Proceedings of ICBioMed 2025 Symposium: Extended Reality (XR) Applications in Medical Imaging
DOI: 10.54254/2753-8818/2025.PJ25364

A Review of Metformin's Anti-aging Mechanisms

Zixuan Zhao

Jincheng No.1 High School, Jincheng, China
zzhao3@fandm.edu

Abstract. Metformin is a frequently prescribed pharmaceutical for the therapeutic management of type 2 diabetes mellitus. Recent discoveries indicate that metformin possesses potential anti-aging properties, leading to further investigation of the mechanisms behind this action. This research examines the intrinsic processes by which metformin demonstrates its anti-aging effects, based on existing literature and data. The discourse commences with an exploration of the etiology of aging, succeeded by a comprehensive analysis of the fundamental mechanisms that underpin metformin's anti-aging properties. Subsequently, the paper reviews animal experiments related to metformin's anti-aging properties, categorized into animal models and cellular models. Finally, the paper concludes with a discussion on non-core mechanisms of metformin, clinical trials, and future research directions. Metformin delays the aging process through direct or indirect activation of AMP-activated protein kinase (AMPK), which subsequently modulates multiple longevity-associated pathways. Specifically, it activates sirtuins (SIRTs), inhibits the mammalian target of rapamycin (mTOR) pathway, and suppresses NF-κB signaling. Additionally, metformin enhances the antioxidant response by promoting the activation of the Nrf2 pathway, thereby reducing reactive oxygen species (ROS) accumulation. Furthermore, it contributes to metabolic regulation by inhibiting gluconeogenesis. Together, these mechanisms collectively mediate metformin's anti-aging effects.

Keywords: Metformin, Anti-aging, Signaling pathways, Mechanisms, Review

1. Introduction

Metformin was initially identified as a guanidine derivative isolated from Galega officinalis (French lilac or goat's rue), where it exists as a natural biguanide compound. Historically, this medicinal plant was employed in traditional treatments for various ailments including fever, snakebites, and plague. The modern therapeutic application of metformin was established in 1957 when French physician Jean Sterne pioneered its clinical use for diabetes management, marking the first major milestone in the development of metformin as an antidiabetic agent. In 1995, due to concerns over lactic acidosis, the United States discontinued the use of phenformin and buformin while adopting metformin as the preferred biguanide for diabetes treatment [1]. To date, metformin has been clinically utilized in the management of type 2 diabetes for over six decades, maintaining its position as a first-line therapeutic agent. Recent studies employing long-term metformin administration in model organisms (including rodents, Drosophila melanogaster, and Caenorhabditis

DOI: 10.54254/2753-8818/2025.PJ25364

elegans) have demonstrated significant lifespan extension, suggesting metformin's potential geroprotective properties [2]. This review systematically examines: (1) the etiological basis of aging through the lens of the nine hallmarks of aging, molecular damage, oxidative stress, and senescence-associated secretory phenotype (SASP); (2) the core mechanisms underlying metformin's anti-aging effects via its molecular targets (including AMPK, mTOR, SIRT1, and FOXO signaling pathways) and modulation of gut microbiota; and (3) concludes with an analysis of metformin's anti-inflammatory mechanisms and future research directions in geroscience. This study provides a valuable foundation for future advancements in the field of anti-aging research.

2. The etiological basis of aging

Aging is a complex physiological process driven by multifactorial mechanisms. The classification of aging as a disease entity remains a contentious issue in academia. Proponents argue that the biomolecular alterations observed in aging mirror those in certain pathological conditions, thereby warranting its categorization as a treatable disease. Opponents, however, maintain that aging represents a natural biological progression. This debate underscores the inherent challenge in distinguishing between cellular-level pathological changes and physiological decline. López-Otín et al. originally classified nine hallmarks of aging into primary (genomic instability, epigenetic shifts, proteostasis decline, telomere shortening), antagonistic (nutrient-sensing dysregulation, mitochondrial ROS overproduction), and integrative (cellular senescence/SASP, stem cell depletion, disrupted cell signaling). In 2023, they augmented this model with chronic inflammation, microbiome dysbiosis, and impaired autophagy [3].

Aging is evident through interrelated microscopic and macroscopic events. Molecularly, it signifies the accumulation of post-reproductive entropy that undermines molecular fidelity, while systemically, ROS-mediated mitochondrial dysfunction, indicated by mtDNA depletion, serves as a crucial factor in lifespan and degenerative pathology, as evidenced by lifespan extension through ROS reduction [4].

The SASP phenomenon describes how growth-arrested senescent cells secrete bioactive factors (cytokines, chemokines) that initially aid tissue repair but chronically promote carcinogenesis and inflammation through genetic dysregulation [5]. Evidence suggests dietary control (reducing glucose uptake/insulin resistance) and body fat management represent effective anti-aging strategies by inducing beneficial energy restriction [6].

3. Core mechanisms of metformin in anti-aging interventions

High-throughput screening of ~300,000 compounds identified 41 potential metformin targets including mitochondrial complex I, mGPDH, and KDM6A/UTX demethylase, directly linking to its metabolic and epigenetic regulatory functions [1].

3.1. The AMP-Activated Protein Kinase (AMPK) signaling pathway

Metformin can exert several biologically beneficial effects independently of AMPK. Specifically, metformin directly inhibits cancer cell growth by suppressing the electron transport chain in mitochondrial complex I; it also reduces blood glucose levels in rats lacking AMPK activation targets through direct action. Furthermore, the metformin-nuclear pore complex (NPC)-Rag C-mTORC1-ACAD10 signaling pathway directly promotes growth in Caenorhabditis elegans, while metformin directly inhibits the mTORC1 signaling pathway via Rag GTPase [1]. Conversely, the

DOI: 10.54254/2753-8818/2025.PJ25364

anti-aging process requires coordinated action between the cellular energy sensor AMPK and metformin. AMPK consists of α, β, and γ subunits, with the phosphorylation activation site located on the α subunit. Metformin inhibits mitochondrial complex I, leading to reduced cellular energy levels, decreased ATP synthesis, and an elevated AMP/ATP ratio, thereby activating AMPK through phosphorylation. The activated AMPK then indirectly suppresses the mTOR signaling pathway by promoting the tumor suppressor genes TSC1 and TSC2 to inhibit Rheb, a protein essential for mTOR pathway activation. This results in the suppression of phosphorylation at S772 and S792 sites, ultimately inhibiting cellular metabolism, curbing cancer cell proliferation and metastasis, and enhancing autophagy. This autophagic process facilitates the delivery of senescent or damaged organelles to autophagosomes, providing nutrients during cellular starvation [5].

AMPK promotes the production of nicotinamide riboside (NAD^+), which activates the silent information regulator (SIRT1), also known as the longevity protein. This activation enables PGC-1α and FOXO to undergo SIRT1-mediated deacetylation, thereby enhancing cellular resistance to mitochondrial-derived free radical damage while simultaneously facilitating DNA repair, promoting autophagy, and augmenting antioxidant enzyme activity. The global regulatory effects of AMPK are further mediated through downstream mTORC1 and SIRT1 pathways, ultimately achieving multi-organ synergistic anti-aging effects.

3.2. The mammalian Target Of Rapamycin (mTOR) signaling pathway

The mTORC1 signaling pathway serves as a "dual safeguard system," primarily regulating cellular growth, metabolism, and energy homeostasis. mTORC1 is governed by two molecular "switches": the TSC-Rheb pathway (responding to cellular energy status) and the Rag GTPase pathway (sensing amino acid availability). The TSC-Rheb pathway consists of TSC (comprising TSC1 and TSC2) and Rheb (a small GTPase). Under energy-sufficient conditions, growth factors activate the PI3K-AKT signaling pathway. AKT-mediated phosphorylation of TSC2 inhibits the TSC complex. Activated Rheb binds GTP to promote mTORC1 activation. Under energy-deficient conditions, AMPK phosphorylates TSC2. The activated TSC complex promotes GDP-bound Rheb. mTORC1 becomes inhibited. The Rag GTPase pathway consists of Rag A/B and Rag C/D. During amino acid sufficiency, Rag GTPase becomes activated. Rag A/B binds GTP while Rag C/D binds GDP. mTORC1 translocates to lysosomes. Then, mTORC1 integrates Rheb signals for full activation. During amino acid deficiency, Rag A/B binds GDP while Rag C/D binds GTP. The Rag GTPase system becomes inactivated. mTORC1 dissociates from lysosomes. mTORC1 remains inhibited. Metformin directly inhibits mTOR, reducing cellular ATP levels and consequently impairing NPC-mediated transport. This prevents nuclear entry of Rag C (a GTPase)—an essential prerequisite for complete mTOR activation—thereby suppressing mTOR [2]. mTOR typically inhibits the autophagy kinase ULK1; hence, mTOR prevents the activation of ULK1, thereby inhibiting autophagy, cancer cell development, proliferation, and metabolism, reducing the buildup of misfolded proteins, alleviating cellular stress, and mitigating cellular senescence. Misfolded proteins are linked to neurodegenerative illnesses, such as Parkinson's, and this process efficiently mitigates age-related pathologies[5].

3.3. The Silent Information Regulator (SIRT1) signaling pathway

SIRT1 was initially identified in yeast (Saccharomyces cerevisiae) where it demonstrated lifespan regulation and mitochondrial bioactivity repair functions [7].

DOI: 10.54254/2753-8818/2025.PJ25364

Activated AMPK promotes phosphorylation of NAD^+-dependent deacetylase SIRT1, thereby facilitating epigenetic protein modifications. This process relieves mTORC1 inhibition, and the activated mTORC1 subsequently enhances NAD^+ biosynthesis, creating a positive feedback loop that further amplifies SIRT1 activity. Additionally, metformin reduces the Michaelis constant (KM)—a kinetic parameter reflecting enzyme-substrate affinity (where lower KM indicates higher affinity)—of NAD^+, leading to SIRT1 activation. This method repairs functionally deficient or injured mitochondria, reduces intracellular reactive oxygen species (ROS) levels, and alleviates ROS-induced cellular damage [5].

3.4. The anti-aging mechanism of Forkhead Box Protein O (FOXO)

The Forkhead box protein O (FOXO) transcription factor implements its anti-aging effects via two principal pathways. On one side, the overexpression of antioxidant enzymes, such as superoxide dismutase (SOD), enhances the elimination of detrimental intracellular molecules and mitigates oxidative damage. Conversely, the stimulation of autophagy serves to remove defective mitochondria and sustain proteostasis [8]. Specifically, the previously stated AMPK/SIRT1/mTORC1 signaling circuit.

3.5. The anti-aging effects of gut microbiota

Metformin alters the composition of intestinal microbiota by increasing the abundance of beneficial bacterial species, including Akkermansia muciniphila (a mucin-degrading bacterium) and short-chain fatty acid (SCFA)-producing bacteria. Cabreiro et al. first demonstrated that metformin extends lifespan in Caenorhabditis elegans through microbial-mediated modulation of folate and methionine metabolism. However, the exact microbial targets of metformin and the underlying host-microbiome crosstalk in its anti-aging effects require further investigation [1].

4. Animal experiments on metformin's anti-aging

4.1. Sex differences in metformin's extension of healthy lifespan

Previous studies have suggested that metformin may extend lifespan in model organisms, but the effects of sexual dimorphism remain unclear. In 2022, Vinciguerra M. et al. conducted a study aiming to evaluate the effects of metformin on lifespan and healthspan in genetically heterogeneous UM-HET3 mice (both males and females) and to investigate the underlying mechanisms. The experiment utilized 4-month-old (young adulthood) UM-HET3 mice divided into four groups: male controls on a standard diet (n=60), a male metformin group (0.1% in feed ≈100 mg/kg/day, n=60), a female controls (n=60), and female metformin group (n=60). Mice had ad libitum access to food and water throughout the study, with regular monitoring of body weight and health status. Metformin was administered continuously via diet from 4 months of age until natural death at 0.1% feed concentration (a dose determined by prior dose-response studies to have no significant side effects), avoiding gavage-induced stress. Physiological assessments included motor activity and muscle strength, while metabolic health was evaluated through oral glucose tolerance tests (OGTT, every 6 months) and fasting insulin levels (ELISA). Aging and inflammatory markers were assessed via plasma IL-6 and TNF-α (inflammatory cytokines, ELISA) and hepatic/skeletal muscle p16INK4a (senescence-associated protein, Western blot). Lifespan was determined by recording time of death for each mouse and calculating median and maximum lifespan (90th percentile survival). Results showed female metformin-treated mice exhibited a significant 8% extension in median lifespan

DOI: 10.54254/2753-8818/2025.PJ25364

($p<0.01$), while males showed no statistical difference. Key female-specific improvements included: a significant delay in age-related motor decline (25% longer rotarod performance), reduced fasting insulin (-30%, $p<0.05$), improved insulin sensitivity, and decreased inflammatory cytokines (40% reduction in IL-6), with no significant changes in males. Mechanistically, in the AMPK/mTOR pathway, female mice showed increased AMPK phosphorylation and decreased mTORC1 activity (measured by p-S6) in liver and skeletal muscle. Autophagy assessment revealed an elevated LC3-II/LC3-I ratio (indicating enhanced autophagic flux) only in females. Additionally, 16S rRNA sequencing showed increased abundance of the beneficial gut bacterium Akkermansia muciniphila in metformin-treated females, correlating with reduced inflammation [9].

4.2. Research on metformin's improvement of tendon degeneration in aging mice

In the 2022 study by Zhang et al. investigating metformin's improvement of tendon degeneration in aging mice, 130 female C57BL/6J mice (Jackson Laboratory) were divided into two primary groups: young controls (2.5-4.5 months old) and aged groups (14-19 months old, natural aging model). The 19-month-old mice were further subdivided into metformin-treated (daily 50 mg/kg body weight IP injection for 8 weeks) and control (equivalent saline injection for 8 weeks) groups. Tendon aging markers included significantly increased SAβ-gal-positive cells, elevated expression of macrophage marker CD68, and upregulated senescence-associated proteins (p53, p16, and CCN1). In vitro experiments (aged tendon cell culture), metformin demonstrated dose-dependent (0-1000 μg/ml) effects: reducing Sβ-gal-positive cells, inhibiting HMGB1 cytoplasmic translocation, and increasing stem cell markers (NS and CD73). In vivo (IP metformin), treatment significantly decreased dsHMGB1 and inflammatory marker CD68 (demonstrating anti-inflammatory effects), reduced senescence markers (p16, CCN1), decreased rounded cell numbers, improved collagen fiber alignment, and enhanced structural organization. Mechanistically, metformin directly bound and inhibited proinflammatory dsHMGB1 release, reduced senescent cell burden, increased tendon stem cell populations (NS+, SSEA-1+, CD73+), and improved tissue architecture, collectively reversing degenerative changes [10].

5. Discussion

The principal cause of inflammation arises from the instability of cytoplasmic homeostasis and elevated levels of pro-inflammatory cytokines. These inflammatory mediators activate various pro-inflammatory signaling pathways, including NF-κB, mTOR, RIG-I, and JAK/STAT, which subsequently induce cell cycle arrest and promote the senescence-associated secretory phenotype (SASP). This process results in inflammatory aging (inflammaging) and ultimately immunosenescence, predominantly characterized by mitochondrial dysfunction, compromised autophagy, increased levels of reactive oxygen species (ROS) leading to oxidative stress, and the buildup of ROS-damaged lysosomes. These damaged lysosomes activate NLRP3 inflammasomes, which further stimulate NF-κB signaling, creating a detrimental vicious cycle. Metformin exerts anti-inflammatory effects through DICER1 and NF-κB signaling pathways. Independent of AMPK activation, metformin directly inhibits NF-κB signaling, suppresses proliferation and differentiation of macrophages and lymphocytes ($CD8^+$ and $CD4^+$), and consequently reduces secretion of inflammatory cytokines including TNF-α, IL-6, and IL-1, thereby delaying the aging process [2].

The UK Prospective Diabetes Study (UKPDS) led by Turner and Holman, through long-term randomized controlled trials in patients with type 2 diabetes mellitus (T2DM), compared the effects of metformin versus conventional sulfonylurea treatment. The results demonstrated that metformin

DOI: 10.54254/2753-8818/2025.PJ25364

significantly reduced mortality risk. Compared to patients treated with sulfonylureas, those receiving metformin exhibited a 20% lower risk of heart disease and a 42% reduction in diabetes-related mortality, achieving survival rates comparable to healthy populations [8]. In the United States, the Targeting Aging with Metformin (TAME) trial, now in its sixth year, represents a landmark clinical investigation evaluating metformin as an anti-aging therapeutic. This study's primary objective is to validate whether metformin can delay aging and prevent multiple age-related diseases by targeting fundamental biological mechanisms of aging. The trial has enrolled 3,000 participants aged 65-75 years, including both diabetic and non-diabetic individuals, employing metformin as monotherapy to assess its impact on aging biomarkers and clinical outcomes [11]. The TAME trial constitutes a pioneering endeavor in geroscientific medicine. Its success could fundamentally transform our understanding of and intervention strategies against aging. Despite facing scientific and regulatory challenges, the trial outcomes will provide critical evidence for anti-aging drug development, potentially ushering in a new era of "healthy aging." Importantly, the results may support FDA recognition of "aging" as a legitimate target for drug development, thereby accelerating the advancement of novel anti-aging interventions.

6. Conclusion

Metformin activates AMPK by increasing the AMP/ATP ratio through mitochondrial complex I inhibition. Activated AMPK subsequently promotes SIRT1 activation, which facilitates DNA repair, enhances autophagy, and boosts antioxidant enzyme activity to counteract mitochondrial ROS production. Simultaneously, AMPK inhibits mammalian mTOR—since mTOR suppresses ULK1, AMPK-mediated mTOR inhibition ultimately promotes autophagy. The activated AMPK signaling pathway also suppresses gluconeogenesis, thereby reducing cancer risk. Through NF-κB pathway inhibition, metformin exerts anti-inflammatory effects while activating Nrf2 to reduce ROS accumulation. Moreover, metformin improves FOXO expression and alters gut microbiota composition, which together diminish mitochondrial damage, stimulate antioxidant enzymes, and promote autophagy—mechanisms that facilitate lifetime extension and the avoidance of degenerative illnesses. Clinical data illustrates metformin's advantages for diabetes patients, encompassing extended lifetime, diminished cardiovascular disease risk, and prevention of neurodegenerative disorders such as Parkinson's and Alzheimer's diseases. Nonetheless, its possible preventive effects in healthy persons remain ambiguous. Future study should aim to clarify the specific molecular processes of metformin and explore potential synergistic interactions with other anti-aging therapies.

References

[1] Soukas A A, Hao H, Wu L. 2019. Metformin as anti-aging therapy: is it for everyone? [J]. Trends in Endocrinology & Metabolism, 30(10): 745-755.
[2] Torres W, Nava M, Galbán N, et al. 2020. Anti-aging effect of metformin: a molecular and therapeutical perspective [J]. Current Pharmaceutical Design, 26(35): 4496-4508.
[3] López-Otín C, Blasco MA, Partridge L, Serrano M, Kroemer G. 2023. Hallmarks of aging: An expanding universe. Cell. 186(2): 243-278. doi: 10.1016/j.cell.2022.11.001. Epub 2023 Jan 3. PMID: 36599349.
[4] Hayflick L. 2007. Biological aging is no longer an unsolved problem [J]. Annals of the New York Academy of Sciences, 1100(1): 1-13.
[5] Wang C, Chen B, Feng Q, et al. 2020. Clinical perspectives and concerns of metformin as an anti-aging drug [J]. Aging Medicine, 3(4): 266-275.
[6] Harman D. 1981. The aging process [J]. Proceedings of the National Academy of Sciences, 78(11): 7124-7128.

DOI: 10.54254/2753-8818/2025.PJ25364

[7] Kulkarni A S, Gubbi S, Barzilai N. 2020. Benefits of metformin in attenuating the hallmarks of aging [J]. Cell metabolism, 32(1): 15-30.
[8] Mohammed I, Hollenberg M D, Ding H, et al. 2021. A critical review of the evidence that metformin is a putative anti-aging drug that enhances healthspan and extends lifespan [J]. Frontiers in endocrinology, 12: 718942.
[9] Vinciguerra, M. 2022. Urolithin A improves physical performance in middle age. NatAging 2, 460. https: //doi.org/10.1038/s43587-022-00239-3
[10] Zhang J, Brown R, Hogan MV, Onishi K, Wang JH. 2023. Metformin improves tendon degeneration by blocking translocation of HMGB1 and suppressing tendon inflammation and senescence in aging mice. J Orthop Res. 41(6): 1162-1176. doi: 10.1002/jor.25470. Epub 2022 Nov 7. PMID: 36262012; PMCID: PMC10113400.
[11] American Federation for Aging Research. (n.d). Targeting the Biology of Aging. https: //www.afar.org/tame-trial.