S K O D A R E S E A R C H H U B

White Paper | Cellular Longevity Series | Pillar 4 — Precision Supplementation

NAD+ and the Biology of Aging:

The Coenzyme at the Center of Cellular Longevity, and a Tripartite Delivery Protocol for Maximum Bioavailability

NMN Supplementation • Transdermal Patch • Subcutaneous Injection

Plus: TMG as the Essential Methyl Donor for Sustained NAD+ Optimization

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1. Executive Summary

Nicotinamide Adenine Dinucleotide — NAD^+ — is not a supplement trend. It is a coenzyme so fundamental to cellular life that without it, no human cell can generate energy, repair its DNA, regulate its gene expression, or defend itself against oxidative damage. It is, in the most literal biochemical sense, the molecule of life. And by age 70, most humans have lost 50-60% of the NAD^+ levels they carried at age 20 — a decline now understood to be a primary driver of the aging phenotype itself, not merely a correlate of it.

This white paper makes the scholarly case for NAD^+ restoration as one of the most scientifically grounded interventions in the longevity toolkit — and documents a specific tripartite delivery protocol deployed by a 71-year-old male subject who has already achieved a documented biological age reversal of approximately 15 years through the broader Skoda Longevity Protocol. The addition of a three-channel NAD^+ optimization system — oral NMN supplementation, transdermal patch delivery, and subcutaneous injection — represents the most bioavailability-optimized approach currently achievable outside of clinical infusion settings.

The NAD^+ Tripartite Protocol — Overview

CHANNEL 1 — Oral NMN (Nicotinamide Mononucleotide): Immediate NAD^+ precursor; oral bioavailability; sustained baseline elevation; foundational layer of the protocol.
CHANNEL 2 — Transdermal NAD^+ Patch: Bypasses first-pass hepatic metabolism; delivers NAD^+ directly through the dermis into systemic circulation; sustained slow-release delivery profile.
CHANNEL 3 — Subcutaneous NAD^+ Injection (initiated ~15 days ago): Direct delivery of NAD^+ into subcutaneous tissue for rapid systemic absorption; bypasses all gastrointestinal and hepatic barriers; the highest bioavailability non-IV delivery route available.
METHYL SUPPORT — TMG (Trimethylglycine / Betaine): Essential methyl donor that prevents NAD^+ precursor metabolism from depleting SAM (S-adenosylmethionine) pools; protects the methylation cycle; converts homocysteine to methionine; a non-negotiable co-factor for sustained NAD^+ supplementation.
PROTOCOL STATUS: NMN and patch established; subcutaneous injections initiated approximately 15 days prior to this publication; full tripartite protocol now active.

The scientific rationale for prioritizing NAD^+ — specifically in injectable form — above other longevity-oriented injectables and peptides is grounded in a fundamental biological distinction: NAD^+ is not a pharmacological agent. It is a coenzyme. It is a molecule the body already makes, already uses in every cell, and already depends on for survival. Restoring what time has depleted is categorically different from introducing an exogenous pharmaceutical signal. This distinction carries profound implications for both safety and mechanism of action.

2. The Biochemistry of NAD+: What It Is and Why It Matters

2.1 NAD+ as Coenzyme — Not Peptide, Not Drug

The biochemical identity of NAD+ deserves precise clarification, because the category distinction matters enormously for understanding both its safety profile and its mechanism of action. NAD+ is a coenzyme — a small organic molecule that must bind to an enzyme to enable that enzyme to catalyze a biochemical reaction. It is not a hormone. It is not a peptide. It is not a synthetic pharmaceutical with a novel mechanism introduced into the body from outside.

Category	Definition	Examples	Mechanism of Action	NAD+ Comparison
Hormone	Signaling molecule produced by endocrine glands; acts on distant target cells via receptors	Testosterone, estradiol, cortisol, insulin	Binds cell surface or nuclear receptors; triggers gene expression changes	NAD+ is not a hormone — it does not signal; it participates directly in enzymatic reactions
Peptide / Peptide Therapy	Short chain of amino acids; acts as signaling molecules or growth factors	BPC-157, TB-500, GHK-Cu, ipamorelin, CJC-1295	Bind specific receptors; modulate growth factor, inflammatory, or repair pathways	NAD+ is not a peptide — it has no amino acid structure and no receptor-binding signaling function
Pharmaceutical Drug	Synthetic or natural compound introduced to produce a specific pharmacological effect	Statins, metformin, SSRIs, GLP-1 agonists	Inhibit enzymes, block receptors, alter neurotransmitter reuptake; exogenous mechanism	NAD+ is not a drug — it is endogenous; the body synthesizes it and depends on it for baseline function
Coenzyme (NAD+)	An organic non-protein molecule that binds to enzymes and is essential for their catalytic activity	NAD+, NADH, FAD, CoA, ATP	Accepts and donates electrons in redox reactions; activates dehydrogenase, ligase, and transferase enzymes	NAD+ IS a coenzyme — the body's own molecule, depleted by aging, restored by supplementation

2.2 The Molecular Architecture of NAD+

NAD+ (Nicotinamide Adenine Dinucleotide, oxidized form) is a dinucleotide — two nucleotides joined by a phosphate bridge. Its molecular structure consists of an adenine nucleotide and a nicotinamide nucleotide linked through their 5'-phosphate groups. This structure enables NAD+ to function as an electron carrier: the nicotinamide ring accepts a hydride ion (H^-, one proton

and two electrons) to become NADH (reduced form), and this reversible redox cycling is the fundamental currency of cellular energy metabolism.

What makes NAD+ uniquely central to biology — rather than simply one of many metabolic cofactors — is the extraordinary breadth of enzyme families that depend on it. NAD+ is not a specialist molecule serving a single pathway. It is a universal currency operating across energy metabolism, genome maintenance, immune regulation, and cellular stress response simultaneously:

Enzyme Family	NAD+ Role	Biological Function	Aging / Disease Relevance
Dehydrogenases (>400 enzymes)	Electron acceptor — converts NAD+ to NADH	ATP synthesis via glycolysis, Krebs cycle, beta-oxidation of fatty acids	Declining NAD+ directly reduces cellular energy production — the basis of age-related fatigue and metabolic decline
Sirtuins (SIRT1-7)	NAD+ is consumed as substrate (not just cofactor)	Deacetylation of histones and proteins; gene silencing; DNA repair; mitochondrial biogenesis; inflammation regulation	Sirtuins are the primary longevity enzymes; low NAD+ = sirtuin inactivity = accelerated aging phenotype
PARP enzymes (PARP1-17)	NAD+ consumed to synthesize poly-ADP-ribose chains	DNA strand break repair; genome stability maintenance; chromatin remodeling	PARP activation during DNA damage depletes NAD+; competition with sirtuins for NAD+ is a central aging mechanism
CD38 / CD157 (NADases)	Hydrolyze NAD+ to produce cyclic ADP-ribose	Calcium signaling; immune cell activation; inflammatory response	CD38 expression increases with aging and inflammation — a major driver of NAD+ decline; the 'CD38 problem'
NMNAT enzymes	Synthesize NAD+ from NMN (final biosynthetic step)	NAD+ production; axonal integrity; neuroprotection	NMNAT2 decline in neurons contributes to neurodegeneration; NAD+ precursors bypass NMNAT bottleneck

2.3 The NAD+ Biosynthetic Pathways

The body synthesizes NAD+ through three primary routes, each with distinct efficiency and regulatory characteristics. Understanding these pathways is essential for appreciating why external supplementation is necessary and why precursor selection matters:

The Three NAD+ Biosynthetic Pathways

PATHWAY 1 — DE NOVO SYNTHESIS (from tryptophan): The complete biosynthesis of NAD^+ from dietary tryptophan through the kynurenine pathway. An 8-step enzymatic process producing one NAD^+ molecule per tryptophan molecule consumed. Highly inefficient; provides baseline but insufficient for therapeutic restoration. This pathway declines significantly with age.
PATHWAY 2 — PREISS-HANDLER PATHWAY (from nicotinic acid / niacin): Conversion of niacin (vitamin B3) through NaMN and NaAD to NAD^+. More efficient than de novo synthesis; historically the primary dietary NAD^+ support mechanism. Limited by niacin availability and flushing side effects at therapeutic doses.
PATHWAY 3 — SALVAGE PATHWAY (from nicotinamide / NMN / NR): The most efficient pathway; recycles nicotinamide back to NAD^+ via NAMPT (the rate-limiting enzyme). NMN and NR (nicotinamide riboside) enter directly into this pathway. NAMPT activity declines with age — the primary bottleneck. Supplemental NMN bypasses the NAMPT bottleneck by entering downstream as a direct NAD^+ precursor.
SUPPLEMENTATION LOGIC: NMN supplementation feeds directly into the salvage pathway's most efficient segment, bypassing the NAMPT rate-limiting step and converting to NAD^+ via NMNAT enzymes in a single reaction. This is why NMN is the preferred precursor for therapeutic NAD^+ restoration.

3. The NAD+ Aging Decline: Mechanisms and Consequences

3.1 The Quantified Decline

NAD+ levels decline with age in a pattern that is now well-documented across multiple tissues and species. The magnitude of this decline is not subtle — it is a 40-60% reduction in circulating and intracellular NAD+ between young adulthood and the seventh decade. This is not normal metabolic variation. It is a clinically significant depletion of a molecule on which every cell depends for fundamental function:

50-60%	~71	>400	3
NAD+ decline from age 20 to age 70	Subject age — at peak depletion risk	Enzymes requiring NAD+ for function	Delivery channels in tripartite protocol

Decade	Approximate NAD+ Level (Relative to Age 20 = 100%)	Primary Depletion Drivers	Clinical Manifestations
20s	100% (reference)	Baseline production; minimal PARP/CD38 activity	Peak energy, DNA repair, cognitive function
30s	~85-90%	Mild increase in oxidative stress; early PARP demand	Subtle fatigue patterns; first metabolic efficiency reduction
40s	~70-75%	Increasing DNA damage burden; rising CD38 expression	Noticeable fatigue; metabolic slowing; early mitochondrial decline
50s	~60-65%	Accelerating sirtuin competition; NAMPT decline	Significant mitochondrial dysfunction; insulin resistance trends; cognitive fog
60s	~45-55%	High CD38 activity; chronic inflammation (inflammaging); PARP saturation	Sarcopenia acceleration; cardiovascular risk; sleep architecture disruption
70s (Current)	~40-50%	Peak depletion; multiple competing NAD+ consumers; minimal de novo synthesis	Full aging phenotype; all downstream sirtuin and PARP pathways compromised

3.2 The Four Mechanisms of Age-Related NAD+ Decline

3.2.1 The CD38 Problem — Inflammation as a NAD+ Consumer

CD38 is a transmembrane glycoprotein and NADase enzyme — meaning its primary function is to break down NAD+. CD38 expression increases dramatically with aging, driven by chronic low-grade inflammation (the 'inflammaging' phenotype). In older adults, CD38 activity can account for the majority of NAD+ degradation, overwhelming the salvage pathway's capacity to replenish what is consumed.

Research by Camacho-Pereira et al. (Cell Metabolism, 2016) identified CD38 as a primary driver of the NAD+ decline in aging tissues. In mice, CD38 knockout prevented the age-related NAD+ decline entirely — a powerful demonstration that inflammation-driven NAD+ consumption, not reduced production, may be the dominant mechanism. Apigenin (a flavonoid) and quercetin are CD38 inhibitors that can complement NAD+ restoration protocols.

3.2.2 NAMPT Decline — The Rate-Limiting Bottleneck

NAMPT (Nicotinamide Phosphoribosyltransferase) is the rate-limiting enzyme of the NAD+ salvage pathway — the enzymatic gateway through which nicotinamide is recycled back to NMN and then to NAD+. NAMPT activity declines with age in most tissues, creating a biosynthetic bottleneck that restricts NAD+ replenishment even when precursor substrates are available. NMN supplementation strategically bypasses this bottleneck by entering the pathway downstream of NAMPT, at the NMN-to-NAD+ conversion step.

3.2.3 PARP Competition — DNA Damage as a NAD+ Drain

PARP enzymes (Poly-ADP-Ribose Polymerases) repair DNA strand breaks by consuming NAD+ to synthesize poly-ADP-ribose chains that recruit repair machinery to damage sites. This is an essential protective function — but it directly competes with sirtuins for the available NAD+ pool. As DNA damage accumulates with age (from oxidative stress, UV exposure, metabolic byproducts), PARP activation increases, depleting NAD+ and leaving sirtuins substrate-starved. The sirtuin-PARP competition for NAD+ is one of the most important molecular dynamics in the aging process.

3.2.4 Mitochondrial Dysfunction — The Vicious Cycle

Mitochondrial dysfunction reduces NAD+ production (the electron transport chain generates NADH which is oxidized back to NAD+); and NAD+ depletion reduces SIRT3 activity (the primary mitochondrial sirtuin), which in turn accelerates mitochondrial dysfunction. This creates a self-reinforcing spiral: less NAD+ → less SIRT3 activity → more mitochondrial dysfunction → less NAD+ recycling → further depletion. Breaking this cycle via NAD+ precursor supplementation is one of the most mechanistically compelling rationales for the intervention.

3.3 The Downstream Consequences: What NAD+ Depletion Actually Does

Biological System	NAD+ Depletion Effect	Clinical Manifestation	Restoration Benefit

Mitochondrial Function	SIRT3 inactivation; Complex I/III dysfunction; reduced ATP synthesis	Fatigue; exercise intolerance; metabolic inefficiency	Restored SIRT3 activity; improved mitochondrial biogenesis; Zone 2 cardio synergy
DNA Repair (PARP/SIRT1)	Reduced PARP1 capacity; SIRT1 inactivity; genomic instability accumulation	Accelerated cellular aging; increased cancer risk; epigenetic dysregulation	Restored PARP and SIRT1 function; improved genome maintenance; epigenetic rejuvenation
Insulin Sensitivity	SIRT1 deacetylation of IRS-1 impaired; reduced GLUT4 expression; mitochondrial glucose oxidation reduced	Insulin resistance; T2DM progression; metabolic syndrome	Improved insulin signaling; synergistic with resistance training GLUT4 upregulation
Neurological Function	SIRT1/SIRT3 in neurons impaired; NAD^+-dependent axonal maintenance reduced; neuroinflammation elevated	Cognitive decline; memory impairment; increased neurodegeneration risk	NMNAT-dependent axonal protection; SIRT1 neuroprotection; cognitive function support
Cardiovascular System	Endothelial SIRT1 activity reduced; vascular inflammation; reduced NO production	Endothelial dysfunction; arterial stiffening; cardiovascular risk elevation	Restored endothelial SIRT1; improved NO signaling; vascular health support
Skin and Dermal Integrity	NAD^+-dependent PARP1 impaired in keratinocytes; SIRT1 reduced in fibroblasts; DNA repair impaired	Accelerated skin aging; wrinkle formation; reduced collagen synthesis; UV damage accumulation	Restored keratinocyte DNA repair; SIRT1-driven collagen support; anti-photoaging effect
Immune Function	$CD38^+$ immune cells over-activate; NAD^+-dependent immune regulation impaired	Chronic inflammation (inflammaging); impaired adaptive immunity; delayed wound healing	NAD^+ restoration modulates CD38 activity; supports regulatory T-cell function; reduces inflammaging
Sleep Architecture	SIRT1 circadian clock regulation impaired; NAD^+ oscillation pattern disrupted	Disrupted circadian rhythm; poor sleep quality; reduced slow-wave sleep (SWS)	Restored SIRT1 circadian function; improved sleep architecture; synergy with sleep optimization pillar

4. Delivery Modalities: Why Injection Is the Preeminent Route

4.1 The Bioavailability Problem

NAD+ biology is well established. The more complex clinical question has been delivery: how do you meaningfully elevate intracellular NAD+ levels through supplementation? The answer is not as simple as oral administration of NAD+ itself, because NAD+ is a large, charged molecule that does not cross cell membranes intact — it must be either transported by specific carriers or converted to precursors that can enter cells and be resynthesized. This creates a hierarchy of delivery approaches, each with distinct bioavailability characteristics:

Delivery Route	Bioavailability	Mechanism	Onset	Duration	Protocol Role
Oral NAD+ (direct)	Very low — degrades in GI tract; minimal intact absorption; first-pass hepatic metabolism	Largely converted to nicotinamide before reaching target tissues	Slow	Limited	Not used — superseded by precursor approach
Oral NMN	Moderate — intact absorption demonstrated in human trials (Yoshino et al. 2021); enters directly as NMN in plasma	Absorbed via intestinal Slc12a8 transporter; circulates as NMN; intracellular conversion to NAD+ via NMNAT	1-2 hours	6-8 hours	CHANNEL 1 — Foundational baseline layer
Oral NR (Nicotinamide Riboside)	Moderate — but converted to nicotinamide in plasma before intracellular conversion	Less efficient than NMN for direct precursor delivery; additional conversion step	1-2 hours	6-8 hours	Alternative to NMN; not used in current protocol
Transdermal Patch	Moderate-High — bypasses GI and hepatic first-pass; sustained systemic delivery through dermis	Lipid-soluble carrier systems enable dermal penetration; slow sustained release into circulation	2-4 hours	12-18 hours	CHANNEL 2 — Sustained slow-release layer; complements oral NMN peaks

Subcutaneous Injection	High — direct delivery to subcutaneous tissue; rapid vascular absorption; no GI or hepatic barrier	Subcutaneous tissue is highly vascularized; NAD+ or NMN absorbed directly into systemic circulation	15-30 minutes	Rapid peak; 4-6 hours	CHANNEL 3 — Highest bioavailability non-IV route; most direct systemic delivery
IV Infusion (clinical)	Very High — 100% systemic delivery; used in clinical NAD+ therapy protocols	Direct intravenous NAD+; requires clinical setting; 2-4 hour infusion typically	Immediate	4-8 hours	Clinical gold standard; impractical for daily protocol; subcutaneous injection approximates benefit

4.2 The Coenzyme Argument: Why NAD+ Injection Is Categorically Different

When discussing injectable longevity interventions, NAD+ occupies a unique position in the hierarchy. The distinction is not merely pharmacological — it is categorical. Consider the contrast with other injectable longevity agents:

NAD+ vs. Other Injectable Longevity Agents: The Categorical Difference

PEPTIDE THERAPIES (BPC-157, TB-500, ipamorelin, CJC-1295, etc.): Synthetic or semi-synthetic peptides that act as signaling molecules — binding specific receptors to trigger growth factor release, tissue repair pathways, or anabolic hormone secretion. They introduce a novel pharmacological signal. The body does not normally circulate these molecules at therapeutic concentrations. They work by telling the body to do something it would otherwise do less of.

GLP-1 AGONISTS (semaglutide, tirzepatide): Receptor agonists that mimic incretin hormone signaling. Pharmacological mimics of endogenous hormones at supraphysiological receptor activation levels.

NAD+ INJECTION: Delivers a molecule the body already produces, already uses in every cell, and already depends on for fundamental enzymatic function. It does not introduce a novel signal. It does not activate a receptor. It restores a substrate that has been depleted. The body's own enzymatic machinery uses it immediately and precisely as evolution designed.

THE SAFETY IMPLICATION: Because NAD+ is endogenous and is consumed by existing enzymatic pathways rather than activating novel receptors, the safety profile is fundamentally different from pharmacological injectables. There is no receptor saturation, no agonist tolerance, no downstream signaling dysregulation. Excess NAD+ is metabolized by the same pathways that normally process it.

THE EFFICACY IMPLICATION: Restoring an endogenous coenzyme to levels present in young adulthood enables the body's own sirtuin, PARP, and metabolic enzymes to function at their designed capacity — rather than in the substrate-starved state of NAD+ depletion. This is precision restoration, not pharmacological override.

4.3 The Subcutaneous Route: Pharmacokinetic Rationale

Subcutaneous injection delivers NAD^+ (or NMN) into the well-vascularized subcutaneous adipose tissue, from which it is absorbed into the systemic circulation without passing through the gastrointestinal mucosa or undergoing first-pass hepatic metabolism. This pharmacokinetic profile offers several specific advantages over oral delivery:

- Bypasses intestinal degradation: Oral NAD^+ and NMN are subject to enzymatic degradation in the intestinal lumen and mucosal cells. Subcutaneous delivery circumvents this degradation entirely, delivering the intact molecule to systemic circulation.
- Bypasses hepatic first-pass metabolism: Oral compounds absorbed from the gastrointestinal tract pass through the portal circulation and liver before reaching systemic circulation — where significant metabolic conversion can occur. Subcutaneous delivery enters the systemic venous circulation directly.
- Rapid onset: Subcutaneous tissue is highly vascularized, and molecular absorption into capillary beds typically occurs within 15-30 minutes — significantly faster than oral delivery's 1-2 hour peak.
- Dose precision: Unlike oral delivery where intestinal absorption efficiency varies with food intake, hydration, and gut motility, subcutaneous injection delivers a precise, consistent dose with predictable absorption kinetics.
- Daily protocol compatibility: Subcutaneous self-injection is the established route for insulin administration in diabetics worldwide — a technique that is safe, learnable, and compatible with a daily protocol. The subject's prior experience with insulin self-administration provides direct procedural familiarity.
- Approximates IV benefit: While IV infusion provides 100% systemic bioavailability instantly, subcutaneous delivery achieves a high percentage of this benefit with the practical advantage of self-administration in a non-clinical setting.

5. The Skoda NAD+ Tripartite Protocol: Design and Implementation

5.1 Protocol Architecture: Three Channels, One Objective

The tripartite protocol is designed around a single pharmacokinetic objective: maintain elevated systemic and intracellular NAD+ levels continuously across the full 24-hour cycle. No single delivery channel achieves this objective alone. Each channel occupies a distinct pharmacokinetic niche that, in combination, creates a sustained elevation profile without the peaks and valleys of any single-channel approach:

The 24-Hour NAD+ Coverage Model

MORNING — Subcutaneous Injection (daily): Rapid onset (15-30 min); creates the day's peak NAD+ elevation; directly bioavailable; sets the intracellular NAD+ tone for peak morning cognitive and metabolic function. Duration: 4-6 hours primary effect.

SUSTAINED — Transdermal Patch: Applied once daily; slow-release dermal absorption over 12-18 hours; maintains baseline elevation between injection peaks and overnight; prevents the trough that would follow injection alone.

FOUNDATIONAL — Oral NMN: Taken daily; moderate bioavailability but consistent precursor delivery; contributes to the steady-state NAD+ pool; feeds the salvage pathway continuously; provides tissue-level precursor availability that complements systemic delivery from injection and patch.

OVERNIGHT — Patch continuation: The transdermal patch's 12-18 hour release profile covers the overnight period — when SIRT1-mediated circadian regulation, DNA repair, and growth hormone-driven cellular restoration are at their highest activity and NAD+ demand is significant.

NET RESULT: No 24-hour window without meaningful NAD+ elevation. The tripartite system creates a pharmacokinetic floor below which NAD+ levels cannot drop significantly — approximating the sustained NAD+ availability of young adulthood.

5.2 Protocol Parameters: Current Implementation

Channel	Agent	Form	Dose	Timing	Route	Status	Primary Mechanism
Channel 1	NMN (Nicotinamide Mononucleotide)	Oral capsule / powder	Per product protocol	Morning, fasted or with light meal	Oral	Active — established	Direct NAD+ precursor via salvage pathway; Slc12a8 intestinal absorption
Channel 2	NAD+	Transdermal patch	Per patch specification	Applied daily; 12-18h wear	Transdermal (skin)	Active — established	Bypasses GI dermal absorption into systemic circulation; sustained slow release

Channel 3	NAD+	Subcutaneous injection	Per protocol dosing	Daily — morning preferred	Subcutaneous injection	Active — initiated ~15 days ago	Highest non-IV bioavailability direct systemic absorption; immediate peak elevation
Co-factor	TMG (Trimethylglycine)	Oral capsule	1-3g daily typical	With NMN — morning	Oral	Active — essential co-protocol	Methyl donor protects SAM pool; supports methylation cycle; homocystein clearance

5.3 Protocol Initiation Sequence and Rationale

The sequential initiation of the three channels — rather than simultaneous introduction — reflects a deliberate protocol design philosophy grounded in both safety monitoring and pharmacological logic:

- Phase 1 (NMN + TMG established first): Oral NMN with TMG was the foundational layer — lowest risk profile, allows the body to adapt to elevated NAD+ precursor availability, establishes the salvage pathway's operating efficiency before higher-bioavailability channels are added.
- Phase 2 (Transdermal patch added): The patch extends the coverage profile, adding sustained systemic delivery that oral NMN's 6-8 hour peak cannot provide. Additive effect is confirmable through subjective energy and cognitive metrics before the injection phase introduces a confounding variable.
- Phase 3 (Subcutaneous injection initiated ~15 days ago): The highest-bioavailability channel is introduced last — allowing the foundational protocol to be established and any individual response patterns to be characterized before adding the most potent delivery mechanism. The injection represents the current leading edge of the protocol; its full adaptive effects will be assessable at the June 15, 2026 benchmark.
- TMG as non-negotiable concurrent initiation: TMG was initiated simultaneously with NMN and has remained continuous throughout all three phases. This is not optional — it is a metabolic necessity (see Section 6).

5.4 Integration with the Broader Seven-Pillar Protocol

NAD+ optimization does not operate in isolation — it functions as a molecular amplifier for several other pillars of the Skoda Protocol, creating synergies that exceed what either intervention produces independently:

Protocol Pillar	NAD+ Synergy Mechanism	Combined Effect
Pillar 1: Intermittent Fasting (18h)	Fasting activates SIRT1 and AMPK; both require adequate NAD+ for full activity; fasting also upregulates NAMPT expression	Fasting-induced SIRT1 activation is amplified by restored NAD+ substrate availability — the two interventions are mechanistically co-dependent
Pillar 2: Precision Nutrition	Protein adequacy supports tryptophan availability for de novo NAD+ synthesis; anti-inflammatory diet reduces CD38-driving inflammaging	Dietary anti-inflammation reduces the primary consumer (CD38) of NAD+; nutrition supports both production and conservation
Pillar 3: Resistance Training	Exercise activates SIRT1, SIRT3, and AMPK — all NAD+-dependent; SIRT3 drives mitochondrial biogenesis post-resistance training	Resistance training creates the demand for NAD+-dependent sirtuin activation; supplementation ensures the substrate is available to meet that demand
Pillar 3: Zone 2 Cardio	Zone 2 drives PGC-1alpha (mitochondrial biogenesis master regulator); PGC-1alpha requires SIRT1 deacetylation for activation; SIRT1 requires NAD+	The mitochondrial biogenesis signal from Zone 2 cardio is completed by SIRT1; NAD+ restoration ensures SIRT1 can respond to the Zone 2 stimulus fully
Pillar 7: Cold Hydrotherapy	Cold activates SIRT3 in mitochondria and upregulates PGC-1alpha via a partially NAD+-dependent pathway	Cold-induced mitochondrial adaptation is enhanced by NAD+ availability; thermogenic BAT activation is supported by SIRT3 activity
Sleep Optimization	SIRT1 regulates BMAL1 and CLOCK — the core circadian clock proteins; circadian NAD+ oscillations coordinate metabolic timing	NAD+ restoration re-synchronizes the circadian NAD+ rhythm, improving sleep architecture and the metabolic benefits of circadian alignment

6. TMG: The Essential Methyl Donor

6.1 Why NAD+ Supplementation Creates a Methylation Demand

The biochemical relationship between NAD+ precursor supplementation and methylation metabolism is among the most important — and most frequently overlooked — considerations in NAD+ protocol design. Understanding it requires a brief exposition of one-carbon metabolism:

When NMN is converted to NAD+ and then consumed by sirtuins (SIRT1-7), the primary byproduct is nicotinamide. Nicotinamide is recycled back to NMN via the salvage pathway — but a competing pathway methylates nicotinamide to form methylnicotinamide, which is then excreted. This methylation reaction consumes SAM (S-adenosylmethionine) — the body's universal methyl donor. At high NAD+ supplementation doses, the demand for SAM to methylate nicotinamide byproducts can deplete the SAM pool, creating downstream methylation deficits that affect:

- DNA methylation: SAM is required for epigenetic methylation of cytosine residues — critical for gene silencing and genome stability
- Neurotransmitter synthesis: SAM methylates precursors for dopamine, serotonin, and norepinephrine synthesis
- Homocysteine clearance: SAM-dependent methylation of homocysteine to methionine; SAM depletion allows homocysteine to accumulate — a cardiovascular risk factor
- Myelin synthesis: SAM-dependent methylation of phosphatidylethanolamine to phosphatidylcholine supports neurological myelin integrity
- Creatine synthesis: SAM provides the methyl group for creatine biosynthesis — relevant to the resistance training component of the protocol

6.2 TMG as the Solution: Mechanism of Action

How TMG Protects the Methylation Cycle During NAD+ Supplementation

STEP 1 — TMG donates a methyl group: Trimethylglycine (betaine) directly donates one of its three methyl groups in the BHMT (betaine-homocysteine methyltransferase) reaction, converting homocysteine to methionine.

STEP 2 — Methionine regenerates SAM: Methionine is converted to SAM (S-adenosylmethionine) by the enzyme MAT (methionine adenosyltransferase). SAM is then available for all downstream methylation reactions.

STEP 3 — SAM pool is preserved: By providing an alternative methyl donor pathway that does not compete with the SAM pool, TMG ensures that high NAD+ turnover does not deplete the methylation currency required for all other methylation-dependent processes.

STEP 4 — Homocysteine is managed: TMG simultaneously reduces homocysteine accumulation — the primary cardiovascular risk factor associated with methylation cycle disruption. This is particularly relevant given the subject's active cardiovascular risk management protocol (pravastatin, Omega-3).

NET RESULT: TMG acts as both a methyl donor and a homocysteine-clearing agent simultaneously — making it the ideal co-supplement for any serious NAD+ supplementation protocol.

6.3 TMG Protocol Specifications

Parameter	Specification	Evidence Basis
Dose	1-3g daily; typically 500mg-1g per gram of NMN	Effective homocysteine reduction documented at 1.5-6g/day in clinical trials; lower doses sufficient as methyl donor support
Timing	Concurrent with NMN — morning	Synchronizes methyl donor availability with peak NAD+ precursor metabolism
Form	Oral capsule or powder (TMG / Betaine anhydrous)	Betaine anhydrous is the anhydrous form — more concentrated than betaine HCl; equivalent efficacy
Primary function in protocol	Methyl donor; SAM pool protection; homocysteine clearance	BHMT pathway; methionine cycle; direct cardiovascular benefit
Secondary benefits	Osmolyte (cellular hydration support); potential performance benefits; liver health (hepatoprotective)	Betaine supplementation associated with improved body composition, power output, and liver fat reduction in clinical studies
Interaction considerations	Compatible with all current supplements; no significant contraindications identified	No interaction with pravastatin, DIM, CDG, Omega-3, or other current protocol supplements

6.4 TMG and Cardiovascular Relevance

The cardiovascular significance of TMG in the context of the Skoda Protocol extends beyond its role as a methyl donor for NAD+ supplementation. Elevated homocysteine is an independent cardiovascular risk factor — associated with endothelial damage, arterial stiffness, and thrombosis risk. Given that the subject's cardiovascular management protocol already includes pravastatin (LDL management) and Omega-3 (triglycerides, anti-inflammatory), the addition of TMG-mediated homocysteine clearance represents a logical and additive cardiovascular protective measure. The June 15 SiPhox panel will assess homocysteine as part of the 57-biomarker evaluation — providing the first quantitative confirmation of TMG's effect on this marker.

7. NAD+ Restoration: The Multi-System Benefit Profile

7.1 Mitochondrial Restoration — The Energy Foundation

The mitochondrial benefit of NAD+ restoration is the most mechanistically direct of all the system-level effects. SIRT3 — the primary mitochondrial sirtuin and the key regulator of mitochondrial enzyme activity — is completely dependent on NAD+ for function. In the NAD+-depleted state of a 70-year-old, SIRT3 is functionally impaired regardless of the mitochondrial protein machinery's structural integrity. Restoring NAD+ restores SIRT3 activity, which in turn restores mitochondrial enzyme acetylation status, improving the function of Complex I (NADH dehydrogenase), Complex II, and the ATP synthase.

The synergy with Zone 2 cardiovascular training here is precise and mechanistically elegant: Zone 2 cardio activates PGC-1alpha, which drives mitochondrial biogenesis (new mitochondria). NAD+ restoration activates SIRT3, which optimizes the function of the existing and newly created mitochondria. Together, they address both the quantity and quality dimensions of mitochondrial health simultaneously.

7.2 Genomic Stability and Anti-Aging — The SIRT1/PARP Axis

Perhaps the most profound longevity benefit of NAD+ restoration operates at the level of genome maintenance. SIRT1 and the PARP enzymes collectively manage the body's DNA repair capacity — and both are NAD+-dependent. As NAD+ declines with age, genome instability accumulates: epigenetic marks drift from their youthful patterns (a process David Sinclair identifies as the primary mechanism of aging itself in the 'Information Theory of Aging'), and DNA damage accrues faster than it is repaired.

Sinclair's foundational research at Harvard demonstrates that restoring NAD+ levels in aged mice via NMN supplementation produces measurable epigenetic rejuvenation — reversal of the epigenetic clock — along with restoration of vascular function, muscle endurance, and DNA repair capacity. While the degree to which these findings translate to humans remains an active research question, the mechanistic basis is compelling and the human trial data is accumulating consistently in the same direction.

7.3 Skin Health and Dermal Restoration

The dermatological benefits of NAD+ restoration represent one of the most visually appreciable and mechanistically well-understood aspects of the intervention. The skin is among the most NAD+-demanding organs — continuously exposed to UV radiation that drives PARP activation for DNA repair, and dependent on SIRT1 for collagen gene expression and fibroblast function:

- UV damage repair: PARP1 activity in keratinocytes (skin cells) is among the highest in the body due to continuous UV exposure. NAD+ restoration directly enables this repair capacity, reducing the accumulation of UV-induced DNA damage that drives photoaging.

- Collagen synthesis support: SIRT1 in dermal fibroblasts regulates collagen gene expression. NAD+-restored SIRT1 activity supports the collagen synthesis machinery that maintains skin structural integrity and reduces wrinkle depth.
- Barrier function: NAD+ supports the ceramide synthesis pathway in epidermal cells, maintaining the skin barrier that prevents transepidermal water loss and pathogen entry.
- Anti-inflammatory effect: Skin inflammaging — the chronic low-grade dermal inflammation that accelerates visible aging — is partly driven by NAD+-deficient immune cells in the dermis. Restoration modulates this inflammatory environment.
- Transdermal delivery clinical observation: The patch delivery channel creates a local, high-concentration NAD+ environment in the dermis directly at the delivery site — potentially providing localized dermal SIRT1 and PARP activation at the skin level, in addition to systemic effects.

7.4 Cognitive Function and Neuroprotection

The neurological stakes of NAD+ depletion are among the most clinically significant for the senior demographic. The brain is extraordinarily metabolically active — consuming approximately 20% of the body's total energy despite comprising only 2% of body weight — and is correspondingly dependent on NAD+-mediated energy metabolism and DNA repair. The NAD+-dependent enzyme SIRT1 in neurons regulates neuroplasticity, cognitive function, and neuroprotection against oxidative stress:

- NMNAT2 and axonal integrity: NMNAT2 is an NAD+-synthesizing enzyme whose activity is essential for axonal survival. NMNAT2 depletion in neurons is associated with axonal degeneration — a feature of multiple neurodegenerative diseases. NMN supplementation has been shown to restore axonal integrity in animal models of neurodegeneration.
- SIRT1 and BDNF: SIRT1 in neurons promotes BDNF (Brain-Derived Neurotrophic Factor) expression — the growth factor responsible for neuroplasticity, memory consolidation, and neuronal survival. Cognitive exercise alone cannot maximize BDNF without adequate SIRT1 activity — which requires NAD+.
- Alzheimer's disease relevance: Reduced NAD+ levels have been documented in Alzheimer's brain tissue; NMN administration in animal models reduces amyloid-beta accumulation and improves cognitive performance. Human trials are ongoing.
- Executive function: The prefrontal cortex — the brain region most dependent on sustained mitochondrial energy and most sensitive to oxidative stress — may be among the primary beneficiaries of NAD+ restoration in the context of executive function, decision quality, and sustained cognitive performance under stress.

7.5 Cardiovascular Health

SIRT1 activity in vascular endothelial cells is among the most clinically important cardiovascular protective mechanisms in the NAD+ biology. Endothelial SIRT1 deacetylates eNOS (endothelial nitric oxide synthase), increasing nitric oxide production — the primary vasodilatory and anti-inflammatory signaling molecule in the vasculature. NAD+ depletion leaves endothelial SIRT1 substrate-starved, reducing NO bioavailability and promoting endothelial dysfunction — the

earliest detectable cardiovascular pathology. NAD+ restoration directly addresses this mechanism.

8. The Current Research Landscape

8.1 Key Human Clinical Trials

Study	Population	Intervention	Key Finding	Significance
Yoshino et al., Science 2021	Postmenopausal women with overweight / prediabetes	300mg NMN daily x 10 weeks	NMN increased muscle NAD+ metabolome; improved muscle insulin signaling; NMN transported intact in plasma as NMN (not nicotinamide)	First definitive human evidence that NMN is absorbed intact and reaches target tissues as NMN — validating the precursor approach
Liao et al., Nature Aging 2021	Older adults (65-80 years)	250mg NMN daily x 12 weeks	Improved walking speed; improved muscle strength; increased NAD+ metabolites in blood	Direct functional benefit in the elderly demographic most relevant to this protocol
Irie et al., NPJ Aging 2020	Healthy men (40-60 years)	100-500mg NMN daily x 12 weeks	Safe and well-tolerated across all doses; increased blood NMN and NAD+ metabolites dose-dependently; no serious adverse events	Safety profile established for oral NMN in human subjects
Mills et al., Cell Metabolism 2016	Aged mice (22 months)	NMN in drinking water x 12 months	Reversed age-associated physiological decline in energy metabolism, physical activity, eye function, and insulin sensitivity; reduced body weight	Foundational animal study demonstrating comprehensive NAD+ restoration reverses aging phenotype — mechanistic basis for human translation
Elhassan et al., Cell Reports 2019	Aged men (70-80 years)	NR (nicotinamide riboside) daily x 6 weeks	Increased skeletal muscle NAD+ metabolome; increased SIRT1 and SIRT3 activity in muscle	Direct evidence of sirtuin activation in aged human muscle tissue from NAD+ precursor supplementation
Camacho-Pereira et al., Cell Metabolism 2016	CD38 knockout and wild-type mice	CD38 KO vs. normal aging	CD38 KO prevented NAD+ decline with aging; identified CD38 as primary driver of age-related NAD+ depletion	Mechanistic identification of CD38 as the 'NAD+ drain' — explains why supplementation must overcome this ongoing depletion

8.2 David Sinclair and the Information Theory of Aging

No scholarly treatment of NAD+ biology is complete without engaging with the work of Dr. David Sinclair, Professor of Genetics at Harvard Medical School, whose research has done more than

any other to bring NAD+ to the center of longevity science. Sinclair's Information Theory of Aging proposes that aging is fundamentally a loss of epigenetic information — a corruption of the methylation and acetylation patterns that determine which genes are expressed in each cell — rather than an accumulation of DNA sequence mutations per se.

In this framework, sirtuins (particularly SIRT1) function as the information maintenance system — continuously restoring correct epigenetic patterns in response to DNA damage. But sirtuins can only perform this function when NAD+ is available as substrate. As NAD+ declines with age and PARP enzymes compete for the remaining supply to manage increasing DNA damage, sirtuins become progressively substrate-limited — unable to maintain epigenetic fidelity. The epigenetic clock advances. Aging accelerates.

Sinclair's laboratory has demonstrated that restoring NAD+ levels — and by extension sirtuin activity — can reset epigenetic markers toward younger patterns in animal models, reversing measurable indicators of biological age. The NAD+ tripartite protocol documented in this paper is, at its molecular core, an attempt to provide the NAD+ substrate that enables the body's own sirtuin machinery to maintain and restore epigenetic integrity — to give SIRT1 the currency it needs to do its job.

9. Protocol Experience: The Skoda NAD+ Journey

9.1 Protocol Timeline

Phase	Period	Intervention	Observed Response	Protocol Notes
Pre-Protocol Baseline	July 2025	No NAD+ supplementation; sedentary; insulin-dependent; 255 lbs	Fatigue consistent with estimated 40-50% NAD+ depletion for age; metabolic dysfunction	Biological age estimated equivalent to chronological age or above
Phase 1 — NMN Foundation	Early protocol (2025)	Oral NMN initiated; TMG concurrent	Subjective: improved morning energy over first 4-6 weeks; clearer cognitive initiation; reduced 'morning fog'	Foundational layer established; methylation protection active from initiation
Phase 2 — Transdermal Addition	Mid-protocol	NAD+ patch added to NMN + TMG	Extended energy sustain through afternoon; reduced 2-4pm energy dip; improved sleep onset	Coverage gap from oral NMN 6-8h peak addressed by patch sustained release
Phase 3 — Injection Initiation	~15 days prior to May 2026	Subcutaneous NAD+ injection added (daily)	Early observations: notably sharper morning cognitive clarity; stronger training sessions; subjective wellbeing elevation	Too early for biomarker confirmation; June 15 benchmark will provide first objective data
Current State	May 2026	Full tripartite protocol active: NMN + Patch + Injection + TMG	Subjective: most cognitively acute period of the protocol; gym performance sustained at 20/21-day pace; cold plunge integration smooth	Tripartite protocol in first full cycle; compound benefit expected to build over next 60-90 days

9.2 Biomarker Integration: What the Data Shows

The NAD+ protocol was initiated within the context of an already comprehensive biomarker monitoring system. While direct NAD+ blood level measurement is not yet part of the routine SiPhox panel, several proxy markers are assessable and will be compared at the June 15, 2026 benchmark:

Marker	NAD+ Relevance	Current Status (May 2026)	June 15 Target
HbA1c	NAD+ restoration improves insulin sensitivity via SIRT1 and SIRT3; glucose	6.0% — significantly improved from insulin-dependent baseline	5.7-5.9% — approaching normal range; NAD+ may contribute additional improvement

	metabolism directly NAD+-dependent		
hsCRP (inflammation)	CD38 is inflammation-activated; reducing inflammation reduces NAD+ consumption; NAD+ supports anti-inflammatory SIRT1	Low-normal — managed via protocol	Maintain or improve; first read post-cold plunge addition
Homocysteine	TMG-dependent clearance; elevated homocysteine = methylation cycle stress; reflects TMG protocol efficacy	Not recently assessed	Target: < 10 µmol/L; TMG protocol should produce clear result
Metabolic Age (Renpho)	Mitochondrial function proxy; NAD+-SIRT3 axis directly drives mitochondrial efficiency	70 (vs. chronological 71)	Target: 68-70; continued improvement expected
Subjective energy / cognitive drive	Dopamine and mitochondrial energy both NAD+-sensitive; self-report proxy	Elevated post-injection initiation (15 days)	Structured self-report tracking per cold plunge protocol
Skeletal Muscle Mass	SIRT3 in muscle drives mitochondrial efficiency; NAD+ supports muscle protein synthesis signaling	95.4 lbs — HIGH category	Maintain > 93 lbs during ongoing fat loss

9.3 A Note on the Injection Experience

Fifteen days into the subcutaneous injection phase, the most notable subjective observation is the quality of the morning cognitive state. The combination of the cold hydrotherapy dopamine surge and the injection-delivered NAD+ peak creates a morning neurological environment that is qualitatively different from either intervention alone — characterized by an immediacy of mental clarity and motivational readiness that has directly translated to more productive early-morning work sessions and a more engaged training quality.

This is subjective data. It will be contextualized against objective biomarkers at the June 15 benchmark. But subjective quality of cognitive function is not irrelevant data — it is, for a senior executive managing the complexity of multiple concurrent ventures, one of the most operationally significant metrics in the entire protocol.

"At 71, with insulin-independent glycemic control, a 45-pound transformation, and now a daily NAD+ injection protocol, I am not managing my age. I am actively reversing it — one coenzyme at a time." — Mark A. Skoda, May 2026

10. Conclusions: The NAD⁺ Imperative at 71

10.1 The Scientific Case

NAD⁺ depletion is not a peripheral feature of aging. It is, by the weight of current evidence, a central mechanism — the substrate limitation that disables the body's own sirtuin-based longevity maintenance system, degrades mitochondrial function, reduces DNA repair capacity, and allows the epigenetic drift that defines the aging phenotype to accelerate unchecked. Restoring NAD⁺ does not introduce a foreign pharmacological signal. It removes a substrate limitation that aging has imposed on the body's own machinery.

The tripartite delivery protocol — oral NMN, transdermal patch, subcutaneous injection — addresses the bioavailability problem that has historically limited NAD⁺ supplementation's efficacy. By providing continuous 24-hour systemic NAD⁺ elevation through three complementary pharmacokinetic channels, and by protecting the methylation cycle with concurrent TMG supplementation, the Skoda Protocol approximates the sustained intracellular NAD⁺ availability of young adulthood as closely as current non-clinical technology permits.

Final Summary: The Skoda NAD⁺ Protocol

WHAT: Tripartite NAD⁺ delivery — oral NMN + transdermal patch + subcutaneous injection — with TMG methyl donor support.
WHY: NAD⁺ decline of 50-60% by age 70 disables sirtuins, impairs mitochondria, degrades DNA repair, and drives the aging phenotype at the molecular level.
HOW IT'S DIFFERENT: NAD⁺ is a coenzyme — not a peptide, not a drug, not a hormone. Restoring it enables the body's own enzymatic machinery to function as designed. It is the most physiologically grounded injectable longevity intervention available.
THE INJECTION ADVANTAGE: Subcutaneous delivery bypasses GI degradation and hepatic first-pass metabolism; provides the highest non-IV bioavailability; delivers direct systemic NAD⁺ elevation within 15-30 minutes daily.
THE TRIPARTITE ADVANTAGE: No 24-hour window without elevated NAD⁺. Injection creates the peak; patch provides sustained coverage; NMN maintains the salvage pathway and precursor pool continuously.
TMG: Non-negotiable. Protects the methylation cycle, prevents homocysteine accumulation, and enables sustained NAD⁺ supplementation without depleting the SAM pool on which dozens of other critical reactions depend.
PROTOCOL STATUS: Full tripartite protocol active as of May 2026. First objective biomarker assessment: June 15, 2026 SiPhox 57-biomarker panel.
EXPECTED TRAJECTORY: Compound benefit building over 60-90 days as mitochondrial density increases, sirtuin activity is sustained, and epigenetic restoration has time to accumulate.

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