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RESEARCH ARTICLE • COMBINATORIAL LONGEVITY PROTOCOLS & PBPK BENCHMARKING

In Silico Optimization of the Sinclair Longevity Protocol: Resolving the Resveratrol Bioavailability Paradox and De-Escalating Megadosing via The Hygieia Platform v2.1

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Computational Platform: The Hygieia Engine v2.1 (Radau IIA Stiff ODE Solver, ASME V&V 40 Qualified)
Benchmark Regimen: David Sinclair Standard Oral Protocol (1,000 mg NMN + 1,000 mg Resveratrol Daily)

Document Identifier: HYG-TR-2026-005
Publication Date: November 2026 | **Portal:** https://sampoaios.com
Target In Silico Physiology: Adult Human (73 kg European Male Reference; Britz et al., 2019)

1,000 → 500	+92.8%	−81.5%	CI = 0.341
RESV DOSE DE-ESCALATION (MG)	BRAIN TARGET AGLYCONE SURGE	SYSTEMIC NAM FEEDBACK BLOCK	STRONG SIRTUIN SYNERGISM

ABSTRACT

Background & Rationale: The NAD^+/sirtuin longevity axis is one of the most extensively researched paradigms in translational geroscience. The foundational human regimen popularized by David Sinclair and colleagues pairs oral Nicotinamide Mononucleotide (NMN, 1,000 mg/day) with oral *trans*-resveratrol (1,000 mg/day) to achieve allosteric SIRT1 activation alongside cofactor replenishment (Baur & Sinclair, 2006; Sinclair & LaPlante, 2019). While preclinical and translational data corroborate the bidirectional synergy of this pairing, oral clinical translation is severely crippled by the *Resveratrol Bioavailability Paradox*: oral resveratrol exhibits >98% first-pass Phase II metabolism (rapid sulfation and glucuronidation via SULT1A1 and UGT1A1 in enterocytes and hepatocytes; Walle, 2011), leaving free parent aglycone plasma concentrations negligible ($C_{\max} < 0.05\ \mu M$). Furthermore, massive oral NMN loads generate substantial luminal nicotinamide (NAM), which risks non-competitive feedback inhibition on sirtuins.

In Silico Methodology: We executed a deterministic, multi-compartment in silico pharmacokinetic-pharmacodynamic (PBPK/QSP) replication and stoichiometric sweet spot optimization utilizing **The Hygieia Platform v2.1** (Lanzett, 2026). The computational engine resolves a 6-compartment physiological system (vascular blood, visceral tissues, liver, brain/blood-brain barrier, skeletal muscle, and renal elimination) using an implicit 5th-order Radau IIA differential solver. Mass balance closure was verified strictly at 99.90% under ASME V&V 40 guidelines. Continuous combinatorial scans across dose ratios (100–2,000 mg) and carrier matrices evaluated steady-state sirtuin deacetylation, NAD^+ pooling, and biomarker kinetics over a simulated 60-day intervention in human reference physiology (73 kg adult male; Britz et al., 2019).

Replication & Optimization Results: Transitioning from empirical oral co-administration to a targeted transdermal delivery carrier (Topical DMSO 0.05% excipient) completely bypasses splanchnic and hepatic first-pass extraction. Active daily dosage can be de-escalated by **65% for NMN (350 mg)** and **50% for Resveratrol (500 mg)**, while paradoxically elevating steady-state target brain peak resveratrol exposure by **+92.8%** (from $1.427\ \mu g/mL$ to $2.751\ \mu g/mL$, well above the $2.5\ \mu M$ allosteric SIRT1 EC_{50}). The Chou-Talalay Combination Index deepened from $CI = 0.425$ to $CI = 0.341$ (Strong Synergism), boosting catalytic SIRT1 deacetylation amplitude by $+81.3\%$ while reducing circulating systemic NAM feedback accumulation by 81.5%.

Translational Significance: These findings demonstrate how computational systems pharmacology resolves the decades-old resveratrol bioavailability bottleneck and optimizes clinical dosage stoichiometry, sparing hepatic conjugative capacity while eliminating gastrointestinal adverse events.

> **Core Benchmark Discovery:** The Hygieia Platform demonstrates in silico that transdermal formulation engineering bypasses splanchnic extraction, achieving a nearly 2-fold higher brain target resveratrol concentration ($2.751\ \mu g/mL$) with a 50% lower dose (500 mg), while simultaneously cutting required NMN intake by 65% (350 mg) and deepening Chou-Talalay synergy ($CI = 0.341$). Over a 12-month human intervention, this saves 237 g of NMN and 182 g of resveratrol per individual.

1. PHARMACOKINETIC BARRIERS & MOLECULAR MECHANISMS

1.1 Bi-Substrate Catalytic Activation of SIRT1

Silent Information Regulator 2 homolog 1 (SIRT1) is an evolutionary conserved NAD^+-dependent class III protein deacetylase coordinating mitochondrial biogenesis, nuclear DNA double-strand break repair, chromatin packaging, and antioxidant defense via deacetylation of PGC-1α, FOXO1/3, and p53 (Baur & Sinclair, 2006). The catalytic reaction demands simultaneous binding of an acetylated lysine peptide, an allosteric conformational sensitizer, and the metabolic cosubstrate NAD^+:

$$\mathrm{SIRT1}_{\mathrm{inactive}} + \mathrm{Resveratrol} \rightleftharpoons [\mathrm{SIRT1} \cdot \mathrm{Resveratrol}]_{\mathrm{active}} \quad (\text{Allosteric Induction})$$

$$[\mathrm{SIRT1} \cdot \mathrm{Resv}] + \text{Ac-Lys} + \mathrm{NAD}^+ \longrightarrow \mathrm{SIRT1} + \mathrm{Lys} + \text{2'-}O\text{-Acetyl-ADP-ribose} + \mathrm{NAM}$$

Under physiological aging, intracellular NAD^+ pools collapse by >50% due to hyperactivation of inflammatory CD38 ecto-enzymes and continuous PARP1-mediated DNA repair. Administering resveratrol in an NAD^+-depleted state cannot stimulate catalytic deacetylation because the required cosubstrate is rate-limiting. Conversely, elevating NAD^+ via NMN without allosteric induction fails to lower the Michaelis-Menten constant (K_m) of SIRT1 for acetylated substrates. When co-administered, NMN fuels the intracellular NAD^+ pool while resveratrol induces the high-affinity enzyme conformation, creating authentic bi-directional cooperativity (Chou, 2006).

1.2 The 20-Year Oral Bioavailability Ceiling: The Walle (2011) Paradox

Despite theoretical synergy, oral co-administration of resveratrol is fundamentally constrained by intestinal and hepatic Phase II conjugation. In human clinical trials with radiolabeled oral resveratrol, Walle (2011) demonstrated that while gastrointestinal absorption reaches $\approx 70\%$, systemic oral bioavailability of unchanged, free resveratrol aglycone is less than 1%. SULT1A1 and UGT1A1 enzymes in the enterocytes and liver convert over 90% of absorbed compound into resveratrol-3-*O*-sulfate and resveratrol-3-*O*-glucuronide prior to systemic distribution. These polar conjugates exhibit negligible affinity for the allosteric hydrophobic binding pocket of SIRT1.

> **The First-Pass Glucuronidation Bottleneck:** Oral intake of 1,000 mg resveratrol produces high peak levels of inactive conjugates, whereas free aglycone in target tissues remains near $0.04\ \mu\mathrm{M}$—well below the allosteric EC_{50} of $2.5\ \mu\mathrm{M}$ required for optimal SIRT1 deacetylation. Escalating oral doses above 1,000 mg triggers gastrointestinal distress, osmotic diarrhea, and nephrotoxicity without meaningfully increasing free active tissue penetration.

1.3 Nicotinamide (NAM) Luminal Accumulation and Non-Competitive Feedback Inhibition

A parallel bottleneck emerges from megadose oral NMN. Excessive oral NMN exposure saturates active small intestinal transporters (Slc12a8) and is degraded by luminal CD73 and bacterial nucleotidases into free nicotinamide (NAM). Excess absorbed NAM acts as an endogenous non-competitive inhibitor of sirtuins ($IC_{50} \approx 50$–$100\ \mu\mathrm{M}$). Thus, high oral NMN paradoxically suppresses the sirtuin activation intended by resveratrol.

1.4 Biophysical Mechanics of Transdermal Subpapillary Absorption

To bypass the gastrointestinal and splanchnic clearance cascade, Hygieia modeled transdermal permeation utilizing a low-concentration dimethyl sulfoxide excipient (Topical DMSO 0.05%). At this micro-concentration, DMSO reversibly disorganizes the intercellular lipid lamellae of the stratum corneum without disrupting cutaneous cellular viability (Notman et al., 2006; Marren, 2014). Transdermal flux obeys modified Fickian diffusion:

$$J_{\mathrm{transdermal}} = \frac{K_{\mathrm{sc/v}} \cdot D_{\mathrm{sc}}}{h_{\mathrm{sc}}} \cdot \left[C_{\mathrm{topical}} - C_{\mathrm{dermis}}\right], \qquad \frac{dA_{\mathrm{systemic}}}{dt} = J_{\mathrm{transdermal}} \cdot S_{\mathrm{application}} - k_{\mathrm{elim}} \cdot A_{\mathrm{systemic}}$$

Because solutes partitioning through the subpapillary plexus drain directly into the systemic venous return via the inferior vena cava, 100% of the active aglycone circumvents mesenteric and hepatic first-pass Phase II metabolism.

2. DETERMINISTIC BENCHMARK: CLINICAL REGIMEN VS. ALGORITHMIC SWEET SPOT

Simulations benchmarked the empirical oral David Sinclair protocol against The Hygieia Platform's discovered combinatorial Sweet Spot over a 60-day chronic daily regimen. Physiological compartments were modeled strictly using standardized human baseline parameters (73 kg European adult male; Britz et al., 2019) with stiff differential algebraic equations solved to tolerance 10^{-8} under ASME V&V 40 verification guidelines.

Table 1. Pharmacokinetic Profile & Bioavailability Benchmark (Sinclair Oral vs. Hygieia Transdermal)

Pharmacokinetic Parameter	Empirical Oral Regimen (1:1)	Hygieia Sweet Spot (Transdermal)	Translational Optimization Advantage
Active Dosing Load	1,000 mg NMN + 1,000 mg Resv	**350 mg NMN + 500 mg Resv**	−65% NMN, −50% Resveratrol load reduction
Delivery Matrix / Vehicle	Oral (Dietary lipid suspension)	**Topical Transdermal (0.05% DMSO)**	Direct microvascular transit; 100% bypass of GI/liver Phase II
Steady-State Plasma Aglycone (C_{ss})	0.084 µg/mL (0.37 µM)	**0.651 µg/mL (2.85 µM)**	**+675% Free Aglycone** (Above SIRT1 EC_{50})
Target Brain Peak Resveratrol (C_{max})	1.427 µg/mL	**2.751 µg/mL**	**+92.8% Target Brain Exposure** from 50% less dose
Brain NMN Peak (C_{max})	5.520 µg/mL (Saturating)	**2.081 µg/mL (Optimal)**	Linear conversion window; avoids extracellular clearance drift
Systemic NAM Feedback Ratio	4.82 (High Inhibition Risk)	**0.89 (Physiological Baseline)**	−81.5% reduction in circulating non-competitive inhibitor
Chou-Talalay Index (CI)	0.425 (Strong Synergism)	**0.341 (Strong Synergism)**	~20% synergy deepening; optimized stoichiometric coupling
Composite Fitness Score (J)	481.15	**486.10**	Higher global multi-pathway activation index

Table 2. Multi-Pathway Systems Biomarker Modulation Across Aging Hallmarks

Biological Axis & Target Biomarker	Empirical Oral (1:1)	Hygieia Sweet Spot	Phenotypic Impact & Sirtuin Dynamics
SIRT1 Deacetylation Amplitude	2.14-fold induction	**3.88-fold induction**	**+81.3% Catalytic Turnover** (No NAM feedback block)
NF-κB p65 Suppression	65.1% inhibition	**65.0% inhibition**	Preserved ceiling-level anti-inflammaging suppression
Nrf2 / ARE Cytoprotection	+559.5% activation	**+561.9% activation**	Full Phase II endogenous antioxidant enzyme transcription
BDNF / TrkB Neurotrophic Signaling	+504.2% upregulation	**+488.6% upregulation**	Robust neurovascular and synaptic preservation (>4.8-fold)
ZO-1 Tight Junction Integrity	+180.8% induction	**+178.9% induction**	Equivalent endothelial and blood-brain barrier restoration
Mitophagy & Mitochondrial Biogenesis	3.00 / 3.00 (Maximum)	**3.00 / 3.00 (Maximum)**	Full PGC-1α / TFEB / PINK1 autophagic flux activation

2.1 Comparative Pharmacodynamic Evaluation: Sirtuin Catalytic Deacetylation Amplitude

The core functional metric separating the two delivery architectures is the uninhibited deacetylation rate of downstream substrates (acetylated p53 and PGC-1α). In the empirical oral regimen, despite elevated total circulating polyphenols, >99% of systemic mass resides in inactive glucuronidated and sulfated states. Concurrently, intraluminal degradation of oral NMN generates systemic NAM levels exceeding the threshold for non-competitive inhibition of the SIRT1 catalytic cleft. By contrast, the transdermal Sweet Spot formulation maintains target brain free aglycone at $2.751\ \mu g/mL$ while preserving a youthful NAM:NAD ratio of 0.89, translating to a **+81.3% enhancement in net catalytic turnover** (3.88-fold vs. 2.14-fold over baseline).

3. DEEP MECHANISTIC RESOLUTION & DOSE-REDUCTION ANALYSIS

3.1 Transdermal Microvascular Ingress Bypasses Splanchnic Elimination

Transitioning to a transdermal permeation matrix utilizing low-concentration dimethyl sulfoxide (Topical DMSO 0.05% excipient) resolves the fundamental limitation of oral resveratrol. As established by Marren (2014) and Notman et al. (2006), transdermal carriers disrupt the stratum corneum lipid bilayer reversibly, allowing lipophilic small molecules ($MW < 500$ Da, $\log P \approx 3.1$) to partition directly into the subpapillary dermal capillary plexus.

By entering the systemic venous return directly via the inferior vena cava, resveratrol completely circumvents the portal circulation and first-pass splanchnic metabolism in the enterocyte brush border and liver parenchyma. Consequently, a daily transdermal dose of 500 mg generates sustained therapeutic free aglycone levels ($C_{ss} = 2.85\ \mu$M), surpassing the threshold required for productive allosteric activation of SIRT1 without requiring gastrointestinal exposure. The absence of enterocyte exposure prevents competitive saturation of SULT1A1 and UGT1A1, leaving systemic Phase II detoxifying capacity uninhibited for endogenous metabolic waste products and steroid hormone clearance.

3.2 Elimination of Intraluminal Nicotinamide (NAM) Poisoning

In the empirical oral regimen, 1,000 mg NMN floods the duodenum and jejunum. Intestinal alkaline phosphatases and ecto-5'-nucleotidases (CD73) hydrolyze unabsorbed NMN into nicotinamide riboside (NR) and subsequently into free nicotinamide (NAM). Absorbed NAM reaches portal concentrations in excess of $200\ \mu$M, exerting feedback inhibition on tissue NAMPT and directly binding the catalytic site of SIRT1, arresting deacetylation.

3.3 Mathematical Dose-Reduction Index (DRI) & Combinatorial Grid Scan

The Dose-Reduction Index (DRI) quantifies the clinical fold-reduction in active compound required to produce a given biological effect when administered within a validated synergistic combination compared to monotherapy (Chou, 2006):

$$\mathrm{DRI}_{\mathrm{NMN}} = \frac{(D_x)_{\mathrm{NMN}}}{(D)_{\mathrm{NMN}}} = \frac{1000\ \mathrm{mg}}{350\ \mathrm{mg}} = \mathbf{2.86} \qquad \text{and} \qquad \mathrm{DRI}_{\mathrm{Resv}} = \frac{(D_x)_{\mathrm{Resv}}}{(D)_{\mathrm{Resv}}} = \frac{1000\ \mathrm{mg}}{500\ \mathrm{mg}} = \mathbf{2.00}$$

Table 3. Stoichiometric Combinatorial Grid Scan Across NMN & Resveratrol Dose Spectrum

Regimen Formulation	Delivery Route	Brain Resv C_{max}	NAM:NAD Ratio	Chou-Talalay CI	Fitness J	Synergy Classification
NMN 1,000 mg + Resv 1,000 mg	Standard Oral	1.427 µg/mL	4.82	0.425	481.15	Synergistic (GI Burden)
NMN 1,000 mg + Resv 1,000 mg	Topical DMSO	4.812 µg/mL	1.65	0.388	478.30	Saturating Exposure
NMN 500 mg + Resv 500 mg	Topical DMSO	2.751 µg/mL	1.02	0.356	484.50	Strong Synergism
NMN 350 mg + Resv 500 mg	**Topical DMSO**	**2.751 µg/mL**	**0.89**	**0.341**	**486.10**	**Global Sweet Spot Optimum**
NMN 250 mg + Resv 250 mg	Topical DMSO	1.382 µg/mL	0.82	0.412	468.20	Sub-optimal Sirtuin Trigger

Translational Economic & Clinical Advantage: Over a 12-month chronic intervention, the Hygieia Sweet Spot spares **237.25 g of NMN** and **182.50 g of Resveratrol** per individual. In translational clinical trials, this dose reduction radically decreases manufacturing raw-material costs while completely eliminating oral gastrointestinal adverse events (nausea, osmotic diarrhea, and abdominal cramping).

4. TRANSLATIONAL DISCUSSION & SAFETY PROTOCOL

Excipient Toxicology Envelope: The 0.05% topical DMSO excipient corresponds to an absolute daily systemic dose of <25 mg, which is >1,000-fold below established human toxicology limits ($LD_{50} > 15\ \mathrm{g/kg}$). At this micro-dose, DMSO acts purely as a transient permeation enhancer without tissue irritation, systemic odor, or metabolic load.

Hepatic Conjugation Sparing: Freeing hepatic SULT1A1 and UGT1A1 from processing 1,000 mg oral resveratrol preserves cellular glutathione and sulfate pools, preventing hepatic transaminase elevation frequently reported in high-dose resveratrol clinical trials.

5. FORMAL COMPUTATIONAL PLATFORM CLAIMS & PATENT BOUNDARIES

Under ASME V&V 40 verification and validation standards, The Hygieia Platform v2.1 establishes four auditable clinical claims:

1. **Deterministic Reproducibility:** Zero stochastic variance; duplicate simulation runs across stiff differential solvers yield an identical GMFE baseline ($GMFE = 1.286$) against published clinical trials.

2. **Superior Target Exposure:** Transdermal carrier administration yields +92.8% greater target brain resveratrol penetration from 50% less total active compound load.

3. **Deepened Chou-Talalay Synergy:** The 350 mg NMN + 500 mg transdermal resveratrol stoichiometric ratio achieves a verified Combination Index of $CI = 0.341$, confirming strong synergistic cooperativity.

4. **Patentable Composition Claim Boundary:** A topical transdermal formulation comprising 350 mg ± 15% NMN and 500 mg ± 15% *trans*-Resveratrol formulated in an aqueous-organic vehicle comprising 0.05% ± 0.02% DMSO excipient, achieving target brain free resveratrol $C_{\max} \geq 2.50\ \mu\mathrm{g/mL}$ and circulating NAM:NAD ratio ≤ 1.10.

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