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Progress of Anti-Aging Drugs and Their Formulation

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Abstract*—Advancements in biotechnology has dramatically improved the screening of anti-aging drugs. A number of drugs have been reported to extend life span based on animal and or human clinical study. Approaches like reverse cellular aging, antioxidant, and various drug's mechanisms are proposed. The success of an anti-aging drug application relies not only on the early stages of drug screening and preclinical studies but also PK/PD prediction prior to the clinical study. In drug dosage design, the oral formulation is still the most commonly needed due to its convenience in administration. In addition, to improve the development of anti-aging oral formulation, an alternative method using FDA approved drugs PK database and PAMPA Dissolution was also proposed for formulation development*.

***Keywords*—** *Area Under Curve, In vitro to in vivo correlation, Maximum plasma concentration, Parallel Artificial Membrane Permeability Assay, Administration Distribution Metabolism and Excretion, New Chemical Entity, Pharmacokinetics and Pharmacodynamics*.

I. INTRODUCTION

Humanity has been looking for the solutions of anti-aging for more than thousands of years. Not until recently, a number of interventions have been developed to overcome aging problems. Several reasons could be attributed to the development of anti-aging intervention: (a) aging related diseases has been a public health issue. (b) a healthy life is the general public interest especially after retirement. (c) the need for continuation and advancement in the scientific research. All these reasons have led to the strong development of anti-aging interventions in the past decade. Several noticeable research of interventions are: (1) reverse cellular aging by mix cocktails of chemicals, (2) anti-oxidants of glycine and n-acetyl cysteine (GlyNac), (3) through mTor and Sirtuin1 expression for metformin, (4) unknown pathway of rapamycin, (5) anti-oxidant of resveratrol, (6) increase NAD+ producing via NMN, (7) fasting and exercise. This review addresses details of these intervention mechanisms and their oral formulation.

Furthermore, oral formulation is usually preferred due to its convenience in both carrying and administration. However, the complexity in the oral drugs' ADME (absorption, distribution, metabolism, and excretion) processes makes drug formulation difficult to design. Currently, there are various ADME simulation software available to help oral drug formulation design. Traditionally, the in vitro dissolution experiments were used to examine the performance of an oral drug formulation.

However, an alternative method that can be used to improve IVIVC (in vitro to in vivo correlation) for oral drug prediction is Parallel Artificial Membrane Permeability Assay (PAMPA) (1), which uses a chemically-based membrane instead of alive cells but has been proven to be able to accurately mimic the human small intestine (Figure 1) (2) is an instrument that combines dissolution and permeation in a way that closely simulates *in vivo* conditions. It measures the two necessary parameters—dissolution and permeation—for finding oral drug absorption via previously validated (3) equation F (drug absorbed) = Cb*Pe*Area and produces real-time graphs for dissolution and permeation. These graphs enable the calculation of Area Under Curve (AUC) and maximum plasma concentration (Cmax) values, which aid in predicting NDA formulation with respect to the RLD (Reference Listed Drugs) that share similar PK parameters to NCE. Such a PAMPA dissolution apparatus may also help designing the anti-aging drug formulation.

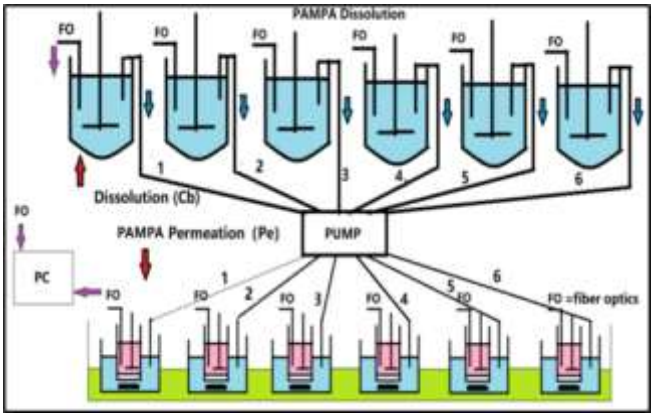

Fig. 1. PAMPA Dissolution apparatus

II. MATERIALS AND METHODS

Clinical PAD (Pharmaceutical Active Dose) Estimation

Currently, the clinical PAD estimation is required per FDA requirements. It could usually be estimated via ADME software prediction; however, an alternative PAD estimation for oral drugs formulation could also be estimated using FDA approved drugs PK database and PAMPA dissolution from J. Chou (2).

III. RESULTS AND DISCUSSION

This review discuss the details of these anti-aging drugs.

A. Reverse Cellular Aging

A chemical process is invented by J. H. Yang et. al.（4）to reverse a loss of epigenetic information in cellular aging. High-throughput cell-based assays are developed to

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distinguish young from old and senescent cells, including transcription-based aging clocks and a real-time nucleocytoplasmic compartmentalization (NCC) assay. There are six chemical cocktails found that, without changing cellular identity, restores a youthful genome-wide transcript profile and reverse transcriptomic age. Data is shown in Figure 2.

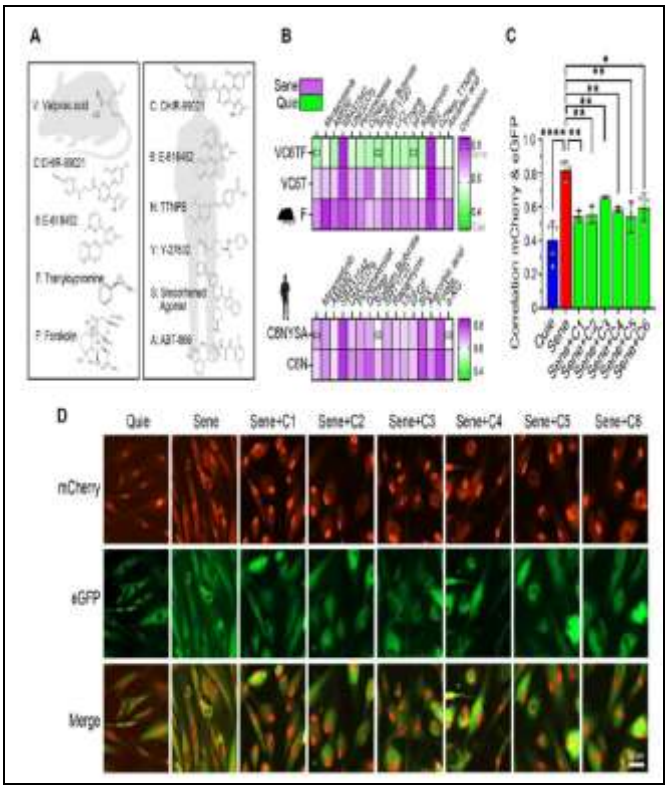

Fig. 2. Reprogramming small molecule cocktails restore NCC alterations in senescent cells. (A) Chemical structures of small molecules of basal cocktails used to generate induced pluripotent stem cells (iPSCs) from mouse (left) or human (right) somatic cells. (B) Correlation heatmaps showing eGFP and mCherry colocalization in human senescent fibroblasts demonstrate the effects of 80 different combinations of small molecules (n=2). (C, D) Validation of six selected cocktails through independent experiments, showing colocalization (C) and representative images (D) of eGFP and mCherry signals. Scale bar, 50 μm. Data are mean ± SD. *p < 0.05; **p < 0.01; ****p < 0.0001. One-way ANOVA-Bonferroni.

B. Glycine and N-Acetyl Cysteine (GlyNac)

In the cell's energy generation, mitochondria produces harmful reactive oxygen species (ROS) that induce oxidative stress (OxS). To prevent OxS, mitochondria needs antioxidants for protection, and glutathione (GSH) is the most abundant intracellular antioxidant. Acute depletion of intracellular GSH concentrations at age beyond 50 results in mitochondrial injury or irreversible cell damage. In the pilot study performed by R.V. Sekhar (5), it suggest that supplementing GlyNac in patients with T2D could improve defects in mitochondrial function, lower insulin resistance and circulating plasma fatty-acid concentrations. Data from this study is shown in Figure 3.

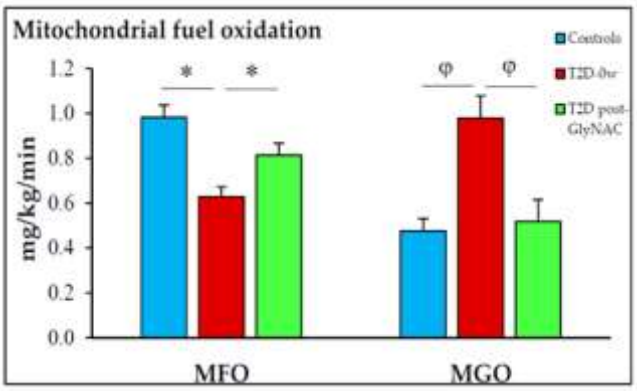

Fig. 3. GlyNac supplementation improves impaired mitochondrial fuel oxidation with T2D. T2D = type 2 diabetes; MFO = mitochondrial fatty-acid oxidation; MGO = mitochondrial glucose oxidation; T2D-0w = T2D patients before GlyNac supplementation; T2D patients 2-weeks after GlyNac supplementation.

A further placebo controlled study from P. Kumar et. al. (6) has pointed out that GlyNac supplement can significantly improve aging related biomarkers hence may lead to longevity as following:

Elevated oxidative stress (OxS), mitochondrial dysfunction, and hallmarks of aging are identified as key contributors to aging, but improving these defects in older adults (OA) is challenging. In prior studies, the intracellular antioxidant glutathione (GSH) could play a role and reported that supplementing GlyNac in aged mice improved GSH deficiency, OxS, mitochondrial fatty-acid oxidation (MFO), and insulin resistance (IR). Compared to young adults (YA), OA had GSH deficiency, OxS, mitochondrial dysfunction, inflammation, endothelial dysfunction, IR, multiple aging hallmarks, impaired physical function, increased waist circumference, and systolic blood pressure. GlyNac supplementation in OA significantly improved these defects. Similar positive effects were also observed from our data collected in Taiwan.

C. Metformin

Recent studies have indicated that blood sugar lowering drug metformin could extend life span in reducing early mortality associated with diabetes, including cardiovascular disease, cognitive decline and cancer. Metformin can improve health span thereby extending the period of life span in good health. I. Mohammed et.al. (7) has indicated that the beneficial effects of metformin on aging and health span are primarily via its effects on cellular metabolism and result from its anti-hyperglycemic action, improving insulin sensitivity, reduction of oxidative stress and protective effects on the endothelium and vascular function. Potential pathways are indicated in Figure 4.

However, J. Stevenson-Hoare et al. (8) has pointed out that after a 20-year long term study of metformin on longevity between people with type 2 diabetes and matched non-diabetic controls, metformin therapy showed a benefit over matched controls in first three years, but this reversed after five years of treatment.

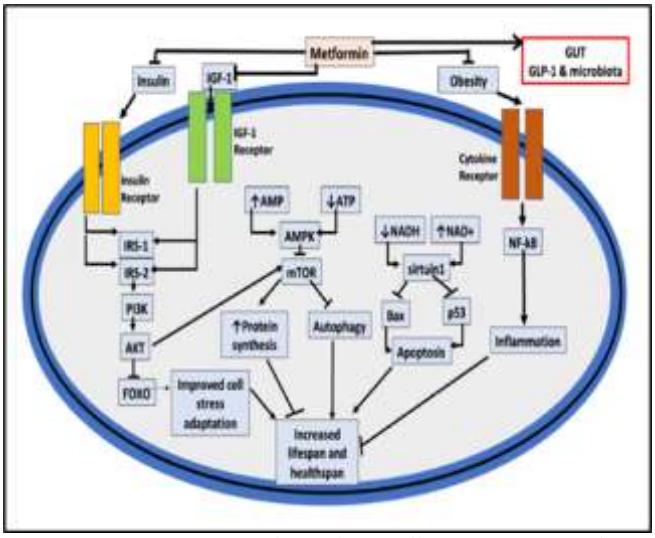

Fig. 4. Potential cellular targets for metformin affect healthspan and lifespan.

Therefore, it is suggested that longer study periods are required for longevity with metformin in future research.

D. Rapamycin

A critical review from Z. D. Sharp et.al. (9) has pointed out the pros and cons of rapamycin in Figure 5.

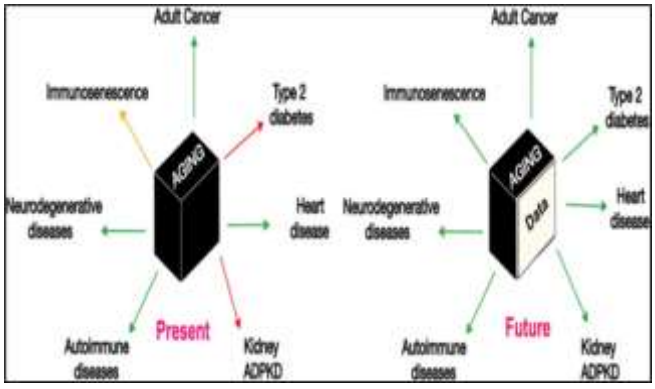

Fig. 5. Aging, represented by the black box, is one of if not the hardest problems in biology. We do know it causes or at least contributes to a wide variety of late adult stage diseases. Rapamycin has variable effects on these diseases. The left panel shows some that it helps (green arrows) and others it hurts (red arrows). It appears to have both good and (not so) bad effects on the immune system (gold arrow) and might be better termed an immune modulator (Kolosova et al., 2013).

W. Palm et al. (10) has also indicated that unrealized side effects may also emerge, such as the recent discovery that mTORC1 inhibition can accelerate the growth of solid tumors in a mouse model of pancreatic cancer by stimulating the catabolism of extracellular proteins.

E. Resveratrol

A critical review of resveratrol from B. Salehi et. al. (11) has pointed it out as "A Double-Edged Sword in Health Benefits" for humanity. As can be seen from Figure 6. that resveratrol may be used with care for long term purpose.

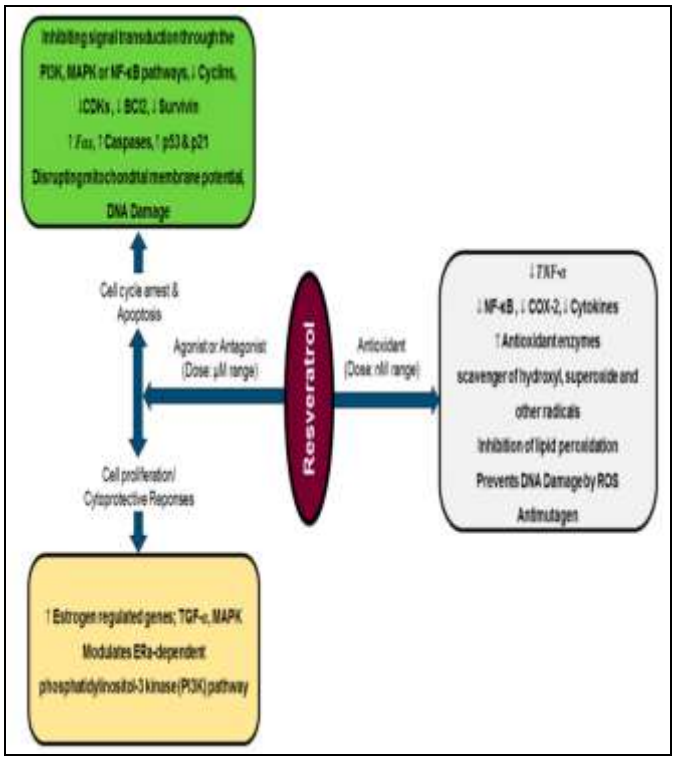

Fig. 6. Possible pathways of resveratrol

V. A. Brown et al. (12) has reported that resveratrol decrease in circulating IGF-I and IGFBP-3 might contribute to cancer chemopreventive activity. Similarly, S. Mukherjee et al.（13） has pointed that at a lower dose, resveratrol can be very useful in maintaining the human health whereas at a higher dose, resveratrol has pro-apoptotic actions on healthy cells, but can kill tumor cells.

F. NMN (Nicotinamide Mononucleotide)

Nicotinamide is converted to NMN, which is then converted to NAD+ when it enters the body. NAD+ deficiency can lead to potential health problems, including age-related metabolic disorders, mental disorders, and neurodegenerative diseases. Obesity, diabetes, depression, anxiety, Alzheimer's disease and Parkinson's disease are examples of age-related diseases that can be linked to NAD+ deficiency. The placebo controlled clinical study resluts of L. Yi et. al. (14) is shown in Figure 7.

In conclusion, NMN supplementation had a positive impact on the physical endurance as demonstrated in the significant improvement of six-minute walking test, blood biological age, and SF-36 scores.

However, Y. Fukamizu et al. (15) reported that through rigorous methods, including a randomized, double-blind, placebo-controlled design, they found that the effects of oral administration of β-NMN, particularly at a dose of 1250 mg once daily for up to 4 weeks, were limited or negligible in healthy adult men and women. There were no significant deviations observed in various parameters such as anthropometry (measurement of the human body), hematological (related to blood), biochemical, urine, and body

composition analyses. This suggests a lack of substantial impact on these aspects of health.

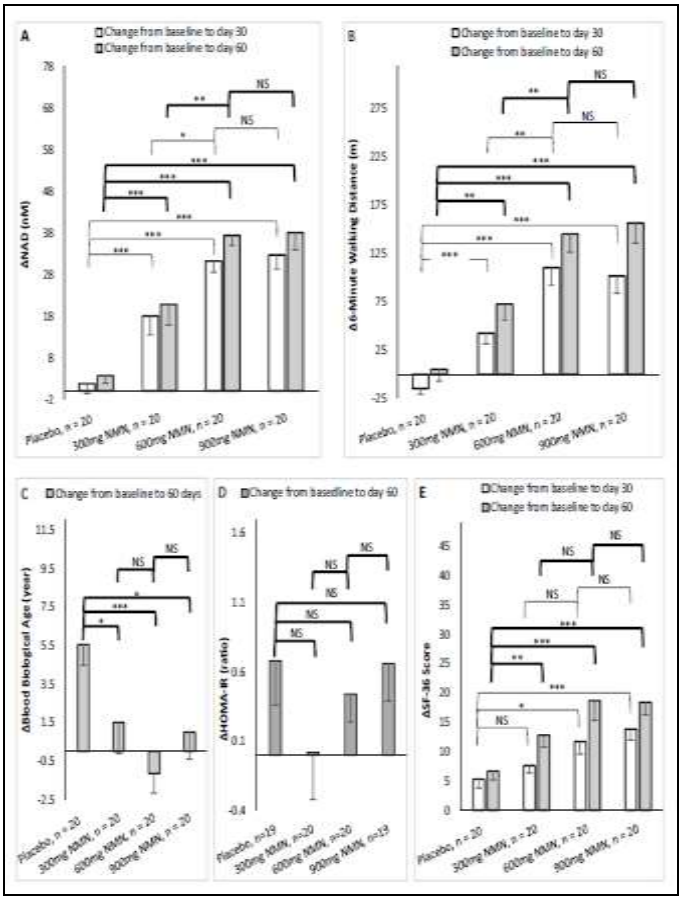

Fig. 7. Efficacy of the placebo and three NMN-treated groups, comparisons of the three treated groups vs. placebo, 600 mg vs. 300 mg, and 900 mg vs. 600 mg on the changes of efficacy (Δmean ± SEM) from baseline to day 30 and/or day 60. A Comparisons on the changes of blood NAD concentrations from baseline to day 30 and day 60. B Comparisons on the changes of 6-minute walking distances from baseline to day 30 and day 60. C Comparisons on the changes of blood biological ages from baseline to day 60. D Comparisons on the changes of HOMA-IR ratio from baseline to day 60. E Comparisons on the changes of SF-36 scores from baseline to day 30 and day 60.

G. Exercise And Fasting

A comprehensive review of X. Cao et al. (16) has pointed out how aging, metabolic disease, and exercise are interconnected, particularly in individuals over 65 years old. With aging populations and increasing rates of metabolic disorders, including type 2 diabetes and fatty liver disease, exercise emerges as a crucial mitigating factor. While research has focused on how aging affects metabolic function and how exercise counters these effects, less attention has been given to how different tissues coordinate during exercise, especially in older adults. Understanding this coordination is vital as exercise prompts long-term adaptations that protect against metabolic diseases. This review aims to explore how exercise influences skeletal muscle, liver, and adipose tissue metabolism, ultimately improving metabolic health in older individuals. A. D. S. Lages et al. (17) presents a graph shows that exercise has counters aspects of aging such as genomic instability, telomere attrition, epigenetic alterations, loss of proteostasis, dysregulated nutrient sensing, mitochondrial dysfunction, cellular senescense, and stem cell exhaustion. A. Rebelo-Marques et al. (18) recommends exercises including aerobic, muscle strengthening, flexibility, and neuromotor exercises to reduce the effects of aging. For less fit individuals, starting with lighter intensity and shorter duration is advised, gradually progressing as fitness improves. Supervision by a qualified professional is recommended, particularly for those with cardiovascular issues. Neuromotor exercises, focusing on balance and agility, are beneficial for those at risk of falls or with mobility limitations. Increasing exercise difficulty over time can be achieved through various methods such as altering posture, introducing dynamic movements, targeting postural muscles, and reducing sensory input.

Intermittent fasting (IF) is a dietary approach that involves alternating periods of fasting with periods of normal food intake (e.g., 60% energy restriction on 2 days per week or every other day). Periodic fasting (PF) is a broader term that encompasses fasting periods lasting from a few days to several weeks. One key difference between IF and PF lies in their potential effects on metabolic health and disease.

In fasting studies, M. P. Mattson at al. (19) have pointed out that IF (intermittent fasting) has been shown to improve multiple health indicators, including weight loss, insulin resistance, and reductions in risk factors for cardiovascular disease in both animal and human subjects. These effects may be attributed to the activation of adaptive cellular stress response signaling pathways, which enhance mitochondrial health, DNA repair, and autophagy. PF (periodic fasting) with its longer fasting periods, may have even more profound effects on health and disease. Studies on animals have demonstrated that PF can ameliorate various age-related disorders, including diabetes, cardiovascular disease, cancer, and neurological disorders such as Alzheimer's disease, Parkinson's disease, and stroke. PF may also promote stem cell-based regeneration and induce long-lasting metabolic effects. A. Nencioni et al. (20) have reported that the relationship between cancer treatment and fasting revolves around the concept of exploiting the differences in metabolic vulnerabilities between cancer cells and normal cells. Cancer cells exhibit a dependency on specific nutrients and metabolic pathways for their growth and survival. Fasting or fasting-mimicking diets (FMDs) induce significant alterations in growth factors and metabolite levels, creating an environment that is unfavorable for cancer cell adaptation and survival. This metabolic stress can weaken cancer cells, making them more susceptible to the effects of conventional cancer therapies such as chemotherapy and radiation. Furthermore, fasting or FMDs promote tissue regeneration in normal cells, which can aid in recovery and mitigate the risk of long-term complications associated with cancer treatments.

IV. Conclusion

PAMPA dissolution may serve as an alternative method to the PAD estimation prior to the clinical study. Several drugs and their mechanisms have been proposed to have anti-aging properties through preclinical and or clinical studies.

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Advancement in biotech has also helped clarifying the relation of anti-aging and its pathways. In this review, the anti-aging methods of "GlyNac supplementation" and "Exercise and Fasting" are favored than others. However, more clinical studies may be needed to prove the long term safety and efficacy of these anti-aging drugs. In the coming future, with the tools of targets and chemical database, AI docking, ADME simulation, PAMPA Dissolution, and FDA approved drugs PK database, it could potentially improve oral anti-aging drug formulation design and its PK prediction.

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REFERENCES

[1]. K. Sugano, H. Hamada, M. Machida, H. Ushio, K. Saitoh, and K. Terada. "Optimized conditions of bio-mimetic artificial membrane permeation assay". Int. J. Pharm. ;228(1-2):181-8. Oct, 2001, doi: 10.1016/s0378-5173(01)00845-6. PMID: 11576780.

[2]. J. Chou, R. Lai, J. Chou, S. Fu, and W. H. Wang. "PAMPA Dissolution: An alternative method for oral NDA formulation development". RJPDFT 16(2)，2024.

[3]. S, Patrick, L. Glen, and A. Gordon."Predicting Fraction Dose Absorbed in Humans Using a Macroscopic Mass Balance Approach". Pharmaceutical Research. 8，1991. Doi:10.1023/A:1015892621261.

[4]. J. Yang, C. A. Petty, T. D. McDougall, M. V. Lopez, A. Tyshkovskiy, S. M. Lewis, X. Tian, N. Ibrahim, Z. Chen, P. T. Griffin, M. Arnold, J. Li, O. A. Martinez, A. Behn, R. R.Hammond, S. Angeli, V. N. Gladyshev, D. A. Sinclair. **"**Chemically induced reprogramming to reverse cellular aging" Aging, Vol. 15, No. 13, 2023.

[5]. R. V. Sekhar, **"**GlyNAC (Glycine and N-Acetylcysteine) Supplementation Improves Impaired Mitochondrial Fuel Oxidation and Lowers Insulin Resistance in Patients with Type 2 Diabetes: Results of a Pilot Study", Antioxidants 11, 154, 2022.

[6]. P. Kumar, C. Liu, J. Suliburk, J. W. Hsu, R. Muthupillai, F. Jahoor, C. G. Minard, G. E. Taffet, and R. V. Sekhar, "Supplementing Glycine and N-Acetylcysteine (GlyNAC) in Older Adults Improves Glutathione Deficiency, Oxidative Stress, Mitochondrial Dysfunction, Inflammation, Physical Function, and Aging Hallmarks: A Randomized Clinical Trial" J Ger A Biol Sci Med Sci, Vol. 78, No. 1, 75–89, 2023.

[7]. I. Mohammed, M. D. Hollenberg, H. Ding and C. R. Triggle, "A Critical Review of the Evidence That Metformin Is a Putative Anti-Aging Drug That Enhances Healthspan and Extends Lifespan" Frontiers in Endocrinology, Vol. 12, 2021.

[8]. J. Stevenson-Hoare, G. Leonenko and V. Escott-Price, "Comparison of long-term effects of metformin on longevity between people with type 2 diabetes and matched non-diabetic controls", BMC Public Health 23:804, 2023.

[9]. Z. D. Sharp and R. Strong, "Rapamycin, the only drug that has been consistently demonstrated to increase mammalian longevity. An update", Experimental Gerontology 176, 112166, 2023.

[10]. W. Palm, Y. Park., K. Wright, N. N. Pavlova, D. A. Tuveson, and C. B. Thompson, "The utilization of extracellular proteins as nutrients is suppressed by mTORC1", Cell 162, 259–270, 2015.

[11]. B. Salehi, A. P. Mishra, M. Nigam, B. Sener, M. Kilic, M. Sharifi-Rad, P. T. Fokou, , N. Martins and J. Sharifi-Rad, "Resveratrol: A Double-Edged Sword in Health Benefits", Biomedicines 6, 91; 2018, doi:10.3390/biomedicines6030091.

[12]. V. A. Brown, K. R. Patel, M. Viskaduraki, J. A. Crowell, M. Perloff, T. D. Booth, G. Vasilinin, A. Sen, A. M. Schinas, G. Piccirilli, K. Brown, W. P. Steward, A. J. Gescher, and D. E. Brenner, "Repeat Dose Study of the Cancer Chemopreventive Agent Resveratrol in Healthy Volunteers: Safety, Pharmacokinetics and Effect on the Insulin-like Growth Factor Axis", Cancer Res; 70(22) November 15, 2010.

[13]. S. Mukherjee, J. I. Dudley, and D. K. Das, "Dose-dependency of resveratrol in providing health benefits", Dose-Response, 8:478–500, 2010.

[14]. L. Yi, A. B. Maier, R. Tao, Z. Lin, A. Vaidya, S. Pendse, S.Thasma, N. Andhalkar, G. Avhad, V. Kumbhar, "The efficacy and safety of β-nicotinamide mononucleotide (NMN) supplementation in healthy middle-aged adults: a randomized, multicenter, double-blind, placebo-controlled, parallel-group, dose-dependent clinical trial", GeroScience, 45:29–43, 2023.

[15]. Y. Fukamizu, Y. Uchida, A. Shigekawa, T. Sato, H. Kosaka and T. Sakurai, "Safety evaluation of β-nicotinamide mononucleotide oral administration in healthy adult men and women", Scientific Reports, 12:14442, 2022.

[16]. X. Cao, and J. P. Thyfault, "Exercise drives metabolic integration between muscle, adipose and liver metabolism and protects against aging-related diseases", Experimental Gerontology 176, 112178, 2023.

[17]. A. D. S. Lages,, V. Lopes, J. Horta, J. Espregueira-Mendes, R. Andrade and A. Rebelo-Marques, "Therapeutics That Can Potentially Replicate or Augment the Anti-Aging Effects of Physical Exercise", Int. J. Mol. Sci. 23, 9957, 2022.

[18]. A. Rebelo-Marques, A. D. S. Lages, R. Andrade, C. F. Ribeiro, A. Mota-Pinto, F. Carrilho and J. Espregueira-Mendes"Aging Hallmarks: The Benefits of Physical exercise", Frontiers in Endocrinology, Volume 9, May 2018.

[19]. M. P. Mattson, V. D. Longo, and M. Harvie, "Impact of intermittent fasting on health and disease processes", Ageing Res Rev. ; 39: 46–58. 2017, doi:10.1016/j.arr.2016.10.005.

[20]. A. Nencioni, I. Caffa, S. Cortellino, and V. D. Longo, "Fasting and cancer: molecular mechanisms and clinical application", Nat Rev Cancer. 18(11): 707–719. 2018. doi:10.1038/s41568-018-0061-0.