Concept Paper



# Pulsed Reductive Impulses via Exogenous Reducing Equivalents: A Novel Paradigm for Anti-Cancer and Anti-Aging Metabolic Therapy

M. Freschi *

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# Pulsed Reductive Impulses via Exogenous Reducing Equivalents: A Novel Paradigm for Anti-Cancer and Anti-Aging Metabolic Therapy

**M. Freschi**

Independent researcher, Reggio Emilia, Italy; mrfreschi@gmail.com

**Abstract**

Nicotinamide adenine dinucleotide (NAD+/NADH) metabolism holds a central position in both tumor pathogenesis and cellular aging processes. Current therapeutic strategies pursue apparently contradictory objectives: oncology aims to deplete NAD+ in cancer cells, while anti-aging medicine administers NAD+ intravenously to restore levels that decline with age. This work proposes a paradigm shift: the pulsed administration of exogenous reducing equivalents — with NADH (the reduced form of the coenzyme) as the primary but not exclusive vehicle — as an integrated anti-cancer and anti-aging strategy. The rationale is based on intrinsic metabolic selectivity: cancer cells, characterized by mitochondrial dysfunction and dependence on fermentative glycolysis (Warburg effect), are unable to dispose of an acute excess of NADH through the electron transport chain, thereby suffering selectively lethal reductive stress. Healthy cells, endowed with functional mitochondria, can manage the reductive overload by oxidizing excess NADH in the respiratory chain, with respiratory control mechanisms regulating the flux. A protocol of brief, intense pulses (redox press-pulse) followed by recovery phases is proposed, in synergistic combination with glucose restriction (ketogenic diet/fasting) and optimization of intracellular magnesium. This triad — reducing substrate, enzymatic structure, and environment — aims to restore respiratory chain efficiency in healthy cells and selectively destabilize cancer cell metabolism. The convergence between anti-cancer and anti-aging mechanisms mediated by cyclic reductive impulses is also discussed. A speculative appendix explores the implications of quantum biology for understanding the efficiency of mitochondrial electron transfer.

**Keywords:** NADH; reductive stress; Warburg effect; redox impulses; cancer metabolic therapy; anti-aging; mitochondrial respiratory chain; NAD+/NADH; press-pulse; autophagy; quantum biology; reducing equivalents

## 1. Introduction

Cancer and aging share deep metabolic roots that converge on mitochondrial metabolism and cellular redox homeostasis. Despite decades of research, these two conditions have been addressed as separate entities, with therapeutic strategies often in mutual contradiction. Metabolic oncology, inspired by the pioneering work of Otto Warburg [1] and recently revisited by Thomas Seyfried [2], identifies mitochondrial dysfunction as the fundamental characteristic of cancer cells. Molecular gerontology, on the other hand, recognizes the decline in mitochondrial function and NAD+ depletion as two central hallmarks of aging [3,4].

This work proposes a unifying theoretical framework based on pulsed manipulation of the NAD+/NADH redox state as a simultaneously anti-cancer and anti-aging strategy. Unlike current practices — which predominantly administer NAD+ (oxidized form) intravenously in anti-aging clinics [5], or aim to deplete NAD+ in cancer cells through NAMPT inhibitors in oncology [6] — the administration of exogenous reducing equivalents, with NADH (reduced form) as the primary vehicle, in brief and intense cyclic pulses is proposed here.

 doi:10.20944/preprints202604.0237.v1



NADH is not the only possible modality for exogenous electronic delivery. As discussed in Section 6.2, other sources of reducing equivalents — including ubiquinol (reduced coenzyme Q10), molecular hydrogen ($H_2$), and catalytic systems based on metalloporphyrins — represent complementary or alternative routes to achieve the same objective: pulsed perturbation of the intracellular redox state. NADH is proposed as first-line because it is the native substrate of the respiratory chain, has already been used in clinical settings [7,8], and its intravenous administration is technically established.

The rationale rests on three pillars: (i) the intrinsic metabolic selectivity of exogenous reducing equivalents, which exploits the difference in mitochondrial competence between healthy and cancer cells; (ii) the pulse logic (redox press-pulse), derived from Seyfried's therapeutic strategy [9] and the physiology of radiation fractionation; (iii) the convergence between the mechanisms activated by cyclic reductive impulses (autophagy, mitophagy, inflammation reduction, mitochondrial turnover) and the well-known anti-aging pathways (sirtuins, AMPK, mTOR).

It is important to emphasize that, although some clinics already use NADH infusions — notably the Hyperthermia Centre Hannover in the oncological setting [7] and Birkmayer's clinical studies for Parkinson's disease [8], as well as the IntraVita protocol in the United Kingdom for anti-aging — none of these precedents has articulated the metabolic selectivity rationale proposed here, the press-pulse logic applied to redox state, the synergistic combination with glucose restriction, or the unifying anti-cancer/anti-aging framework.

## 2. The Warburg Effect and Tumor Metabolism: The Central Role of NAD+/NADH

### *2.1. Glycolytic Dependence of Cancer Cells*

In 1924, Otto Warburg observed that cancer cells prefer glycolysis followed by lactic fermentation even in the presence of sufficient oxygen for oxidative phosphorylation — a phenomenon known as aerobic glycolysis or the Warburg effect [1]. This observation, initially interpreted as a simple metabolic adaptation, has been reinterpreted by Seyfried as evidence of a primary mitochondrial dysfunction that forces the cell to depend on fermentation for ATP production [2].

In this context, NAD+ plays a critical and dual role. On one hand, glycolysis requires NAD+ as a cofactor in the reaction catalyzed by glyceraldehyde-3-phosphate dehydrogenase (GAPDH). To maintain glycolytic flux, the cancer cell must constantly regenerate NAD+ from NADH, primarily through the conversion of pyruvate to lactate by lactate dehydrogenase (LDH) [10]. On the other hand, NAD+ serves as a substrate for enzymes critical to tumor survival, including PARP (DNA repair), sirtuins (gene expression regulation), and CD38/CD157 (signaling) [11].

Recent work has revealed a third, previously underappreciated source of ATP in cancer cells: mitochondrial substrate level phosphorylation (mSLP) through the glutaminolysis pathway [42,43]. Cancer cells can produce significant ATP at the succinyl-CoA ligase reaction, using glutamine as fuel via the sequence glutamine → glutamate → alpha-ketoglutarate → succinyl-CoA → succinate + ATP/GTP. Succinate and lactate, as end-products of glutamine and glucose fermentation respectively, together acidify the tumor microenvironment [42]. Importantly, neither oxygen consumption nor lactate production are accurate markers for ATP production through OxPhos or glycolysis in cancer cells [42,43]. The mSLP pathway is critically relevant to the hypothesis presented here because the alpha-ketoglutarate dehydrogenase reaction — upstream of succinyl-CoA ligase — requires NAD+ as an electron acceptor, making it vulnerable to the same NAD+ depletion mechanism that blocks glycolysis.

### *2.2. The NAD+ Paradox in Oncology vs Anti-Aging*

Recent oncological literature has clearly identified NAD metabolism as a therapeutic target, demonstrating that the enzyme NAMPT (nicotinamide phosphoribosyltransferase), rate-limiting for

 doi:10.20944/preprints202604.0237.v1



NAD synthesis, is frequently amplified in various cancer types [6,12]. NAMPT inhibitors such as FK866 effectively deplete NAD and suppress tumor proliferation [13].

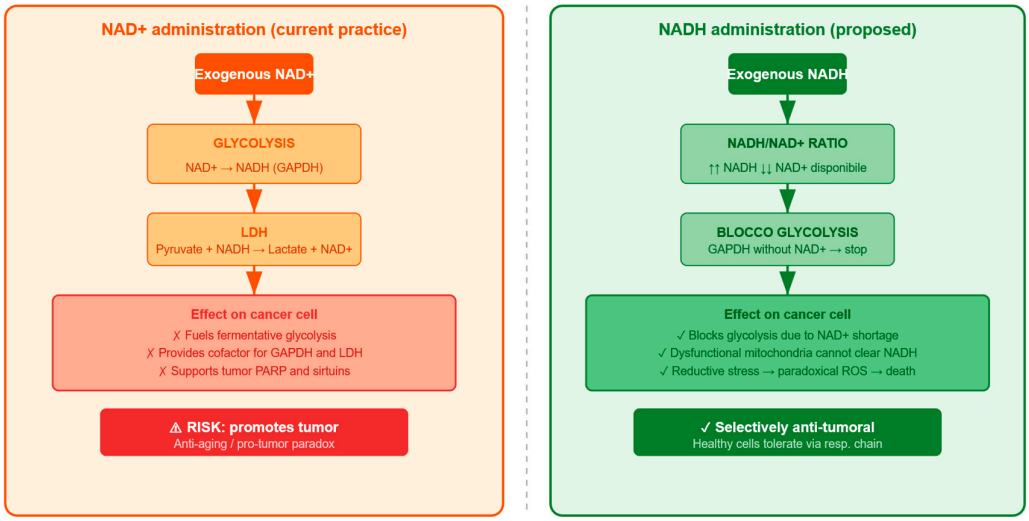
Simultaneously, anti-aging clinics predominantly administer NAD+ intravenously with the opposite objective: to increase NAD+ levels to support sirtuin function, DNA repair, and energy metabolism [5]. This creates a paradox that has been insufficiently discussed: systemic administration of NAD+ could fuel the glycolytic metabolism of subclinical cancer cells, providing them with the essential cofactor for GAPDH and LDH.

### *2.3. Exogenous Reducing Equivalents vs NAD+: A Fundamental Distinction*

This work proposes that the administration of reducing equivalents — primarily in the form of NADH (reduced form) — produces radically different metabolic effects from the administration of NAD+ (oxidized form). While NAD+ fuels tumor glycolysis by providing the necessary oxidizing cofactor, an acute excess of NADH creates the opposite conditions: it saturates the cellular NADH/NAD+ ratio, reducing the availability of free NAD+ and blocking glycolysis due to the lack of an electron acceptor at GAPDH (Figure 1).

The principle is not limited to NADH alone. Any delivery modality that acutely increases the intracellular load of reducing equivalents — exogenous NADH, ubiquinol, dissolved molecular hydrogen, or catalytic redox systems — could produce analogous effects, provided the perturbation is sufficiently rapid and intense to exceed the compensatory capacity of cancer cells without exceeding that of healthy cells.

**Figura 1. Differential metabolic effects of NAD+ vs NADH administration**

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## 3. Redox State and Tumor Microenvironment

### *3.1 The Oxidative-Inflammatory Environment as a Tumor Substrate*

Cancer cells maintain an elevated but controlled level of reactive oxygen species (ROS), which serves as a proliferative signal through the constitutive activation of HIF-1$\alpha$, NF-κB, and mTOR [14]. Chronic inflammation in the tumor microenvironment generates additional ROS through macrophages, neutrophils, and cancer-associated fibroblasts (CAFs), creating a vicious cycle: inflammation → ROS → mitochondrial damage → greater dependence on glycolysis → lactate production → microenvironment acidification → immunosuppression → further inflammation [15].

In this context, the NAD+/NADH ratio is a critical indicator of intracellular redox state. Cancer cells tend to maintain a high NAD+/NADH ratio through intense LDH activity, which regenerates

 doi:10.20944/preprints202604.0237.v1



NAD+ at the expense of NADH [10]. Acute perturbations of this ratio — specifically, a sudden increase in intracellular NADH — destabilize tumor metabolism at multiple levels.

### *3.2. The Transmembrane Redox Interface*

A legitimate objection to the manipulation of extracellular redox state is that the cell membrane could isolate the intracellular compartment. However, redox state is transmitted across the membrane through at least six documented mechanisms:

(a) **Transplasma membrane redox systems (ECTO-NOX):** membrane proteins that transfer electrons from intracellular NADH to extracellular acceptors; in a reduced external environment, these systems are blocked, causing intracellular NADH accumulation [16].

(b) **Cysteine/cystine system (xCT/SLC7A11):** the xCT transporter imports cystine (oxidized form), which is internally reduced to cysteine for glutathione synthesis. A reductive extracellular environment alters the cysteine/cystine ratio, modifying the intracellular glutathione pool [17].

(c) **Redox-sensitive receptors:** EGFR, IGFR, and integrins possess extracellular domains with cysteine residues whose oxidation is required for dimerization and activation. A reductive environment suppresses these proliferative signals [18].

(d) **Direct ROS diffusion:** hydrogen peroxide ($H_2O_2$) crosses the cell membrane, including through aquaporins, acting as a transmembrane redox messenger [19].

(e) **Lactate/pH gradients:** lactate transport through MCT transporters is dependent on the pH gradient; alterations in the extracellular environment modify this gradient and the metabolic shuttle between cancer cells and stroma [20].

(f) **Intercellular communication:** gap junctions (connexins) allow the direct passage of NADH, NAD+, glutathione, and second messengers between adjacent cells [21].

### *3.3. Reductive Stress: A Mirror Concept to Oxidative Stress*

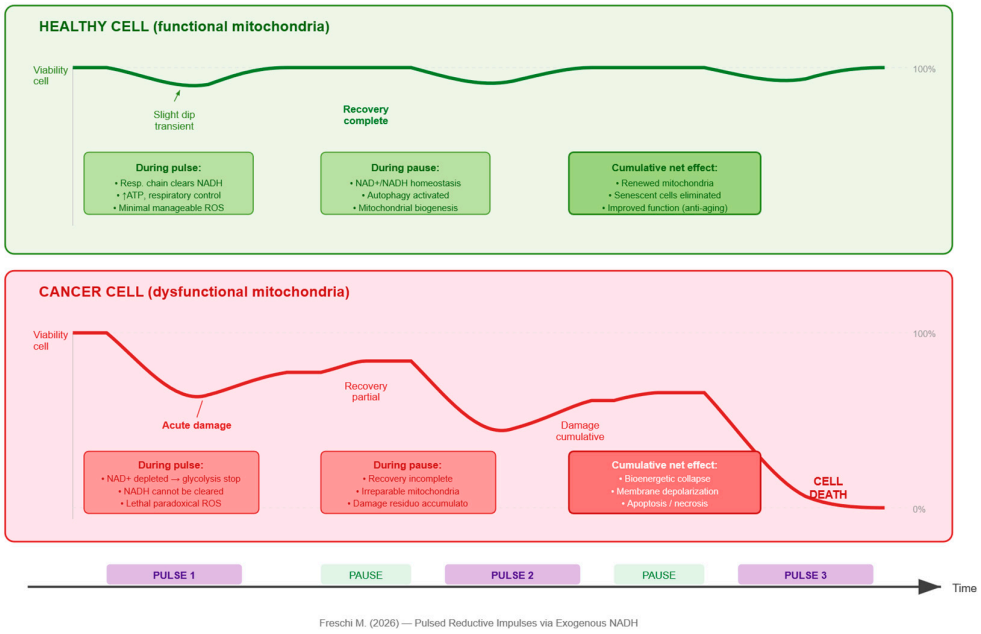
Reductive stress — defined as an excess of reducing equivalents relative to the system's capacity to dispose of them — is a real and documented phenomenon, although less studied than oxidative stress [22]. Under conditions of NADH excess, the respiratory chain becomes saturated and electrons "leak" from complexes I and III, reacting directly with molecular oxygen to form superoxide. Paradoxically, a reductive excess generates secondary oxidative stress [23].

However, this paradoxical response is proportional to mitochondrial competence: cells with functional mitochondria manage a moderate and transient NADH excess by increasing flux through the respiratory chain and ATP production, with minimal ROS generation. Cells with dysfunctional mitochondria — such as cancer cells — cannot compensate and suffer both glycolytic blockade (due to NAD+ shortage) and ROS generation from the saturated respiratory chain: a metabolic double hit (Figure 2).

 doi:10.20944/preprints202604.0237.v1



**Figure 2. Cyclic reductive impulse: differential response healthy vs cancer cell**

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## 4. Limitations of Conventional Antioxidants

### *4.1 Lack of Targeting*

Classical exogenous antioxidants (vitamin C, vitamin E, beta-carotene) distribute uniformly in body fluids without selectively concentrating at sites of greatest oxidative stress or in the tumor microenvironment [24]. This dilution drastically limits their local efficacy.

### *4.2. Secondary Pro-Oxidation*

An antioxidant that donates its electron itself becomes a radical. Oxidized vitamin C (ascorbyl radical/dehydroascorbic acid), in the presence of free iron — abundant in the tumor microenvironment — enters the Fenton cycle generating hydroxyl radical (OH•), the most devastating reactive species [25]. Oxidized vitamin E (tocopheryl radical) can propagate lipid peroxidation in membranes if not rapidly regenerated [26]. This paradoxical pro-oxidation mechanism at least partially explains the negative results of large clinical trials with antioxidants: the ATBC study showed increased tumors in the beta-carotene group [27], the SELECT study revealed increased prostate cancer risk with vitamin E [28].

### *4.3. Inadequate Compartmentalization*

Critical oxidative damage occurs predominantly in the inner mitochondrial membrane, where the respiratory chain transfers electrons. Vitamin C, being water-soluble, does not penetrate lipid membranes effectively; vitamin E, being lipid-soluble, distributes in membranes but does not selectively concentrate in mitochondria [29]. Targeted mitochondrial antioxidants such as MitoQ and SkQ1, conjugated with lipophilic cations that exploit the mitochondrial membrane potential, represent an advancement but do not solve the stoichiometry problem: they are consumed after donating one electron [30].

### *4.4. The Advantage of Reducing Equivalents as Electron Vehicles*

The administration of reducing equivalents — primarily NADH — overcomes the three limitations of conventional antioxidants: (i) it does not require artificial targeting because selectivity emerges from the difference in mitochondrial competence between healthy and cancer cells; (ii)

 doi:10.20944/preprints202604.0237.v1



NADH is the native substrate of the respiratory chain, not an exogenous agent; (iii) it operates at the central metabolic system level (Krebs cycle, respiratory chain) rather than as a peripheral ROS scavenger. Other forms of exogenous electronic delivery — ubiquinol, molecular $H_2$, catalytic metalloporphyrins — share advantage (i) while differing in site of action and specific mechanism.

## 5. Hypothesis: Cyclic Reductive Impulses via Exogenous Reducing Equivalents

*5.1 Rationale for Intrinsic Metabolic Selectivity*

The selectivity of the proposal is based on a fundamental biological asymmetry: the difference in mitochondrial competence between healthy and cancer cells.

In a healthy cell, an acute pulse of exogenous NADH is managed by the respiratory chain: NADH is oxidized to NAD+ at complex I, electrons flow through complexes III and IV to oxygen, generating a proton gradient that powers ATP synthase. However, it is essential to consider the mechanism of **respiratory control**: when intracellular ATP reaches high levels, the increased ATP/ADP ratio inhibits ATP synthase; the proton gradient accumulates on the inner mitochondrial membrane; the respiratory chain slows due to the inability to discharge the gradient; complex I accepts fewer electrons from NADH [31]. This physiological feedback mechanism prevents the healthy cell from oxidizing NADH indefinitely — a regulated ceiling exists. The NADH excess accumulates transiently but is subsequently cleared as ATP is consumed by cellular functions. The net result is a self-regulated cycle: transient NADH excess → chain slowdown → ATP consumption → chain restart → NADH clearance. During this process, ROS generation is contained by endogenous antioxidant systems (glutathione, thioredoxin, superoxide dismutase, catalase).

In a cancer cell with dysfunctional mitochondria, the same NADH pulse cannot be managed through this regulated mechanism. The respiratory chain is structurally compromised and respiratory control does not function properly. It is important to clarify the primary mechanism of action: intravenously administered NADH, being a charged and relatively large molecule, does not readily cross cell membranes. Its primary effect is therefore extracellular — the elevated concentration of NADH in the plasma and interstitial space creates a thermodynamic saturation that abolishes the gradients required for cancer cells to export their metabolic waste products. Specifically, the monocarboxylate transporters (MCT) that export lactate and protons, and the ECTO-NOX systems that transfer electrons to extracellular acceptors, are blocked when the extracellular environment is already saturated with reducing equivalents. The intracellular consequences follow indirectly: because the cancer cell cannot discharge its endogenously produced NADH toward the blocked extracellular environment, endogenous NADH accumulates, the NADH/NAD+ ratio shifts drastically toward the reduced state, and multiple lethal effects occur simultaneously:

(a) **Glycolytic blockade:** NAD+ shortage arrests GAPDH, blocking glycolysis — the primary source of ATP for the cancer cell.

(b) **Blockade of mitochondrial substrate level phosphorylation (mSLP):** the alpha-ketoglutarate dehydrogenase reaction, which converts alpha-ketoglutarate to succinyl-CoA in the glutaminolysis pathway, requires NAD+ as an electron acceptor. The depletion of NAD+ caused by NADH accumulation blocks this reaction, cutting off the substrate supply to succinyl-CoA ligase and halting mSLP-derived ATP production [42,43]. This means that the cancer cell cannot use glutamine as an alternative energy source to bypass the glycolytic blockade.

(c) **Metabolic congestion:** NADH excess allosterically inhibits key Krebs cycle enzymes (isocitrate dehydrogenase, alpha-ketoglutarate dehydrogenase), halting intermediary metabolism.

(d) **Paradoxical ROS generation:** excess electrons leak from the saturated and dysfunctional respiratory chain, generating superoxide that the cancer cell — already with antioxidant systems at their limit — cannot neutralize.

(e) **Bioenergetic collapse:** without ATP from glycolysis, without functional oxidative phosphorylation, and without mSLP from glutaminolysis, the cancer cell undergoes a total energy crisis with consequent plasma membrane depolarization and activation of cell death

 doi:10.20944/preprints202604.0237.v1



pathways. The cancer cell is thus subjected to a simultaneous blockade of all three known ATP-generating pathways.

### *5.2. Press-Pulse Logic Applied to Redox State*

Seyfried proposed the press-pulse strategy for metabolic cancer management, where constant metabolic "pressure" (caloric restriction, ketogenic diet) is integrated with acute "pulses" (fasting, pharmacological agents) that exploit the metabolic vulnerability of cancer cells [9]. It is proposed here to extend this logic to redox state.

The reductive impulse (infusion of reducing equivalents, primarily NADH) creates an acute perturbation of the NAD+/NADH ratio that the healthy cell tolerates — thanks to respiratory control and endogenous antioxidant systems — and the cancer cell does not. The pause between impulses allows healthy cells to fully recover redox homeostasis, while cancer cells accumulate progressive damage with each cycle — analogously to radiation fractionation, where healthy tissue repairs damage between fractions and tumor tissue does not [32].

Intermittency is likely superior to continuous administration for three reasons: (i) it prevents cancer cell adaptation to chronic reductive stress; (ii) it activates beneficial homeostatic mechanisms during the recovery phase (autophagy, mitochondrial biogenesis); (iii) it respects the physiological limits of the Na+/K+-ATPase pump and electrolyte homeostasis, which would not tolerate prolonged redox alteration.

### *5.3. Synergy with Glucose Restriction*

The efficacy of the reductive impulse is maximized when cancer cells are simultaneously deprived of glucose. Under fasting or ketosis conditions, blood glucose is reduced and cancer cells are already metabolically stressed. The addition of a reducing equivalent pulse during this window creates a double attack: the missing glucose blocks glycolysis at the entry point, the NADH excess blocks it at the exit (at GAPDH, due to NAD+ shortage). Healthy cells, which in ketosis utilize ketone bodies as mitochondrial substrate, are less dependent on glycolysis and better tolerate the perturbation.

### *5.4. The Therapeutic Triad: Substrate, Structure, Environment*

An integrated approach with three synergistic components is proposed (Figure 3):

**Substrate (exogenous reducing equivalents):** provides electrons directly usable by the respiratory chain, bypassing endogenous metabolic bottlenecks (degraded enzymes, energy deficit, redox vicious cycle). NADH is the primary vehicle; ubiquinol operates directly in the inner mitochondrial membrane; molecular hydrogen acts as a selective reductant of the most toxic ROS.

**Structure (magnesium):** $Mg^{2+}$ is an essential cofactor for over 600 enzymatic reactions, including stabilization of the Mg-ATP complex, maintenance of iron-sulfur cluster geometry in respiratory complexes, and correct positioning of NADH at dehydrogenase active sites [33]. Subclinical magnesium deficiency, which is epidemically widespread, compromises electron transfer efficiency independently of substrate availability.

**Environment (ROS and inflammation reduction):** the combination with targeted anti-inflammatory and antioxidant compounds (curcumin, EGCG, omega-3 DHA/EPA) reduces oxidative noise in the microenvironment, improving the conditions under which electron transfer occurs in healthy cells and reducing survival signals (NF-κB, HIF-1$\alpha$) in cancer cells.

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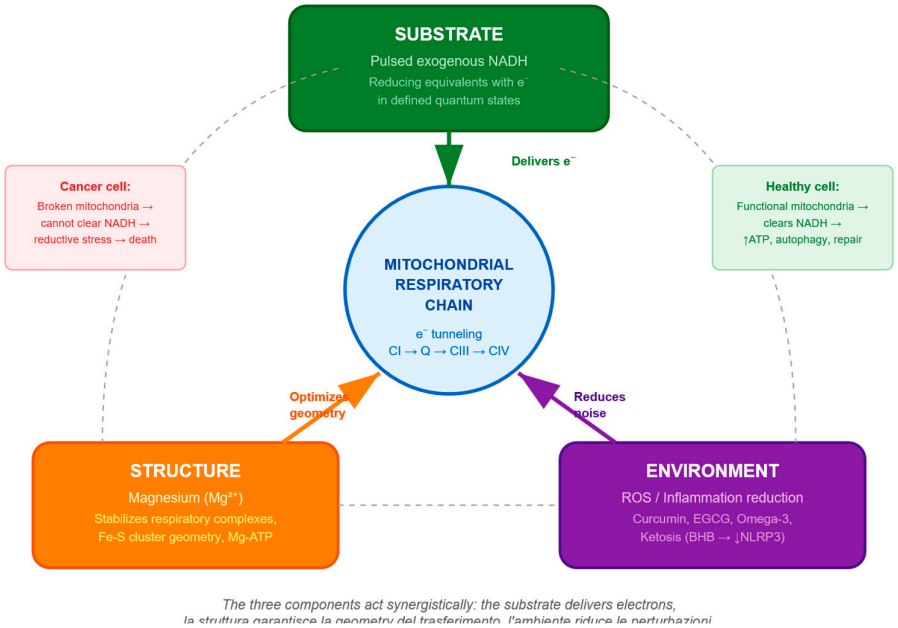
**Figure 3. The therapeutic triad: substrate, structure, environment**




*The three components act synergistically: the substrate delivers electrons, la struttura garantisce la geometry del trasferimento, l'ambiente riduce le perturbazioni*

*5.5. Dual-Compartment Rationale: Extracellular Blockade and Intracellular Stress*

The mechanism described above highlights an important distinction: intravenous NADH acts primarily as an extracellular thermodynamic blockade rather than as a direct intracellular electron donor. The NADH molecule does not need to enter the cancer cell to exert its effect — its presence at high concentration in the extracellular space is sufficient to abolish the export gradients upon which cancer cells depend for metabolic waste disposal and NAD+ regeneration via LDH.

However, the efficacy of the approach could be further enhanced by combining extracellular blockade with agents capable of directly increasing the intracellular reducing load. The principle of dual-compartment redox perturbation — simultaneous saturation of the extracellular environment (blocking waste export) and of the intracellular environment (accelerating NAD+ depletion) — would create a more complete metabolic siege. While NADH is proposed as the primary agent for extracellular blockade, other reducing equivalents with different membrane permeability profiles could complement its action intracellularly. For example, small lipophilic molecules capable of crossing membranes, or agents that diffuse freely into all cellular compartments, could serve this complementary role. The identification and validation of optimal agents for intracellular reducing load augmentation represents an important direction for future experimental work within this framework.

This dual-compartment concept also underscores the synergy with glucose restriction: in the absence of glucose, the cancer cell loses its last efficient mechanism for NAD+ regeneration (the LDH reaction requires pyruvate as substrate). When extracellular blockade (via NADH) is combined with substrate deprivation (via fasting/ketosis), the cancer cell has no remaining pathway to regenerate NAD+ at a rate sufficient to sustain any of its three ATP-generating pathways.

## 6. Proposed Experimental Protocol

*6.1 Preclinical In Vitro Phase*

The following experimental sequence is proposed:

(a) Cell cultures of tumor lines (e.g., PC-3, LNCaP for prostate; HeLa; MCF-7 for breast) and healthy lines (e.g., RWPE-1 for prostate; MCF-10A for breast) exposed to increasing concentrations of

 doi:10.20944/preprints202604.0237.v1



exogenous NADH (0.1–10 mM) for variable durations (1–8 hours), followed by recovery periods in standard medium.

(b) Primary endpoints: cell viability (MTT, trypan blue), intracellular NAD+/NADH ratio (enzymatic assays or NADH fluorescence), lactate production (indicator of glycolytic flux), ATP levels, ROS generation (DCFDA probe), mitochondrial membrane potential (JC-1).

(c) Experimental groups: pulsed NADH (2h exposure + 22h recovery, repeated for 5 cycles) vs continuous NADH (same total dose distributed over 120h) vs control. This comparison is critical to validate the superiority of the pulse logic.

(d) Combination: repeat the experiments in glucose-free medium (ketosis/fasting simulation) to verify the synergistic effect of glucose depletion and NADH overload.

*6.2. Modalities for Reducing Equivalent Delivery*

The central principle — pulsed perturbation of intracellular redox state through exogenous reducing equivalents — can be achieved through various modalities, each with specific advantages and limitations:

**Exogenous NADH:** is the native substrate of the respiratory chain and the most direct vehicle. It can be administered by direct intravenous infusion, as already practiced by the Hyperthermia Centre Hannover [7], Birkmayer's studies for Parkinson's disease [8], and the IntraVita protocol. Advantages: clinical precedent, direct enzymatic recognition. Limitations: molecular instability, limited membrane permeability. Delivery in liposomes or nanoparticles could improve intracellular bioavailability and potentially enable tumor targeting through the EPR (enhanced permeability and retention) effect.

**Ubiquinol (reduced CoQ10):** operates directly in the inner mitochondrial membrane as an electron carrier between complexes I-II and complex III. Advantages: direct access to the site of action, lipid solubility, good safety profile. Limitations: does not bypass blockade at the complex I level, acts downstream in the electron pathway.

**Molecular hydrogen ($H_2$):** a selective reductant that preferentially reacts with the hydroxyl radical (OH•) without neutralizing reactive species with signaling functions ($O_2^{\bullet -}$, $H_2O_2$). Advantages: unique selectivity, small molecular dimensions that allow diffusion into every cellular compartment, clinical trials underway in Japan. Limitations: does not supply electrons to the respiratory chain, acts primarily as a selective scavenger.

**Other modalities:** the principle proposed here — pulsed perturbation of intracellular redox state — is not bound to a specific molecular vehicle. Any technique, molecule, or procedure capable of acutely increasing the intracellular load of reducing equivalents could be evaluated within this framework, with particular interest in systems that deliver electrons with defined quantum states optimized for functional biocompatibility (see Appendix). The future development of catalytic redox vehicles — capable of cycling between oxidation states without being consumed — would represent a significant advancement over stoichiometric vehicles such as NADH. Each of these modalities would merit independent experimental evaluation within the press-pulse framework proposed here. NADH is suggested as first-line due to its nature as an endogenous biological substrate and the existing clinical precedent.

*6.3. Pulse Protocol Parameters (Preliminary Clinical Proposal)*

For a potential Phase I clinical trial, the following indicative parameters are proposed:

- **Pulse duration:** 2–4 hours of slow intravenous infusion
- **Frequency:** 2–3 sessions per week during intensive cycles
- **Cycle duration:** 2–3 weeks
- **Inter-cycle pause:** 4–6 weeks
- **Metabolic combination:** sessions should ideally be conducted during fasting periods (≥16 hours) to maximize the metabolic vulnerability of cancer cells

 doi:10.20944/preprints202604.0237.v1



- **Supplementation:** magnesium glycinate/citrate (400–800 mg/day), reduced CoQ10 (ubiquinol, 200–400 mg/day)
- **Mandatory monitoring:** plasma NAD+/NADH ratio, blood lactate, blood glucose, ketones, continuous ECG during infusion, electrolytes (Na+, K+, $Mg^{2+}$), inflammatory markers (CRP, IL-6), complete blood count

*6.4. Safety Criteria*

NADH is an endogenous molecule with a favorable safety profile in available clinical experience [7,8]. However, the pulse protocol with doses potentially higher than those used for anti-aging requires specific attention to:

- **Cardiac risk:** acute alterations of the NAD+/NADH ratio may affect cardiomyocyte membrane potential. Continuous ECG monitoring during infusion is mandatory.
- **Metabolic risk:** an excessive infusion rate could cause paradoxical lactic acidosis or excessively activate respiratory control, reducing ATP production in healthy cells. Gradual dose titration is recommended.
- **Electrolyte risk:** reductive stress could transiently affect the Na+/K+-ATPase pump. Monitoring of electrolytes before, during, and after infusion is essential.

## 7. Anti-Cancer and Anti-Aging Convergence

*7.1 Shared Mechanisms*

Cyclic reductive impulses activate pathways that are simultaneously anti-tumoral and anti-aging:

**Autophagy and mitophagy:** the transient metabolic stress induced by the reductive impulse, especially in combination with fasting, activates autophagy — the process of recycling damaged cellular components [34]. Mitophagy, in particular, removes dysfunctional mitochondria and replaces them with new mitochondria through PGC-1$\alpha$-mediated mitochondrial biogenesis. This process is fundamental both for eliminating potentially pro-tumoral mitochondria and for rejuvenating the cellular mitochondrial pool [35].

**AMPK/mTOR axis:** the metabolic impulse activates AMPK (energy scarcity sensor) and inhibits mTOR (abundance sensor). This switch activates cellular repair and recycling programs and suppresses proliferation — beneficial both in anti-tumor and anti-aging contexts [36].

**Elimination of senescent cells:** senescent cells — which have ceased dividing but secrete pro-inflammatory cytokines (SASP, senescence-associated secretory phenotype) — are drivers of both aging and tumorigenesis [37]. Cyclic metabolic impulses promote the selective elimination of these cells through autophagy and immune activation.

**Reduction of chronic inflammation (inflammaging):** beta-hydroxybutyrate, the principal ketone body produced during fasting and ketosis, directly inhibits the NLRP3 inflammasome [38], reducing the chronic low-grade inflammation that serves as a substrate for both aging and tumorigenesis.

**Transient immune unmasking of tumor cells:** cancer cells exploit chronic oxidative inflammation in the tumor microenvironment to create an immunosuppressive niche: elevated ROS inactivate infiltrating T lymphocytes and polarize tumor-associated macrophages toward the pro-tumoral M2 phenotype [15]. The reductive impulse, by transiently neutralizing extracellular ROS and reducing the oxidative inflammatory signals in the tumor stroma, may temporarily disrupt this immunosuppressive shield. During the recovery phase following the impulse, the immune system — functionally restored by the transient reduction of oxidative stress — could re-enter a microenvironment where the tumor's immunoevasion mechanisms have not yet been fully re-established, facilitating immune recognition and attack. This potential immunometabolic synergy between reductive impulses and anti-tumor immunity warrants specific experimental investigation.

 doi:10.20944/preprints202604.0237.v1



*7.2. The Nocturnal Window as a Physiological Reductive Impulse*

The natural circadian cycle features a nocturnal window characterized by: maximal melatonin production (a potent endogenous antioxidant), cortisol nadir (anti-inflammatory), parasympathetic nervous system activation, and, under post-prandial fasting conditions, progressive activation of mild ketosis. This window represents a physiological reductive impulse that can be deliberately enhanced and extended through: extended overnight fasting (14–16 hours), complete environmental darkness, curcumin and EGCG supplementation in the evening window, and avoidance of alcohol (whose hepatic metabolism overloads the NAD+/NADH system and generates pro-oxidant acetaldehyde).

*7.3. Integrated Preventive Protocol*

A stratified approach at two levels is proposed:

**Baseline level (daily):** enhanced nocturnal reductive window (8–10 hours) through intermittent fasting, magnesium supplementation, evening antioxidants, sleep in complete darkness.

**Intensive level (cyclic):** cycles of 10–14 days every 2–3 months, with strict ketosis, prolonged fasting (48–72 hours), and potentially pulsed reducing equivalent infusions, followed by refeeding and recovery phases.

## 8. Limitations and Future Directions

*8.1. Limitations of the Present Hypothesis*

The proposal has several limitations that are openly acknowledged:

(a) **Absence of specific clinical data:** no clinical trials exist that test pulsed NADH administration with the metabolic selectivity rationale described here. The experiences of the Hyperthermia Centre Hannover [7] and Birkmayer [8] use NADH in a different therapeutic framework (general support) without press-pulse logic.

(b) **Pharmacokinetics of exogenous NADH:** the intracellular bioavailability of intravenously administered NADH is not fully characterized. NADH could be metabolized in plasma before reaching target cells. Dedicated pharmacokinetic studies are necessary.

(c) **Tumor heterogeneity:** not all tumors exhibit the same degree of mitochondrial dysfunction. Tumors with partially preserved oxidative phosphorylation might better tolerate the reductive impulse. Patient selection based on metabolic imaging (FDG-PET, as an indicator of the Warburg effect) could improve selectivity.

(d) **Therapeutic window:** the distance between the dose effective against cancer cells and the dose toxic to healthy cells — considering the respiratory control mechanism that also limits the clearance capacity of healthy cells — could be narrow. Only preclinical experimentation can define this window.

(e) **Interaction with conventional therapies:** the effect of the reductive impulse on cells treated with chemotherapy, radiotherapy, or immunotherapy is unknown and requires specific study. Of particular interest would be the combination with PARP inhibitors (e.g., olaparib), which deplete intracellular NAD+ through a complementary mechanism.

(f) **Need for structural turnover:** the administration of exogenous reducing equivalents does not repair damaged respiratory complexes. Its effect is to bypass metabolic bottlenecks and, through cyclic impulses, to stimulate the mitophagy and mitochondrial biogenesis that replace structurally compromised components.

 doi:10.20944/preprints202604.0237.v1



*8.2. Future Directions*

(a) Preclinical in vitro studies with the protocol described in Section 6.1 to validate the selectivity of the reductive impulse, with specific measurement of the simultaneous blockade of glycolysis, OxPhos, and mSLP.
(b) Development of NADH formulations in liposomes or nanoparticles to improve intracellular delivery.
(c) Combined study of pulsed NADH + fasting + PARP inhibitors to maximize NAD+ depletion in cancer cells through complementary mechanisms.
(d) Experimental validation of the dual-compartment redox blockade concept: systematic comparison of extracellular-only perturbation (NADH) versus combined extracellular + intracellular perturbation using agents with different membrane permeability profiles, to determine whether dual-compartment approaches enhance selectivity and efficacy.
(e) Quantum biology studies (see Appendix) to characterize the quantum states of electrons in exogenous vs endogenous NADH and their functional relevance.
(f) Development of predictive response biomarkers based on tumor metabolic profile (NAD+/NADH ratio, complex I expression, LDH activity, succinate export as indicator of mSLP dependence).
(g) Comparative evaluation of different modalities for exogenous reducing equivalent delivery (NADH, ubiquinol, $H_2$, and future catalytic redox vehicles) within the press-pulse protocol, with attention to the coherence of the quantum states of the delivered electrons.
(h) Investigation of the potential immunometabolic effects of reductive impulses on tumor-associated macrophage polarization and T lymphocyte function in the tumor microenvironment.

## 9. Conclusions

This work proposes a paradigm shift in the manipulation of NAD+/NADH metabolism: from the administration of NAD+ (oxidized form, prevailing practice in anti-aging clinics) to the pulsed administration of reducing equivalents — primarily NADH (reduced form) — with a rationale of intrinsic metabolic selectivity based on the mitochondrial dysfunction of cancer cells.

The theoretical framework unifies anti-cancer metabolic therapy and anti-aging strategy in a single paradigm centered on cyclic reductive impulses, overcoming the current contradiction between the oncological approach (deplete NAD+) and the gerontological approach (supplement NAD+).

NADH is proposed as first-line among various possible modalities of exogenous electronic delivery, due to its nature as an endogenous biological substrate and the existing clinical precedent. The general principle — pulsed perturbation of redox state that exploits the differential in mitochondrial competence — is applicable to a broader spectrum of reducing vehicles.

The proposal is experimentally testable with available technologies and relies on clinical precedents (Hyperthermia Centre Hannover, Birkmayer studies, IntraVita protocol) which, while using NADH in different frameworks, provide preliminary data on the safety and tolerability of infusions.

If validated, this strategy could open a new class of metabolic interventions — targeted redox impulses — with applications ranging from oncology to preventive medicine and longevity.

## Appendix A. Quantum Biology Perspectives

*A.1. Preamble*

This appendix explores speculative hypotheses at the frontier between biochemistry and quantum physics. The proposals advanced here are not supported by direct experimental evidence in the specific context of exogenous reducing equivalents and are presented as future research directions. However, they are grounded in well-documented quantum biology phenomena in other biological systems.

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*A.2. Quantum Tunneling in the Respiratory Chain*

Electron transfer in the mitochondrial respiratory chain occurs through quantum tunneling between redox centers (iron-sulfur clusters, heme groups, coenzyme Q) separated by distances of 7–14 Å [39]. The tunneling probability decays exponentially with distance according to the Marcus-Moser-Dutton relationship, and is sensitive to site geometry, energy barrier, and dielectric environment [40].

*A.3. Quantum coherence in biological systems*

The demonstration of quantum coherence in energy transfer in photosynthetic complexes at room temperature [41] has opened the possibility that analogous phenomena operate in the respiratory chain, which is structurally related to photosynthetic complexes. If quantum coherence contributes to the efficiency of mitochondrial electron transfer, its degradation (decoherence) — caused by structural damage to proteins, perturbation of membrane lipids, and paramagnetic noise from ROS — could contribute to the decline in mitochondrial function associated with aging and tumorigenesis.

*A.4. Quantum States of Electrons in Exogenous vs Endogenous Reducing Equivalents*

Although all electrons are identical fundamental particles, their quantum state — defined by orbital energy, symmetry, spin, and wave function phase — is determined by the molecular and environmental context. A NADH synthesized ex vivo under controlled conditions presents reducing electrons in a quantum state defined by the molecular electronic configuration in the ground state, unperturbed by interactions with the aged biological microenvironment.

It is hypothesized that this "freshness" of quantum states may have functional relevance: a NADH produced metabolically in a cellular environment with high paramagnetic noise (ROS), oxidized proteins, and free iron could exhibit shorter decoherence times (T2) compared to synthetic NADH, with consequent reduction in the efficiency of quantum tunneling at complex I.

It is necessary to emphasize that NADH is not the only possible source of electrons in defined quantum states. Any reducing equivalent synthesized under controlled conditions — including ubiquinol and coordinated metallic redox systems — could present analogous advantages in terms of electronic state coherence.

*A.5. Decoherence as a Component of Aging*

It is proposed, speculatively, that mitochondrial aging is not only a chemical phenomenon (protein oxidation, lipid peroxidation, mtDNA mutations) but also a physical phenomenon of progressive decoherence. A "young" mitochondrion with intact proteins, non-peroxidized lipids, and low paramagnetic noise would maintain conditions favorable to quantum coherence in electron transfer, resulting in high efficiency and low ROS production. An "old" mitochondrion with degraded structure would lose these conditions, with consequent lower tunneling efficiency, greater electron leakage, and greater ROS production — independently of substrate availability.

*A.6. Experimental Verification*

Verification of these hypotheses is technically feasible with currently available instruments:

(a) **Pulsed EPR spectroscopy and ENDOR:** to compare the spin states and relaxation times (T1, T2) of electrons in fresh synthetic NADH vs NADH extracted from young tissues vs NADH extracted from aged tissues.

(b) **High-resolution oximetry (Oroboros O2k):** to measure respiratory chain efficiency in mitochondria isolated from young vs aged tissues, before and after incubation with exogenous NADH.

(c) **Time-resolved fluorescence spectroscopy:** to characterize the dynamics of enzyme-bound NADH in different age and pathology contexts.

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If decoherence times (T2) prove to be significantly longer in synthetic NADH compared to biologically aged NADH, this would provide indirect evidence that the quantum states of electrons are functionally relevant in the context of mitochondrial transfer.

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