

NAD⁺, Senolytics, or Pyruvate for Healthy Aging?

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ABSTRACT: In last decades, healthy aging has become one of research hotspots in life science. It is well known that the nicotinamide adenine dinucleotide oxidized form (NAD⁺) level in cells decreases with aging and aging-related diseases. Several years ago, one of NAD⁺ precursors was first demonstrated with its new role in DNA damage repairing in mice, restoring old mice to their physical state at young ones. The finding encourages extensive studies in animal models and patients. NAD⁺ and its precursors have been popular products in nutrition markets. Alternatively, it was also evidenced that clearance of cellular senescence by senolytics preserved multiorgan (kidney and heart) function and extended healthy lifespan in mice. Subsequent studies confirmed findings in elderly patients subjected with idiopathic pulmonary fibrosis. The senolytic therapy is now focused on various diseases in animal and clinical studies. However, pyruvate, as both a NAD⁺ substitute and a new senolytic, may be advantageous, on the equimolar basis, over current products above in preventing and treating diseases and aging. Pyruvate-enriched fluids, particularly pyruvate oral rehydration salt, may be a novel intervention for diseases and aging besides critical care. Albeit the direct evidence that benefits healthy aging is still limited to date, pyruvate, as both NAD⁺ provider and senolytic agent, warrants intensive research to compare NAD⁺ or senolytics for healthy aging, specifically on the equimolar basis, in effective blood levels. This review briefly discussed the recognition of healthy aging by comparing NAD⁺ and Senolytics with sodium pyruvate from the clinical point of view.

KEYWORDS: Healthy aging, NAD⁺, pyruvate, senescence, senolytics

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Pyruvate is one of the key metabolites of canonical glycolysis in cytosol, which connects the glucose oxidative phosphorylation in mitochondria, and produced from the lactate oxidation by lactate dehydrogenase (LDH-B) in addition to the transamination reaction from amino acids, including alanine (Figure 1). Nicotinamide adenine dinucleotide oxidized form (NAD⁺) is an essential molecule: a coenzyme coupled with most important enzymatic reactions. It is well known that the cellular NAD⁺ level is going down with aging and diseases and boosting NAD⁺ content or preserving NAD⁺ homeostasis is health protective,^{1,2} so that NAD⁺ is also called the “molecule of youth” in health and nutrition markets. Both pyruvate and NAD⁺ exist in each individual cell throughout the body, being found around a century ago, however, since the last decade both molecules have become robustly attractive in life science, respectively, but their intimate relationship has been insufficiently considered. This review is an attempt to explore the recognition of healthy aging with sodium pyruvate, relative to NAD⁺ and Senolytics (Table 1), as a nutrition component mainly from a clinical point of view, aiming to invoke concerns of potential pyruvate clinical applications in NAD⁺ homeostasis, first as a novel nutrition ingredient.

NAD⁺ in Healthy Aging

Endogenous NAD⁺ is generated from oxidation of its reduced form (NADH) representatively by the pyruvate reduction with LDH-A coupled with a hydrogen (proton, [H⁺]) consumption, producing lactate in cytosol. This LDH reductive reaction with pyruvate free of energy and oxygen is preferable to the LDH oxidative reaction with lactate in cytosol. Further, the

direction of LDH redox reaction occurred spontaneously in each cell depends on the law of mass action of reactive substrates: the ratio of pyruvate/lactate and the LDH activity.³ Pyruvate also suppresses the stimulated sorbitol pathway in diseases, such as diabetes, reducing the NAD⁺ consumption and preserving the NAD⁺/NADH (NAD⁺ reduced form) ratio (Figure 1).⁴ Additionally, pyruvate promotes oxidative phosphorylation, reconverts NADH to NAD⁺ in electron transport chain and producing ATP in mitochondria. The NAD⁺/NADH constitutes one of coenzyme redox potentials involved in a wide range of crucial enzymatic reactions. Therefore, the NAD⁺ deletion brings to metabolic alterations and organ dysfunction in diseases and aging.⁵ Alternatively, NAD⁺ is also synthesized from its precursors: nicotinamide (NAM), nicotinamide mononucleotide (NMN), or niacin (NA, Vitamin B3) and related derivatives, such as nicotinamide riboside (NR), by the NAD⁺ salvage pathway and/or their exogenous supplements. These large molecules as well as Metformin, Rapamycin, and Resveratrol are amongst sirtuin-activating compounds.⁵ In this respect, there have been many systematic detailed reviews in NAD⁺ biomedical effects on diseases and healthy aging,^{1,2,5,6} but the following works are robustly of clinical relevance.

NAD⁺ plays a critical role in DNA repair, via the poly (ADP-ribose) polymerase (PARP) and the sirtuins (SIRT1-7) signal pathways, cellular energy metabolism, and survival in addition to redox reactions.⁶ In 2017, Sinclair et al first demonstrated the speculation of the new role that NAD⁺ directly regulates protein-protein interactions in damaged DNA repairing in the view of cell level.⁷ The NAD⁺ level in the liver



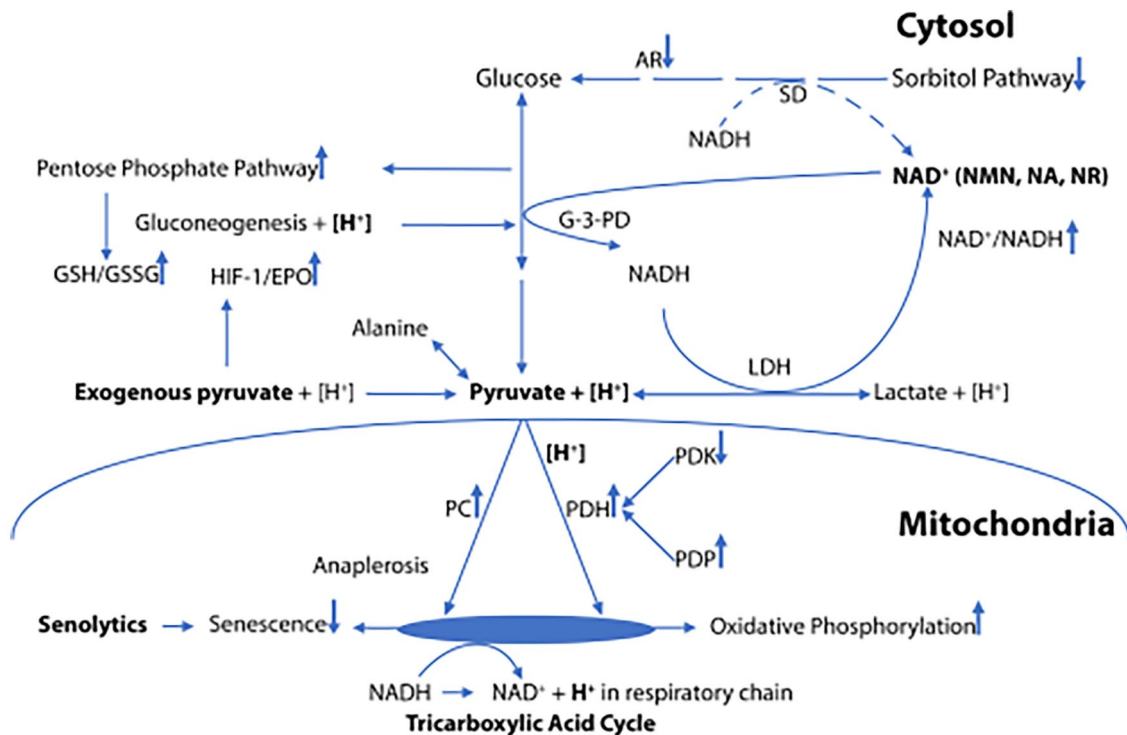


Figure 1. A diagram of post-exogenous pyruvate metabolic profile with NAD^+ and senolytics in healthy aging. Administration of NAD^+ and its precursors (NMN, NA, NR) increases NAD^+ levels in tissues; senolytics inhibit cellular senescence. Exogenous pyruvate with cytosolic $[\text{H}^+]$ reduces to lactic acid (lactate + $[\text{H}^+]$) by LDH, coupled NADH oxidation, increasing NAD^+/NADH ratio and preserving glycolysis at G-3-PD step; pyruvate also inhibits the sorbitol pathway by declining AR activity, decreasing NAD^+ consumption and restores the pentose phosphate pathway in cytosol, raising GSH/GSSG ratio. In addition, exogenous pyruvate stimulates PDH as well as PC activities and promotes the TCA cycle via the regulation with PDK and PDP besides the HIF-1-EPO pathway stimulation. NADH via the electron transport chain converts back to $\text{NAD}^+ + [\text{H}^+]$ and produces ATP in mitochondria. Alternatively, exogenous pyruvate enhances energy metabolism and thus inhibits cellular senescence in addition to NAD^+ restoration.^{4,66} Abbreviations: $[\text{H}^+]$, hydrogen consumed; $[\text{H}^+]$, hydrogen produced; AR, aldose reductase; G-3-PD, glyceraldehyde-3-phosphate dehydrogenase; GSH/GSSG, glutathione (reduced/oxidized form); HIF-1/EPO, hypoxia inducible factor-1/erythropoietin pathway; LDH, lactate dehydrogenase; NA, niacin; NAD^+/NADH , nicotinamide adenine dinucleotide (oxidized/reduced form); NAM, nicotinamide; NMN, nicotinamide mononucleotide; NR, nicotinamide riboside; PC, pyruvate carboxylase; PDH, pyruvate dehydrogenase; PDK, PDH kinase; PDP, PDH phosphatase; SD, sorbitol dehydrogenase.

was decreased with aging and the NMN treatment (500 mg/kg/day) for a week enhanced hepatic NAD^+ concentrations in old mice back to those in young ones (6 vs 22 months); the reduced PARP1 activity in the old mice was also restored by the treatment. Further, a single oral dose of NMN (2000 mg/kg/day) for 8 days significantly improved blood metrics in old and young (23 vs 4 months) mice subjected to the gamma irradiation.⁷ Furthermore, in human primary fibroblast cells, the NMN treatment also declined the phosphorylated Histone H2AX (γH2AX , a DNA damage protein marker) and reduced DNA fragmentation, protecting from Paraquat (a poisonous herbicide)-induced DNA damage.⁷ These results strongly indicate that NMN efficiently protected against the radiation exposure and reduced the adverse effect of chemotherapy, preventing from DNA damage and aging (restoring 2 years old mice to their physical state at a half year⁷), nearly turning a 60 old man back to 20. This novel finding that was recognized by several Nobel laureates encouraged numerous studies, in vitro and in vivo, substantiating that NAD^+ and its precursors are apparently beneficial in preserving organ metabolism and function and postponing aging in animal models as well as patients.⁸⁻¹¹

However, many clinical trials with NAD^+ or its precursors (NMN and NR) showed the indefinite clinical outcomes in past years.¹² Newly, the first clinical test with oral Niacin (NA) displayed remarkable salutary effects on patients with mitochondrial myopathy. With a 10-month therapy, blood NAD^+ increased up to 8-fold and muscle strength and mitochondrial biogenesis enhanced in all subjects. Patients' muscle NAD^+ reached the level of controls and muscle metabolome also shifted toward normal along with liver fat decreased even 50%.¹³ The result was considered advantages of NA over NMN in raising NAD^+ in humans. Alternatively, data reviewed from 8 randomized controlled trials showed that the NA supplementation improved the blood lipid profile, however, did not affect plasma glucose and hemoglobin A1c levels in Type 2 diabetic patients.¹⁴ Nevertheless, a recent study provided a negative result of NR effects on lifespan in both male and female mice at the doses tested.¹⁵

A recent important finding is that the plasma levels of extracellular nicotinamide phosphoribosyltransferase (eNAMPT), a key NAD^+ biosynthetic enzyme located in extracellular vesicles (EVs), were declined with age in both mice and humans, injuring function of specific tissues.

Table 1. Pyruvate clinical advantages relative to NAD⁺ and senolytics in healthy aging.

	MAIN UNDERLYING MECHANISMS	REFERENCES	CLINICAL STUDIES	REFERENCES
NAD ⁺	Improving most enzymatic reactions	Katsyuba et al, ¹ Kang et al, ² Covarrubias et al, ⁵ Bonkowski and Sinclair ⁶	IV NAD ⁺ infusion with safety	Grant et al, ¹⁸ Rutherford et al ¹⁹
NA/NAM/	Promoting energy metabolism	Katsyuba et al, ¹ Kang et al, ² Covarrubias et al, ⁵ Bonkowski and Sinclair, ⁶ Iannetti et al ⁶⁸	NA: mitochondrial myopathy	Pirinen et al ¹³
NMN/NR	Inhibiting oxidative stress and inflammation	Katsyuba et al, ¹ Kang et al, ² Covarrubias et al, ⁵ Bonkowski and Sinclair, ⁶ Iannetti et al ⁶⁸	NAM: acute kidney injury	Poyan Mehr et al ¹¹
	Repairing DNA damage	Li et al ⁷	Controversial outcomes	Radenkovic et al, ¹² Xiang et al, ¹⁴ Harrison et al ¹⁵
			Adverse effects	Radenkovic et al, ¹² Hwang and Song ¹⁷
Senolytics	Inhibiting the SASP	Sabbatinelli et al, ²⁰ Baker et al, ²¹ Gil and Withers, ²² Farr et al ²³	Idiopathic pulmonary fibrosis	Justice et al ²⁴
AP20187	Eliminating senescent cells	Sabbatinelli et al, ²⁰ Baker et al, ²¹ Gil and Withers, ²² Farr et al, ²³ Lehmann et al, ²⁸ Xu et al ²⁹	Systemic sclerosis	Martyanov et al ^{26,27}
Dasatinib/			Diabetes	Hickson et al ³⁰
Quercetin			Osteoporosis, osteoarthritis	Farr et al, ²³ Robbins et al ³⁵
Fisetin			Chronic kidney disease?	Robbins et al ³⁵
			Covid-19?	Derosa et al, ³⁷ Malavolta et al ³⁸
Sodium	Raising NAD ⁺ /NADH ratio	Zhang et al ⁴	Heart failure	Schillinger et al ⁴⁹
Pyruvate	Preserving glycolytic pathways	Zhang et al, ⁴ Gou et al ⁶⁰	Liver cirrhosis	Mateva et al, ⁵⁰ Petkova et al ⁵¹
	Reactivating PHD activity/OXPHOS	Zhang et al, ⁴ Ksiezak-Reding et al, ⁶² Stacpoole ⁶³	Diabetes	Petkova et al, ⁵² Inoue et al ⁵³
	Correcting severe metabolic acidosis	Wang et al, ³ Hu et al, ⁴¹ Flaherty et al ⁴²	Mitochondropathy	Li et al, ⁴⁴ Koga et al, ⁵⁴ Battaglia et al ⁷⁴
	Exerting anti-oxidative, -inflammatory	Liu et al ⁴³	Skin; dermal fibroblasts	Han et al, ⁴⁷ Kim et al ⁶⁷
	Inhibiting apoptosis and senescence	Zhang et al, ⁴⁵ Kaplon et al, ⁶⁵ Olenchock and Vander Heiden ⁶⁶	Leigh syndrome fibroblasts	Iannetti et al ⁶⁸
	Protecting mitochondria	Koga et al, ⁵⁴ Kim et al, ⁶⁷ Battaglia et al ⁷⁴	Covid-19?	Zhou ⁷⁵
	Repairing DNA damage	Han et al, ⁴⁷ McGowan and Bucher, ⁷⁰ Chenoufi et al ⁷¹	Long-term stable solutions	Zhou, ⁴⁸ Zhou ⁷⁵
			Without adverse effects	Schillinger et al, ⁴⁹ Mateva et al, ⁵⁰ Zhou ⁷⁵

Abbreviations: NA, niacin; NAD⁺/NADH, nicotinamide adenine dinucleotide (oxidized/reduced form); NAM, nicotinamide; NMN, nicotinamide mononucleotide; NR, nicotinamide riboside; OXPHOS, oxidative phosphorylation; PDH, pyruvate dehydrogenase; SASP, senescence-associated secretory phenotype.

EV-contained eNAMPT could be internalized into cells and directly enhanced NAD⁺ biosynthesis. Supplementation with EV-contained eNAMPT enhanced wheel-running activities and extended lifespan in aged mice.¹⁶ The results further confirmed the NAD⁺ key role in healthy aging and provided a novel possibility that the pharmaceutical intervention to

increase eNAMPT levels may preserve healthy aging in humans.

Albeit several clinical trials showed positive outcomes, questions still rise from supplements with NAD⁺ and its precursors in several aspects, such as administration routes, enteral absorption, intracellular biosynthesis and transference in various cell

types, drug dose, and treatment period to contribute NAD⁺ homeostasis as well as adverse effects.^{5,12,17}

The preliminary clinical investigation of intravenous (IV) NAD⁺ in healthy males was first documented for its pharmacokinetic properties in 2019.¹⁸ It was just a case report regarding the clinical valuable effect of IV NAD⁺ on a patient subjected with Parkinson's disease to date,¹⁹ though IV Diphosphopyridine nucleotide (DPN) and NAD(P)H were revealed with salutary clinical effects 3 decades ago. In addition, IV NAD⁺ for healthy aging, which cannot be orally administrated due to the degradation, is expensive. It also requires a slow infusion for several hours with mild adverse effects. Current studies indicate that clinical effects of NAD⁺ and its precursors for diseases and healthy aging are still controversial, requiring further validation with high quality and large scale randomized controlled trials.

Senolytics in Healthy Aging

On the other hand, it has been demonstrated for decades that cellular senescence promotes aging with tissue NAD⁺ decline.²⁰ Senescence cells, that is, cells at a stress-induced irreversible growth arrest: naturally generated “zombie cells” in life, but actually are metabolic active with more glycolytic state mimic to cancer cells. The accumulation of these cells in various tissues and organs over time is speculated to intimately associate with organ dysfunction, diabetes, tumorigenesis, and aging. It was van Deursen et al who first demonstrated that clearance of senescence cells by AP20187, one of senolytics, preserved multiorgan (kidney and heart) function and extended healthy lifespan about 25% (17%-35%) in both male and female mice of 2 distinct genetic backgrounds in 2016.^{21,22} It was lately identified that both estrogen deficiency and cellular senescence are independent factors in osteoporosis of aged women.²³

The first human pilot study with oral senolytics (Dasatinib plus Quercetin, DQ) on 14 elderly patients subjected with idiopathic pulmonary fibrosis (IPF with moderate to severe severity) was released by Justice et al in early 2019.²⁴ The DQ treatment over 3-weeks robustly enhanced physical function (6-minutes walk distance, 4-m gait speed, and chair-stands time) with clinical meaningful alterations though pulmonary function tests were not improved. However, correlations were shown between changes in physical function and in senescence-associated secretory phenotype (SASP) factors' expression.²⁴ IPF is a disease associated with senescent cell accumulation in lungs that is irreversible with aging and fatal. These preliminary results evidently indicated the potential and feasibility that intervention with senolytics ameliorated, at least in part, consequences of aging-related diseases in humans, as demonstrated in mice and human cells with IPF, *in vitro*.²⁵

A retrospective investigation of subjects with systemic sclerosis accompanied by fibrotic interstitial lung treated by senolytic Dasatinib (D) for 9 months was reported in 31 patients with drug well-tolerance. Of them, 65% showed no progression

of lung fibrosis and 36% had no progression of total interstitial lung diseases; 12 patients were available with skin gene expression signature before and after the treatment.²⁶ Sooner, reexamination of these 12 patients discovered that the SASP gene expression and its degree were significantly decreased in 3 clinical improvers, compared to 9 non-improvers after the treatment.²⁷ The results are also consistent with the attenuation by DQ treatments in the experimental lung fibrosis and senescent cell transplant in mice.^{28,29}

These clinical pilot studies are considered an unprecedented breakout in the anti-aging area in humans. The second clinical report from Mayo Clinic further illustrated that oral senolytics DQ treatment decreased senescence cells abundance in 9 diabetic patients, as shown by a decrease of cells with senescent markers: p16INK4A-, p21CIP1-expression and SAβgal activity and adipocyte progenitors with reduced replicative potential. Also, abdominal skin epidermal p16INK4A-, p21CIP1-expressing cells were attenuated, as were blood SASP factors, like IL-1α, IL-6, etc.³⁰ These improvements were consistent with findings that the DQ therapy reduced insulin resistance, proteinuria, and renal podocyte dysfunction in obesity mice.³¹ Both strongly indicate that cellular senescence is a causal factor in initiation and progression of obesity/diabetes kidney diseases and senolytic agents hold benefits in treating obesity/diabetes-related metabolic dysfunction and its complications. Recently, senotherapeutics (senolytics and senomorphics) has been greatly focused on various diseases in animal and clinical studies.^{23,32-35} Quercetin with multi-therapeutic benefits is now advocated in the Covid-19 treatment.^{36,37} Elderly people, especially those with comorbidities shows severity and high mortality in Covid-19 pandemic. The virus may efficiently display enhanced replication in senescent cells, indicating that the accumulation of senescent cells with aging and age-related diseases may play a role in the severity in old people and senotherapies may improve the clinical outcome and efficacy of vaccinations of Covid-19 virus infection.^{38,39} A new striking finding in old mice demonstrated that senolytic drugs before or after the Covid-19 virus exposure significantly reduced cellular senescence, leading to increased anti-viral antibodies and declined mortality,⁴⁰ evoking an additive therapeutic approach in elderly with Covid-19 viral infection.

Following the senolytic therapy, the second generation of commercial products as dietary supplements for healthy aging: Synext has been brought to nutrition markets, which modified the first generation of anti-aging products: Niacin (NA) and NAM (both are NAD⁺ precursors) with senolytics and other antioxidants, including Resveratrol, Curcumin, and Q10 in a total of 15 components. However, no clinical research substantiated the reality that Synext, alone or in combination with NAD⁺, prolong lifespan in healthy humans at present. It is just one of dietary supplements in nutrition markets.

Pyruvate in Healthy Aging

The commercial products for healthy aging are now becoming popular in nutrition markets, but their prevailing may be limited due to uncertain efficacy, side effects, and cost, such as Niacin Flush from Synect in nutrition markets. One among potent competitors is sodium pyruvate that holds all merits of current commercial anti-aging products in markets. As mentioned above, exogenous pyruvate spontaneously and directly generates NAD^+ via the LDH reductive reaction free of energy, raising intracellular NAD^+ levels on the equal molecular basis (Figure 1).

Pyruvate outstanding pleiotropic features mainly are (1) enhancement of cellular hypoxia tolerance, reversal of glucometabolic disorder (Warburg effect) by preserving glycolysis, via the enhancement of NAD^+/NADH ratio, and promoting oxidative phosphorylation with reactivation of pyruvate dehydrogenase (PDH) activity, leading to reversal of lethal hypoxia lactic acidosis and the Warburg effect,^{4,41,42} (2) potent (direct and indirect) anti-oxidative/nitrosative stress and -inflammation to inhibit inflammatory mediators and cytokines secretion and inflammatory cells infiltration,^{42,43} and (3) protection of mitochondrial structure and endoplasmic reticulum function as well as against cellular apoptosis and so on.^{44,45} Therefore, pyruvate protects against multiorgan (especially vital organs) dysfunction and improves survival in a diversity of pathogenic injuries, including cardiac, hemorrhagic, traumatic, and septic shock, liver cirrhosis, diabetes, neurodegenerative diseases, mitochondriopathy, and even cancer and aging; clinically, several reports demonstrated pyruvate therapeutic effectiveness and safety in various diseases as well as skin injuries.⁴⁶⁻⁵⁴

Early studies discovered that oxidative/nitrosative stress induces DNA damage. The PARP activation or NAD^+ depletion retardates the repairing DNA damage. It was also demonstrated that pharmacological restoration of NAD^+ by using exogenous pyruvate, NAD^+ , or NAM, attenuated neuronal death with Zn^{2+} -induced injuries in mice *in vitro* and *in vivo*.⁵⁵ Pyruvate not only generates NAD^+ , but also inhibits the PARP activation, as evidenced that systemic pyruvate prevented loss of total NAD^+ content and PARP activation in addition to inhibition of oxidative stress, protecting brain and liver function in swine subjected to severe hemorrhagic shock.⁵⁶

It was found a decade ago that exogenous pyruvate prevented aging of mouse oocytes though NAD^+ and senescence were not concerned in the investigation.⁵⁷ As mentioned above, NAD^+ and eNAMPT are declined with aging, aging compromised NAMPT-mediated NAD^+ biosynthesis, leading to the pathogenesis of Type 2 diabetes; NMN, a product of the eNAMPT reaction and a key NAD^+ precursor, could be effective intervention of age-induced diabetes in mice.⁵⁸ Similarly, oral pyruvate simply raised kidney NAD^+ and attenuated diabetic nephropathy in diabetic mice.⁴ Further, a recent finding showed that the late-onset caloric restriction for 14 days in

middle-aged rats, other than young ones, altered skeletal muscle metabolism by modulating pyruvate metabolism from glycolysis to oxidative phosphorylation with normalized muscle mass.⁵⁹ Furthermore, chronic sodium pyruvate supplementation with experimental chow for 6 months improved brain metabolism, proving benefits in aging-related cognitive dysfunction and inactivity although the NAD^+ level and cellular senescence were not determined, in young and middle-aged mice.⁴⁶ Notably, pyruvate has been demonstrated to preserve energy metabolism and function of matured red blood cells (RBCs) without cellular nucleus and mitochondria, the maximum tissue volume in humans,^{60,61} strongly indicating its survival protection of each individual cell throughout the body in hypoxia or even anoxia. Alternatively, it has been long recognized that the PDH activity is closely pertained in healthy aging and age-related diseases contributed from disturbances of mitochondrial bioenergetics homeostasis,^{62,63} while pyruvate is one of PDH activators, as dichloroacetate (DCA),^{3,4} to reactivate the suppressed PDH activity.^{4,56,64} Importantly, it was first demonstrated several years ago that driving pyruvate oxidation via increase of PDH activity can abrogate tumor growth in BRAF-driven melanoma cell lines by prompting oncogene-induced senescence, a critical defense against tumor progression.^{65,66} Therefore, pyruvate also shows its ant-tumor properties in animals as evidenced by DCA.⁴⁸

These early studies implied that equal to endogenous pyruvate as the key metabolite of glucose metabolism, exogenous one can modulate glucometabolic disorders, specially reverse the Warburg effect predominantly as a PDH stimulator and anti-oxidative, -inflammatory agent to facilitate prolongation of lifespan in healthy aging, as indicated in diabetic mice.^{4,63}

A recent cell investigation demonstrated that pyruvate deprivation induced mitochondrial alterations and cellular senescence in normal human dermal fibroblasts in normal cell cultures; exogenous pyruvate protected against cellular senescence in normal fibroblasts with raising NAD^+ generation, *in vitro*.⁶⁷ Further, pyruvate augments anti-aging phenotypes in skin equivalents, which closely mimics human skin, *in vivo*. The results showed that pyruvate inhibited senescence-associated β -galactosidase staining and expressions of SASP genes and protected mitochondria in the cells.⁶⁷ These data strongly suggest that pyruvate as both an antisenesence metabolite and a NAD^+ substitute may improve natural aging and aging-related diseases in consistence with prior findings that did not monitor cellular senescence mentioned above.

In another cell study, galactose-induced death of Leigh syndrome (mitochondrial complex I deficiency) patient's fibroblast cells can be rescued by both exogenous pyruvate and NAD^+ with different metabolic and molecular mechanisms. Notably, pyruvate is more efficient in the fibroblast cell viability than NAD^+ (1.0-10.0 mM) on an equimolar basis against galactose-induced death.⁶⁸ Although both pyruvate and NAD^+ increased the cellular NAD^+ levels to a different extent,

pyruvate-induced rescue of the cell death was not primarily mediated by the raised NAD^+ . The results displayed more potent anti-oxidative stress of pyruvate relative to NAD^+ ; while less ATP generation with pyruvate compared to NAD^+ in rescuing the cell death, though the cellular senescence was not detected in the study.⁶⁸ These data probably indicate that pyruvate owns a stronger capacity to repair DNA damage compared to equimolar NAD^+ . Alternatively, it was early shown in a specific simple setting of cell experiments that both exogenous pyruvate and NAD^+ in 2.5 mM equally prevented PARP-1-induced mitochondrial depolarization in neuronal death, probably because both identically reversed glycolytic inhibition.⁶⁹ Thus, DNA damage repairing may primarily does less depend on ATP requirement, but inhibition of cellular senescence albeit pyruvate, per se, is an energy substrate in selective conditions. Pyruvate probably as both a NAD^+ substitute and a novel senolytic agent may be more beneficial than NAD^+ /senolytics, per se, due to its multifactorial salutary protection of cell function, including DNA repairing in different molecular mechanisms from NAD^+ (Table 1).^{7,47}

Several pieces of evidence have displayed the pyruvate protection of DNA repairing in rat hepatocytes and mouse and human skin cells.^{47,70,71} However, no research work compares pyruvate to senolytics in this context. It is valuable to differentiate effects of NAD^+ or senolytics from pyruvate on cellular senescence in DNA damage repairing and cell survival and to explore underlying molecular mechanisms on an equimolar basis in various pathogenic assaults and healthy aging.

Importantly, a recent study indicated that the impaired NAD^+ metabolism in diabetes lead to adverse effects from NAD^+ and its precursors.⁷² Thus, chronic oral pyruvate may be advantageous over NAD^+ supplementation in diabetes patients for healthy aging. Further studies are urgently required in comparison of NAD^+ to pyruvate in the elderly with or without diabetes. In fact, pyruvate clinical trials are recently recommended.^{73,74} It is worthy of note that the acidic pyruvate aqueous solution is long-term stable and pyruvate as a dietary supplement is permitted according to US FAD documents.^{48,75}

Intriguingly, ethyl pyruvate (EP, a pyruvate derivative), a well-known high mobility group box 1 (HMGB1, an *inflammatory* cytokine in *later stages*) inhibitor, also reversed the senescent phenotype of bone marrow-mesenchymal stem cells from MRL/lpr (Lupus) mice. Further, it alleviated the pathological alterations of lupus nephritis (significant reduction of albuminuria and renal histological changes) and prolonged survival of the mice.⁷⁶ It was newly further demonstrated that EP with glycyrrhizic acid through the inhibition of HMGB1 release and inflammatory senescence prevented Tau oligomers-induced cellular senescence and tauopathies, such as Alzheimer's disease, in the brain of mice.⁷⁷ In this regard, EP was recently proposed in Covid-19 treatments,⁷⁸ as mentioned above due to susceptibility of cellular senescence to viral infection.³⁷

Similarly, pyruvate is also brain protective against tauopathy and sodium pyruvate was hypothesized in the intervention of Covid-19 viral infection, too.^{46,75}

Data from EP additionally support pyruvate effects on eliminating cellular senescence in animal studies and encourage clinical applications of pyruvate-enriched resuscitation fluids, including IV solutions and oral rehydration salt (Pyr-ORS) in diseases and healthy aging because EP does not work in humans.^{48,64} IV pyruvate solutions would be superior to current fluids in critical care patients with various severe pathogenic injuries in reversal of lethal hypoxic lactic acidosis and diabetic ketoacidosis, improving survival.^{41,42,75} Given the clinical safety with large doses of oral or IV pyruvate administration in severe diseases, such as diabetes and mitochondriopathy, oral pyruvate in Pyr-ORS to efficiently enhance pyruvate absorption from intestine may be preferably as a functional beverage for anti-diabetes and -aging in a large population.^{4,48,75,79} However, current evidence of pyruvate eradication of cellular senescence and exertion of senolytic effects is still limited and largely underdeveloped. Further studies are warranted, particularly lying on the blood effective pyruvate levels absorbed from enteral pyruvate.⁴⁸ Nevertheless, it would be hopeful that the salutary effect of oral pyruvate-enriched fluids for healthy aging is feasible and practicable with clinical safety and low cost.^{48,79}

"Strengthening research, data, and innovation" is one of topics in the United Nations (UN) Decade of Healthy Ageing (2021-2030) that was just launched.⁸⁰ Pyruvate may play a specific role in preventing diseases and improving healthy aging in the UN Decade.

Conclusion

Albert numerous investigations demonstrate that NAD^+ in its precursors and senolytics are protective against diseases and aging, current studies indicate that pyruvate as one of novel senolytics besides a NAD^+ substitute may be a potent agent. Oral Pyr-ORS and IV pyruvate-enriched fluids as a potential nutraceutical intervention may be more beneficial with clinical safety relative to current products in nutrition markets for diseases and healthy aging. Further research and clinical trials to compare pyruvate with NAD^+ , senolytics, specifically on the equimolar basis, or their combination in healthy aging are required.

Author Note

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Author contribution

FQZ prepared the manuscript draft and completed the revised version following the reviewer's comment.

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