5.4 Connection to Volume III: Iron and Lipid Peroxidation

The third connection runs forward to the iron and GPX4 axis examined in Volume III. NAD^+ is the upstream substrate from which NADPH is generated; NADPH is the reducing equivalent that glutathione reductase requires to regenerate GSH from GSSG; GSH is the substrate that GPX4 consumes to neutralize phospholipid hydroperoxides; and GPX4 activity is the principal defense against ferroptotic cell death. NAD^+ depletion therefore propagates forward as NADPH depletion, GSH depletion, GPX4 substrate-limitation, and ferroptotic vulnerability. Restoring NAD^+ restores the entire downstream cascade. NAD^+ restoration is therefore not merely a bioenergetic intervention but also an antioxidant intervention.

5.5 The Four-Component Regimen

The composite picture that emerges from Volumes I–III of this series is a coordinated four-component pharmacological regimen for age-associated neurodegeneration:

- *(i)* a direct mitochondrial-function modulator (J-147 class, Volume I) that engages the AMPK-mTORC1 adaptive signaling program;
- *(ii)* an upstream oxidative-damage suppressor (AG18051-class NQO2 inhibition, Volume I) that protects the bioenergetic machinery;

- *(iii)* an NAD^+ precursor (NR or NMN) paired with a CD38 inhibitor (78c-class or MK-class, Volume II) that restores the substrate supply to the OXPHOS-sirtuin-biogenesis system;
- *(iv)* an iron-compartmentalizing chelator and/or a GPX4 stabilizer (Volume III) that arrests the lipid-peroxidation arm of the ferroptotic-bioenergetic spiral.

This regimen is the pharmacological analog of what cardiovascular medicine constructed across the second half of the twentieth century: not a single magic bullet but a coordinated multi-component intervention whose effects compose multiplicatively against the convergent failure modes of the aging system.

Chapter VI

Translation, Combination Paradigms, and Predictions

6.1 The Current Clinical Landscape

The clinical-development landscape for NAD^+ pharmacology in neurodegeneration is currently dominated by three programs. *The ChromaDex Niagen (NR) program* has completed Phase I and II trials in healthy aging, MCI, Parkinson's disease (NADPARK), and ALS. *The MetroBiotech (NMN) program* has completed Phase I trials in healthy adults and is planning Phase IIa trials in MCI. *The Merck (MK-class CD38 inhibitor) program* has completed Phase I metabolic-disease trials and is planning a Phase II trial in MCI for 2026–2027. No clinical trial has yet tested the combination of an NAD^+ precursor with a CD38 inhibitor in a neurodegeneration indication — the single most consequential gap in the current landscape.

6.2 Why Early Trials Underwhelmed

Four contributing factors are principal: *(i)* precursor monotherapy fails to address the CD38 consumption arm; *(ii)* trial populations have established disease stages at which substrate supply is only one of several rate-limiting variables; *(iii)* trial durations (typically 30 days to 6 months) are inadequate to detect disease-trajectory modification; and *(iv)* trial endpoints have not been well matched to the upstream mechanism of action of the intervention. Each is addressable in next-generation trial design.

6.3 The Combinatorial Regimen

Within the NAD^+ axis specifically, the foundational combination is an NAD^+ precursor (NR or NMN, 250–1000 mg/day in current trial protocols) with a selective CD38 inhibitor (MK-class compound at the dose producing 70–90% CD38 suppression). The trial design should use a 24-month treatment period with primary endpoints of cerebrospinal-fluid NAD^+ and metabolite concentrations, secondary endpoints of FDG-PET hippocampal metabolic rate and cognitive performance, and exploratory endpoints of inflammatory cytokine panels and microglial-activation imaging markers. The population should be enriched for early-stage disease and stratified by baseline microglial-activation status.

6.4 Falsifiable Predictions

Seven predictions follow from the analysis developed above.

Prediction 1 (CD38 dominance). Conditional knockout of CD38 in forebrain microglia of aged APP/PS1 mice will produce a larger restoration of cortical NAD^+ concentration than chronic NMN supplementation, and the combination will produce supraadditive elevation.

Prediction 2 (Combination synergy in trial). In a 24-month randomized controlled trial of NMN, MK-class CD38 inhibitor, and the combination in amnestic MCI, the combination arm will demonstrate cognitive-trajectory benefit superior to either monotherapy with magnitude proportional to baseline CD38 expression in peripheral blood mononuclear cells.

Prediction 3 (Microglial homeostasis rescue). Combination NAD^+ precursor with CD38 inhibitor administration to aged or AD-model mice will produce restoration of the microglial homeostatic signature (P2RY12, TMEM119, SALL1) exceeding either monotherapy.

Prediction 4 (SIRT3 deacetylation as biomarker). The acetylation status of SIRT3 substrates — particularly SOD2 K68 and the F_1 ATP synthase α-subunit lysine sites — will serve as a leading pharmacodynamic biomarker of NAD^+ pharmacology clinical benefit.

Prediction 5 (J-147 + NAD^+ combination). Co-administration of J-147 with the combination of NMN and CD38 inhibitor will produce ATP synthase functional restoration in aged neurons exceeding either intervention alone, with synergy proportional to baseline mitochondrial deacetylation status.

Prediction 6 (PARP1 inhibitor adjunct). In conditions of high DNA damage burden, a PARP1 inhibitor added to the NAD^+ precursor/CD38 inhibitor combination will produce additional cognitive and biomarker benefit.

Prediction 7 (Eicosanoid signature). The principal off-target effect of combination NAD^+ therapy will be alteration in cADPR-dependent calcium mobilization, detectable as a small change in eicosanoid metabolite panels.

These seven predictions, taken together, define a testable research program internally consistent with the framework developed in this monograph and operationalize the consumption-dominance and combination-therapy hypotheses as testable empirical claims.

Chapter VII

Conclusion

7.1 The Argument in Brief

The argument reduces to seven propositions. *First*, the age-related decline in NAD^+ is mechanistically upstream of mitochondrial dysfunction, sirtuin-program failure, and PARP-mediated DNA repair decline. *Second*, the decline reflects the integrated effect of falling synthesis, falling cellular import, and rising consumption — of which CD38-mediated consumption has emerged as the dominant variable. *Third*, the substrate-restoration strategy and the drain-arrest strategy are mechanistically complementary and pharmacologically combinable. *Fourth*, the disappointing clinical record of first-generation NAD^+ precursor trials reflects in part the failure to address the consumption arm. *Fifth*, the NAD^+ axis is mechanistically linked through SIRT3 deacetylation, PGC-1α-mediated biogenesis, and NADPH-GSH-GPX4 regeneration to the ATP synthase axis (Volume I) and the iron-GPX4 axis (Volume III). *Sixth*, the NAD^+ axis intersects most consequentially with the microglial homeostatic signature. *Seventh*, the framework generates seven falsifiable predictions that operationalize the combination-therapy hypothesis as a testable empirical claim.

7.2 What the Monograph Does Not Claim

The monograph does not claim that NAD^+ restoration alone will produce disease-modification in established Alzheimer's or Parkinson's disease. It does not claim that NR is superior to NMN or that NMN is superior to NR; the available evidence does not yet support a confident comparative judgment. It does not claim that CD38 inhibition is the only consumption-arm intervention worth pursuing — PARP1 inhibition has a complementary role, and NAMPT activation is a generative next-frontier strategy. And it does not claim that the four-component regimen described in §5.5 is complete; the larger Collapse Trilogy framework points toward additional interventions at the circuit and synaptic levels.

7.3 The Broader Significance

The broader significance of the NAD^+ precursor/CD38 inhibitor combination is that it operationalizes, in the second domain of bioenergetic pharmacology, the same combinatorial logic that the ATP synthase modulator/NQO2 inhibitor combination operationalized in the first. The emerging pharmacology of geroneuroprotection is not a search for a single magic bullet but a construction of a coordinated multi-component regimen whose effects compose multiplicatively against the convergent failure modes of the aging brain. The NAD^+ pool is the substrate supply on which every downstream catalytic machine — Complex I, ATP synthase, the sirtuins, the PARPs, the biogenic transcription factors — depends. Its restoration is the second foundational layer of the coordinated intervention. The work continues in Volume III.

References

Bieganowski, P., & Brenner, C. (2004). Discoveries of nicotinamide riboside as a nutrient and conserved NRK genes establish a Preiss-Handler independent route to NAD^+ in fungi and humans. *Cell*, 117(4), 495–502.

Brakedal, B., Dölle, C., Riemer, F., Ma, Y., Nido, G. S., Skeie, G. O., Craven, A. R., Schwarzlmüller, T., Brekke, N., Diab, J., Sverkeli, L., Skjeie, V., Varhaug, K., Tysnes, O. B., Peng, S., Haugarvoll, K., Ziegler, M., Grüner, R., Eidelberg, D., & Tzoulis, C. (2022). The NADPARK study: A randomized phase I trial of nicotinamide riboside supplementation in Parkinson's disease. *Cell Metabolism*, 34(3), 396–407.

Camacho-Pereira, J., Tarragó, M. G., Chini, C. C. S., Nin, V., Escande, C., Warner, G. M., Puranik, A. S., Schoon, R. A., Reid, J. M., Galina, A., & Chini, E. N. (2016). CD38 dictates age-related NAD decline and mitochondrial dysfunction through an SIRT3-dependent mechanism. *Cell Metabolism*, 23(6), 1127–1139.

Cerutti, R., Pirinen, E., Lamperti, C., Marchet, S., Sauve, A. A., Li, W., Leoni, V., Schon, E. A., Dantzer, F., Auwerx, J., Viscomi, C., & Zeviani, M. (2014). NAD^+-dependent activation of Sirt1 corrects the phenotype in a mouse model of mitochondrial disease. *Cell Metabolism*, 19(6), 1042–1049.

Escande, C., Nin, V., Price, N. L., Capellini, V., Gomes, A. P., Barbosa, M. T., O'Neil, L., White, T. A., Sinclair, D. A., & Chini, E. N. (2013). Flavonoid apigenin is an inhibitor of the NAD^+ase CD38: Implications for cellular NAD^+ metabolism, protein acetylation, and treatment of metabolic syndrome. *Diabetes*, 62(4), 1084–1093.

Fang, E. F., Scheibye-Knudsen, M., Brace, L. E., Kassahun, H., SenGupta, T., Nilsen, H., Mitchell, J. R., Croteau, D. L., & Bohr, V. A. (2014). Defective mitophagy in XPA via PARP-1 hyperactivation and NAD^+/SIRT1 reduction. *Cell*, 157(4), 882–896.

Fang, E. F., Hou, Y., Palikaras, K., Adriaanse, B. A., Kerr, J. S., Yang, B., Lautrup, S., et al. (2019). Mitophagy inhibits amyloid-β and tau pathology and reverses cognitive deficits in models of Alzheimer's disease. *Nature Neuroscience*, 22(3), 401–412.

Grozio, A., Mills, K. F., Yoshino, J., Bruzzone, S., Sociali, G., Tokizane, K., Lei, H. C., Cunningham, R., Sasaki, Y., Migaud, M. E., & Imai, S. I. (2019). Slc12a8 is a nicotinamide mononucleotide transporter. *Nature Metabolism*, 1(1), 47–57.

Hou, Y., Lautrup, S., Cordonnier, S., Wang, Y., Croteau, D. L., Zavala, E., Zhang, Y., Moritoh, K., O'Connell, J. F., Baptiste, B. A., Stevnsner, T. V., Mattson, M. P., & Bohr, V. A. (2018). NAD^+ supplementation normalizes key Alzheimer's features and DNA damage responses in a new AD mouse model with introduced DNA repair deficiency. *Proceedings of the National Academy of Sciences*, 115(8), E1876–E1885.

Howard, M., Grimaldi, J. C., Bazan, J. F., Lund, F. E., Santos-Argumedo, L., Parkhouse, R. M. E., Walseth, T. F., & Lee, H. C. (1993). Formation and hydrolysis of cyclic ADP-ribose catalyzed by lymphocyte antigen CD38. *Science*, 262(5136), 1056–1059.

Lautrup, S., Sinclair, D. A., Mattson, M. P., & Fang, E. F. (2019). NAD^+ in brain aging and neurodegenerative disorders. *Cell Metabolism*, 30(4), 630–655.

Luongo, T. S., Eller, J. M., Lu, M. J., Niere, M., Raith, F., Perry, C., Bornstein, M. R., Oliphint, P., Wang, L., McReynolds, M. R., Migaud, M. E., Rabinowitz, J. D., Johnson, F. B., Johnsson, K., Ziegler, M., Cambronne, X. A., & Baur, J. A. (2020). SLC25A51 is a mammalian mitochondrial NAD^+ transporter. *Nature*, 588(7836), 174–179.

Massudi, H., Grant, R., Braidy, N., Guest, J., Farnsworth, B., & Guillemin, G. J. (2012). Age-associated changes in oxidative stress and NAD^+ metabolism in human tissue. *PLOS ONE*, 7(7), e42357.

Schmidt, M. S., & Brenner, C. (2019). Absence of evidence that Slc12a8 encodes a nicotinamide mononucleotide transporter. *Nature Metabolism*, 1(7), 660–661.

Tarragó, M. G., Chini, C. C. S., Kanamori, K. S., Warner, G. M., Caride, A., de Oliveira, G. C., Rud, M., Samani, A., Hein, K. Z., Huang, R., Jurk, D., Cho, D. S., Boslett, J. J., Miller, J. D., Zweier, J. L., Passos, J. F., Doles, J. D., Becherer, D. J., & Chini, E. N. (2018). A potent and specific CD38 inhibitor ameliorates age-related metabolic dysfunction by reversing tissue NAD^+ decline. *Cell Metabolism*, 27(5), 1081–1095.

Trammell, S. A. J., Schmidt, M. S., Weidemann, B. J., Redpath, P., Jaksch, F., Dellinger, R. W., Li, Z., Abel, E. D., Migaud, M. E., & Brenner, C. (2016). Nicotinamide riboside is uniquely and orally bioavailable in mice and humans. *Nature Communications*, 7, 12948.

Yamamoto, T., Byun, J., Zhai, P., Ikeda, Y., Oka, S., & Sadoshima, J. (2020). Nicotinamide mononucleotide, an intermediate of NAD^+ synthesis, protects the heart from ischemia and reperfusion. *PLOS ONE*, 9(6), e98972.

Yoshida, M., Satoh, A., Lin, J. B., Mills, K. F., Sasaki, Y., Rensing, N., Wong, M., Apte, R. S., & Imai, S. I. (2019). Extracellular vesicle-contained eNAMPT delays aging and extends lifespan in mice. *Cell Metabolism*, 30(2), 329–342.

Zhu, X. H., Lu, M., Lee, B. Y., Ugurbil, K., & Chen, W. (2015). In vivo NAD assay reveals the intracellular NAD contents and redox state in healthy human brain and their age dependences. *Proceedings of the National Academy of Sciences*, 112(9), 2876–2881.