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Molecular aspects of metformin's anti-aging properties for muscle function and longevity in Drosophila melanogaster

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ABSTRACT

Muscle aging, characterized by the progressive loss of muscle mass and function, presents a significant clinical challenge, contributing to sarcopenia and age-related frailty. Recent research highlights metformin, a widely used anti-diabetic drug, as a promising candidate for mitigating muscle aging by targeting conserved molecular pathways. This review explores metformin's mechanisms in Drosophila melanogaster, emphasizing its activation of AMP-activated protein kinase (AMPK) and inhibition of the mechanistic target of rapamycin (mTOR), pivotal regulators of cellular energy balance and proteostasis. Metformin enhances autophagy, reduces protein aggregation, and preserves muscle integrity by modulating autophagy-related genes, such as Atg1 and Atg8. Furthermore, the drug's suppression of ribosomal S6 kinase (S6K) and eukaryotic initiation factor 4E (eIF-4E) inhibits excessive protein synthesis, mitigating proteostatic stress. Studies in Drosophila reveal that metformin extends lifespan, reduces oxidative stress, and improves muscle function, offering insights into its translational potential for addressing sarcopenia. However, challenges remain in bridging the findings from Drosophila to humans due to species-specific differences and the need for long-term clinical studies. By elucidating the interplay of AMPK, mTOR, and autophagy pathways, this review underscores metformin's therapeutic potential in age-related muscle decline, providing a molecular foundation for its application in geroprotective interventions. Future research should focus on optimizing dosing strategies, exploring synergistic therapies, and advancing biomarkers for muscle aging to fully harness metformin's clinical utility in promoting healthy aging.

Introduction

Aging is an inevitable biological process that affects all organisms, manifesting in various physiological and functional declines, including the deterioration of muscle integrity[1,2]. Muscle aging, often termed sarcopenia, is marked by the progressive loss of muscle mass, strength, and endurance, ultimately impairing mobility and increasing the risk of metabolic disorders and mortality[3–5]. The process of muscle aging is multifaceted and involves a cascade of interconnected molecular, cellular, and systemic changes that disrupt normal homeostasis[6–8].

One of the hallmarks of muscle aging is mitochondrial dysfunction, which impairs the energy production required to maintain muscle function[9,10]. Mitochondria, the powerhouses of the cell, exhibit reduced efficiency with age, leading to decreased ATP generation, increased production of reactive oxygen species (ROS), and subsequent oxidative damage to proteins, lipids, and DNA[11,12]. These changes result in a vicious cycle of cellular damage and energy deficits that accelerate muscle deterioration. Furthermore, chronic low-grade inflammation, often referred to as "inflammaging," exacerbates muscle atrophy by activating catabolic pathways that degrade muscle proteins[13,14]. Simultaneously, dysregulation of proteostasis the balance between protein synthesis and degradation further disrupts muscle homeostasis, compounding the decline in muscle structure and function[15,16]. Another critical factor driving muscle aging is the impaired regenerative

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capacity of muscle stem cells, known as satellite cells[17]. With age, satellite cells lose their ability to effectively repair and regenerate muscle fibers following injury or stress[18,19]. This decline is partly attributed to changes in the systemic environment, such as altered signaling pathways involving insulin-like growth factor 1 (IGF-1) and the activation of the mechanistic target of rapamycin (mTOR) pathway, which contribute to the loss of anabolic signaling necessary for muscle maintenance[20–23]

In recent years, pharmacological interventions targeting these underlying mechanisms have emerged as potential strategies to mitigate the effects of muscle aging[24,25]. Among these, metformin, a well-established anti-diabetic drug, has gained considerable attention for its potential anti-aging properties[26,27]. Originally developed to lower blood glucose levels by improving insulin sensitivity, metformin has since been recognized for its broader biological effects[28,29]. These include activating AMP-activated protein kinase (AMPK), a key energy sensor that promotes catabolic processes such as autophagy, reduces oxidative stress, and inhibits the pro-aging mTOR pathway. Such actions make metformin an attractive candidate for addressing muscle aging by targeting multiple interconnected pathways[30–32].

Baenas and Wagner (2019) have studied model organisms like Drosophila melanogaster has been instrumental in uncovering the mechanisms through which metformin exerts its effects[33,34]. The fruit fly, with its highly conserved genetic pathways and short lifespan, provides a powerful platform for studying the molecular underpinnings of aging and for testing pharmacological interventions[35–37]. Studies on Drosophila have demonstrated that metformin can modulate mitochondrial function, reduce oxidative stress, and improve overall muscle health, thus delaying age-related muscle degeneration[38,39]. These findings raise intriguing questions about the translational potential of metformin for human sarcopenia.

This review explores the complex interaction of cellular and molecular processes underlying muscle aging and examines how metformin influences these pathways in Drosophila melanogaster. By current evidence, we aim to provide insights into metformin's potential as a therapeutic intervention for delaying muscle aging and promoting healthier aging.

Metformin: mechanisms underlying the anti-aging effect

Metformin has promising potential in mitigating the aging process through several molecular mechanisms (Fig. 1). Metformin's primary action involves inhibiting mitochondrial respiratory complex I, reducing cellular respiration and ATP production. This leads to an increased AMP/ATP ratio, which activates AMPK, a key cellular energy sensor that regulates metabolic pathways to restore energy balance[40,41]. In mammals, AMPK activation by metformin leads to decreased hepatic glucose production increased glucose uptake in peripheral tissues, and enhanced insulin sensitivity[42–44]. However, beyond its metabolic regulatory effects, AMPK plays a crucial role in modulating pathways associated with aging and longevity, including mTOR (mechanistic target of rapamycin), FOXO (Forkhead box O transcription factors), and autophagy-related processes[45,46].

One of metformin's notable effects in aging research is its ability to inhibit mTOR, a central regulator of cell growth, proliferation, and metabolism[47,48]. mTOR activity is typically elevated in nutrient-rich environments, promoting protein synthesis and inhibiting autophagy. A cellular degradation process is essential for removing damaged proteins and organelles[49,50]. Through AMPK activation (Figure 2), mTOR inhibition is a hallmark of lifespan extension and is associated with delayed onset of age-related diseases. Metformin's modulation of the AMPK/mTOR axis positions it as a promising agent for targeting aging at the cellular level[51].

Studies in various model organisms, including nematodes, rodents, and *Drosophila melanogaster* (fruit flies), have demonstrated that metformin can extend lifespan and mitigate age-related phenotypes[52–54].

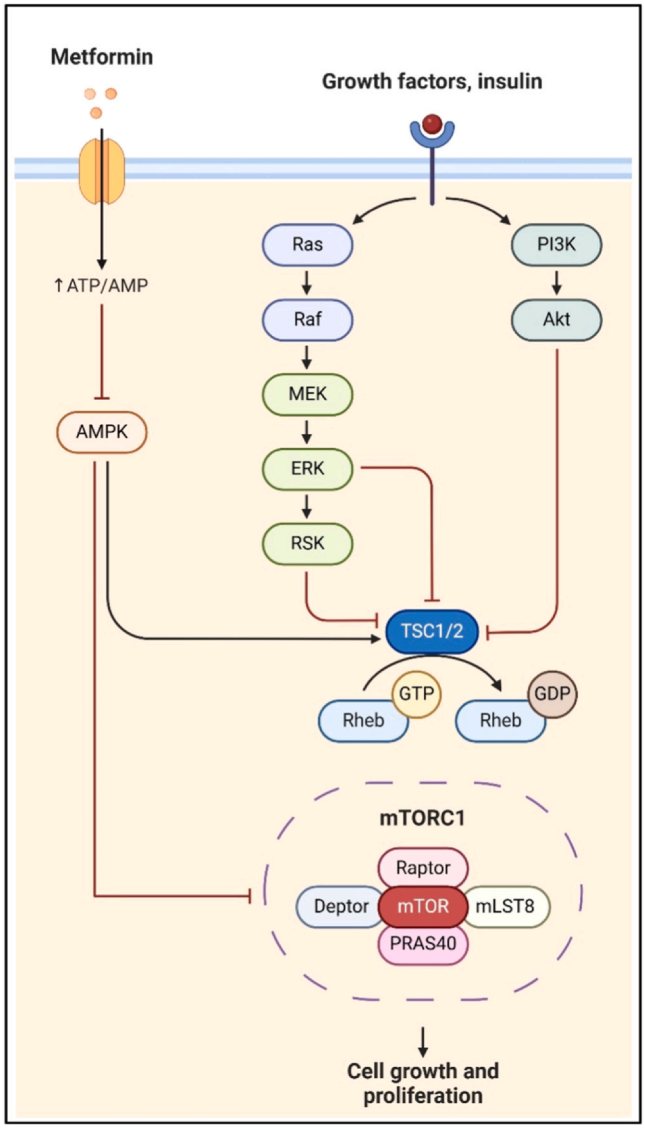

Fig. 1. Metformin-Mediated Modulation of mTORC1 Signaling Pathway. Note: The Fig. 1 illustrates how metformin modulates the mTORC1 signaling cascade to suppress cell growth and proliferation. Metformin increases the AMP/ATP ratio, activating AMP-activated protein kinase (AMPK), which in turn inhibits mTORC1 both directly and indirectly through activation of the TSC1/2 complex. In parallel, growth factors and insulin activate the Ras/Raf/MEK/ERK and PI3K/Akt pathways, which stimulate mTORC1 activity via inhibitory effects on TSC1/2 and activation of Rheb-GTP. Metformin counteracts these effects by enhancing AMPK activity, which inhibits the upstream signaling and prevents Rheb activation, thereby suppressing mTORC1 complex formation and downstream signaling. This leads to reduced cell growth and proliferation, highlighting metformin's potential anti-proliferative and anti-cancer properties.

Drosophila melanogaster provides a powerful model for studying the molecular mechanisms of aging due to its short lifespan, ease of genetic manipulation, and conservation of key cellular pathways with higher organisms, including humans[55,56]. In *Drosophila*, metformin's effects on muscle aging are typically studied using the Gal4/UAS system, a binary genetic tool that allows tissue-specific gene expression. This system enables researchers to manipulate genes specifically in the muscle tissue to study the role of AMPK, mTOR, and autophagy-related genes in response to metformin treatment[57,58]. Lifespan assays in *Drosophila* assess the overall longevity of flies treated with metformin. Additionally, muscle aging is quantified through various physiological assays, including climbing assays, which measure locomotor function, and muscle immunohistochemistry, which detects the accumulation of

ubiquitinated protein aggregates[59–61]. Markers such as ubiquitinated proteins and Atg8, an autophagosomal marker, are used to monitor changes in proteostasis and autophagy in muscle tissue as flies age[62–64]. Specifically, in *Drosophila*, metformin has been shown to prevent the aging-associated accumulation of ubiquitinated proteins in tissues such as the intestine, fat body, and muscle. This suggests that metformin supports the maintenance of proteostasis, which is vital for delaying functional decline during aging[65,66].

In *Drosophila* muscle, aging is accompanied by the accumulation of protein aggregates, impaired proteostasis, and a decline in autophagic processes[67,68]. Metformin's ability to prevent these changes points to its role in preserving muscle integrity during aging. Notably, the stimulation of autophagy, a process that recycles damaged cellular components, is a key feature of metformin's anti-aging effects. Enhanced autophagy helps to maintain cellular homeostasis and delay the progression of age-related muscle degeneration[69,70].

Genetic tools allow for precise control of AMPK activity in the muscle, enabling researchers to dissect the pathway's role in metformin's anti-aging effects[71,72]. Muscle-specific knockdown or overexpression of key genes involved in energy metabolism and protein homeostasis, such as AMPK, mTOR, and autophagy-related genes, provides insights into how metformin influences aging at the molecular level[73].

AMPK activation and lifespan extension

AMPK is a key energy sensor that regulates cellular metabolism and plays a crucial role in the anti-aging effects of metformin[74,75]. Under conditions of low energy, such as those induced by metformin, AMPK is activated through an increased AMP/ATP ratio[76]. In Drosophila melanogaster, AMPK activation is essential for lifespan extension and the suppression of age-related phenotypes, particularly in muscle tissues ATP ratios, which signify low energy states[77–81].

Metformin activates AMPK by directly inhibiting mitochondrial complex I, which leads to reduced ATP production and an elevated AMP/ATP ratio [76]. Activated AMPK phosphorylates a range of downstream targets that are involved in regulating metabolism, protein synthesis, and autophagy[82]. One of its primary effects is the inhibition of the mechanistic target of rapamycin complex 1 (mTORC1), a central regulator of cell growth, proliferation, and protein synthesis[83–85].

In Drosophila muscle, AMPK activation by metformin directly suppresses age-related accumulation of ubiquitinated protein aggregates, which are characteristic of impaired proteostasis during aging[86]. The reduction in protein aggregation is largely due to AMPK's inhibition of mTORC1 signaling, which decreases the activity of ribosomal S6K and eIF-4E. These factors are responsible for initiating cap-dependent protein synthesis, and their downregulation helps limit the excessive production of proteins that are prone to misfolding and aggregation in aged muscle cells[87–89].

Moreover, AMPK promotes autophagy, a process critical for the clearance of damaged organelles and proteins. By inhibiting mTORC1, AMPK relieves the repression of autophagy-related genes, such as Atg1 and Atg8, allowing for enhanced autophagosome formation and degradation of protein aggregates[90]. This increase in autophagy is a major contributor to the maintenance of cellular homeostasis and delayed muscle degeneration in aging flies treated with metformin[91,92].

Genetic evidence from Drosophila demonstrates the importance of AMPK in lifespan extension. Overexpression of a constitutively active form of AMPK (AMPKαT184D) in adult muscle significantly prolongs lifespan and mimics the effects of metformin, even in the absence of the drug. Conversely, muscle-specific depletion of AMPK using RNA interference (RNAi) abrogates metformin's anti-aging effects, leading to increased protein aggregation and shortened lifespan. These findings indicate that AMPK activation is both necessary and sufficient for the lifespan-extending effects of metformin in Drosophila[90,93–96,193].

In addition to its direct effects on mTORC1, AMPK also indirectly regulates other longevity-associated pathways, including FOXO transcription factors. Metformin-mediated AMPK activation promotes the nuclear localization of FOXO, which upregulates the expression of stress-resistance and autophagy-related genes, further contributing to lifespan extension.

Involvement of mTOR and protein synthesis pathways

The mechanistic target of rapamycin (mTOR) is a key regulator of cell growth, proliferation, and protein synthesis, particularly through its involvement in the mTORC1 complex. mTORC1 integrates signals from nutrients, energy status, and growth factors to promote anabolic processes such as protein translation and ribosome biogenesis[97,98]. In Drosophila, mTOR activity is closely linked to aging, with increased mTOR signaling contributing to age-related muscle degeneration due to excessive protein synthesis and reduced autophagic activity[99–101].

Amin et al. (2019) studied that metformin exerts its anti-aging effects on Drosophila muscle primarily by inhibiting mTORC1. This is achieved indirectly by activating AMPK, which phosphorylates and activates TSC2 (tuberous sclerosis complex 2), an upstream inhibitor of mTORC1. The inhibition of mTORC1 leads to the downregulation of protein synthesis and the promotion of autophagy, both critical processes in delaying muscle aging[102]. In normal conditions, mTORC1 promotes the phosphorylation of downstream targets like ribosomal S6K and eukaryotic initiation factor 4E (eIF-4E), both of which are essential for initiating mRNA translation and protein synthesis[103,104]. Metformin-mediated inhibition of mTORC1 reduces the phosphorylation of S6K and eIF-4E, thereby slowing down protein synthesis[105]. This reduction is crucial in aging muscles, where excessive protein accumulation leads to the formation of misfolded or damaged proteins that impair cellular function[106].

Ribosomal S6K is a major downstream effector of mTORC1 that promotes protein synthesis by phosphorylating ribosomal protein S6, facilitating ribosome biogenesis and mRNA translation[107,108]. Overexpression of constitutively active S6K (S6KCA) in Drosophila muscle cancels out metformin's protective effects, indicating that the suppression of S6K activity is essential for reducing age-related protein accumulation[109]. In contrast, inhibition of S6K mimics metformin's anti-aging effects, reducing muscle protein aggregation and preserving muscle integrity[110].

eIF-4E is another critical component regulated by mTORC1, responsible for initiating cap-dependent translation by binding to the 5′ cap of mRNAs. Under normal conditions, mTORC1 phosphorylates and inactivates the eIF-4E binding protein (4E-BP), a repressor of eIF-4E[111,112]. Metformin's inhibition of mTORC1 leads to an increase in 4E-BP activity, which binds to and inhibits eIF-4E, thereby suppressing cap-dependent translation[113]. This reduction in translation initiation limits the production of unnecessary proteins during aging, reducing the burden on proteostasis mechanisms in aging muscle. Experimental studies in Drosophila have provided strong genetic evidence supporting the involvement of mTOR and protein synthesis pathways in metformin's anti-aging effects. Overexpression of wild-type mTOR (TorWT) in muscle fails to extend lifespan or prevent aging, while dominant-negative forms of mTOR (TorTED) mimic the effects of metformin by suppressing mTOR activity, thereby reducing protein synthesis and delaying muscle aging. Similarly, genetic activation of S6K or eIF-4E abolishes metformin's anti-aging effects, further underscoring the critical role of protein synthesis suppression in the drug's mechanism of action[114–117,193].

Metformin's inhibition of mTOR and its downstream effectors, S6K and eIF-4E, highlights its role in balancing anabolic and catabolic processes in muscle aging[118]. By reducing excessive protein synthesis and promoting autophagy, metformin helps to maintain cellular homeostasis, delay the onset of muscle degeneration, and extend lifespan in Drosophila[119,120].

Autophagy and protein homeostasis

Autophagy is a vital intracellular process that regulates the degradation and recycling of damaged organelles, misfolded proteins, and other cellular debris. This pathway is essential for maintaining protein homeostasis, especially in aging tissues, where the accumulation of toxic protein aggregates and damaged organelles can lead to cellular dysfunction[121–124]. In Drosophila melanogaster, autophagy plays a crucial role in muscle health, as protein homeostasis declines with age, leading to muscle degeneration and functional impairment. Metformin's anti-aging effects in Drosophila are strongly linked to its ability to stimulate autophagy, primarily through the activation of AMPK and inhibition of the mechanistic target of rapamycin complex 1 (mTORC1). AMPK is a key energy sensor that promotes autophagy when cellular energy levels are low. Metformin increases the AMP/ATP ratio, which activates AMPK, triggering a cascade of events that ultimately enhance autophagic flux[125].

One of the primary downstream effects of AMPK activation is the inhibition of mTORC1, a central regulator of cell growth and protein synthesis[126]. mTORC1 acts as a negative regulator of autophagy by phosphorylating and inhibiting Unc-51-like autophagy activating kinase 1 (ULK1), which is essential for the initiation of autophagy[127]. When mTORC1 is suppressed by metformin, ULK1 is dephosphorylated and activated, initiating the autophagic process. This leads to the formation of autophagosomes, which engulf damaged proteins and organelles, ultimately delivering them to lysosomes for degradation[99,128].

In Drosophila, age-related muscle degeneration is associated with the accumulation of ubiquitinated protein aggregates, which are markers of impaired proteostasis[129]. Autophagy plays a critical role in clearing these aggregates, thereby maintaining muscle integrity. Studies have shown that metformin-fed Drosophila exhibit a significant reduction in ubiquitinated protein aggregates in their muscle tissue, suggesting enhanced autophagic activity. This reduction in protein aggregates correlates with delayed muscle aging and extended lifespan in the flies[130,131].

Genetic studies in Drosophila further support the essential role of autophagy in metformin's anti-aging effects. Knockdown of key autophagy genes, such as Atg6 (Beclin1 homolog) or Atg8 (autophagosome marker), abrogates the beneficial effects of metformin on muscle aging, leading to the accumulation of protein aggregates and accelerated muscle degeneration[132,133]. Conversely, overexpression of autophagy-related genes enhances the effects of metformin, leading to improved muscle function and longevity[130,134–136].

In addition to promoting autophagy, metformin plays a critical role in maintaining protein homeostasis by downregulating protein synthesis. This is achieved through the suppression of mTORC1 and its downstream targets, including ribosomal S6K and eIF-4E. Both S6K and eIF-4E are essential for initiating cap-dependent translation and promoting protein synthesis. In aging tissues, excessive protein synthesis can contribute to the accumulation of misfolded proteins and cellular stress, which in turn accelerates aging[137].

H. Querfurth et. al. (2021) and Z. Mao et. al (2018) provided that, by inhibiting mTORC1 and reducing the activity of S6K and eIF-4E, metformin decreases overall protein synthesis in Drosophila muscle. This not only reduces the burden of newly synthesized misfolded proteins but also allows the cellular machinery to focus on the degradation of damaged proteins via autophagy, ensuring that protein homeostasis is maintained during aging[138,139].

Anti-aging drugs and their mechanisms: a detailed overview highlighting metformin's superiority

Recent research as shown in Table 1[165–187] has brought attention to several drugs with significant anti-aging potential, each targeting unique biological mechanisms to extend lifespan and promote healthier aging. Rapamycin (sirolimus) functions as an inhibitor of the mTOR pathway, stimulating autophagy and cellular repair processes; however, its long-term use is limited due to its immunosuppressive properties[140,141]. Similarly, alpha-ketoglutarate (AKG) targets mTOR and ATP synthase while influencing DNA and histone demethylation and reducing oxidative stress caused by reactive oxygen species (ROS)[142,143]. Both curcumin and tetramethylpyrazine nitrone (TMPN) also interact with the mTOR pathway, with curcumin mitigating inflammation by suppressing NF-κB and TMPN safeguarding mitochondrial function via the AMPK/mTOR pathway[144–146].

Antioxidant compounds such as, resveratrol and Tinospora cordifolia counteract oxidative stress by neutralizing ROS, thereby preventing cellular damage[147–149]. Spermidine, on the other hand, fosters autophagy, reduces inflammation, and regulates histone acetylation, thereby maintaining cellular equilibrium[150]. Pentagalloyl glucose and sulforaphane have been found to support longevity by promoting daf-16 nuclear translocation, enhancing sod-3 expression, and modulating insulin/IGF-1 signaling pathways. Fisetin and quercetin address cellular senescence through apoptotic regulation, focusing on pathways like PI3K/AKT/NF-κB and HIF-1α, respectively[151–153]In addition, verapamil serves as a calcium channel blocker, while atracurium influences neuromuscular signaling through its effects on the acetylcholine receptor subunit unc-38, contributing to maintaining cellular function[154–157].

Ligustilide has shown potential in countering adriamycin (ADM)-induced immune senescence by preserving thymic function and antioxidant effects by reducing the level of oxidative stress markers, while procyanidin C1 acts as a senolytic by selectively inducing mitochondrial dysfunction and ROS production in senescent cells to facilitate their removal[158–161]. Among these options, metformin stands out as the most versatile and well-studied anti-aging drug. It operates through multiple mechanisms, including the activation of AMPK, inhibition of mTOR, reduction of oxidative stress, and modulation of inflammatory pathways.

Unlike rapamycin, metformin does not significantly suppress the immune system, making it a safer option for extended use. Its affordability, established safety in clinical applications, and metabolic benefits position it as a leading candidate for anti-aging therapies[162]. Ongoing studies, such as the TAME (Targeting Aging with Metformin) trial which a multicenter, randomized, double-blind, placebo-controlled clinical trial designed to investigate metformin's potential to delay aging and extend healthspan by targeting multiple biological pathways associated with age-related diseases, are further exploring its potential to delay the onset of aging-related declines and improve overall healthspan[163,164].

Implications for therapeutic applications

The findings from *Drosophila* studies suggest that metformin holds promise as a therapeutic agent for age-related muscle decline. Its ability to activate AMPK, suppress mTOR signaling, and induce autophagy could potentially be leveraged to treat sarcopenia and other age-associated muscle disorders. Given the conservation of these pathways in mammals, further research is warranted to explore the translational potential of metformin in human aging and muscle maintenance. The molecular pathways influenced by metformin in Drosophila, particularly AMPK, mTOR, eIF-4E, and S6K, play crucial roles in its muscle aging suppression effects. These pathways, along with others such as autophagy and oxidative stress regulators, collectively contribute to the preservation of proteostasis and delay of muscle degeneration. A detailed breakdown of these pathways, their involvement in aging, and experimental evidence in *Drosophila* is provided in Table 2[188–212].

Translating Drosophila findings to clinical applications

The molecular mechanisms by which metformin delays muscle aging in Drosophila melanogaster primarily through AMPK activation, mTOR inhibition, suppression of protein synthesis (eIF-4E/S6K), and enhanced autophagy are highly conserved in mammals. However, translating these findings to humans requires careful consideration of

Table 1
Targeting pathways, in-vivo studies/in-vitro studies, biochemical activities, and evaluation parameters of anti-aging drugs.

Drug	Targeting Pathway	In-vivo studies/In-vitro studies	Evaluation Parameters	Biochemical activity or therapeutic potential	References
Rapamycin (sirolimus)	inhibition of mTORC1 by the rapamycin-FKBP12 complex	male and female C3B6F1 hybrid mice- continuously fed with 42 mg/kg rapamycin or intermittently fed by alternating weekly	Wang Allison test, log-rank test, restricted mean time lost (RMTL) analysis	Immunosuppressive, anti-proliferative, and anti-aging effects	165,166
Alpha-ketoglutarate	by activating the Nrf2/ARE signaling pathway, in which Nrf2 directly ↑ the expression of anti-apoptotic protein, Bcl2	C57BL/6 mice-Dietary-supplemented 2 % AKG (w/w)- two independent cohorts of mice, each consisting of 45 ± 2 females and 45 ± 2 males (total of 182 animals)	Cytokine secretion profile of leuko-cytes, splenocytes	Intracellular antioxidant	167,168
Pentagalloyl glucose	improving *daf−16*, *sod−3*, *ctl−1*, and *gst−4* levels in the DAF−16/FOXO pathway and upregulating *skn−1* and *gst−4* expression in the SKN−1/Nrf2 pathway	*Caenorhabditis elegans* models -RAW 264.7 cells-PGG treatment, MTT (0.5 mg/mL)	Non-fluorescent probe H2DCF-DA	Antioxidant and anti-stress properties	169
Curcumin	by modulating signaling pathways such as AMPK, NRF2, and NF-κB	*Drosophila melanogaster*- 100 μM pretreatment-mean lifespan of approximately 30 days in both males and females	γH2Ax foci in larval wing disc-immunostaining	Antioxidative, anticancer, anti-inflammatory, and antineurodegenerative effects	170,171
Fisetin	by inhibiting the Akt signaling pathway through the suppression of *Stc1* expression, leading to the apoptosis of senescent cells	old p16-3MR mice-$p16^{INK4A}$-positive senescent cells were treated with fisetin in Endothelial Cell Growth Media (EGM)-2 for 48 h	cell viability assay, SA-β-gal staining	Anti-apoptotic	172,173
Quercetin	targeting SIRT1 to regulate cellular senescence and multiple aging-related cellular processes such as SIRT1/Keap1/Nrf2/HO−1 and PI3K/Akt/GSK−3β mediated oxidative stress	*Simocephalus vetulus (S. vetulus)* −1–10 mg/L 1, 2.5, and 5 mg/L quercetin had no significant effect on the intrinsic growth rate 10 mg/L significantly decreased the intrinsic growth rate	one-way analysis of variance (ANOVA) and Tukey's test	Anti-cancer, antibacterial, and anti-inflammatory activities	174,175
Verapamil	signaling pathways (e.g., insulin/IGF−1, mTOR, AMPK, and germline signaling pathways), novel calcineurin-nuclear factor Y signaling pathway	*Caenorhabditis elegans* (*C. elegans*)- 100 μM and 400 μM- lifespan extension of 20.59 % and 19.45 %	The log-rank (Mantel-Cox) test, Sidak multiple comparisons test	Anti-hypertensive	176
Spermidine	by suppressing apoptosis and enhancing autophagy through the mediation of the AMPK-FOXO3a signaling pathway	SAMP8 mice- suppressed oxidative stress in all mice	Novel object recognition test (ORT) and open field test (OFT)	Antioxidant functions, anti-inflammatory properties,	177,178
Sulforaphane	Sulforaphane promotes C. elegans longevity and healthspan via DAF−16/DAF−2 insulin/IGF−1 signaling	*Caenorhabditis elegans*- 100–400 μM - enhanced the mean lifespan significantly from 22.6 days to 27.3 days	brood size assay	Anti-inflammatory and antioxidant properties	179
Isorhapontigenin	PI3K/AKT/mTOR-mediated autophagy pathway	NPCs (nucleus pulposus cells)−10–20 μM-mitigated NPCs aging	One-way ANOVA, Kruskal-Wallis H test, least significant difference (LSD) method and Dunnett's T3 test	Antiinflammatory And anti-aging effects	180
Atracurium	activation or overexpression of the Forkhead Box O (FOXO) family of transcription factors	*Caenorhabditis elegans*-(50 μM) extends lifespan in wild type (N2) C.elegans	log-rank tests	Neuromuscular blocker	181
Resveratrol	by regulating MAPK, COX2, and Nrf2 signaling pathways	premature senescence model of human embryonic lung fibroblasts (HEFs) induced by hydrogen peroxide (H2O2)−10 μmol/L-	Cell viability test, SA-β-gal staining	Anti-inflammatory, antioxidant, and senescence-delaying properties	182
Ligustilide	promoted TEC (thymic epithelial cells) proliferation and reticular differentiation, leading to an increase in $CD4^+$ single positive (CD4SP) T cell proportion	Mouse thymic epithelial cells (TECs) - 2.5 mg/kg ADM injected every 3 days for 10 injections - regulates thymic T cell differentiation and maturation	SA-β-gal staining and fluorescence labeling	Anti-aging	183
Tinospora cordifolia	to target AMPK signalling pathway	Wistar strain female rats aged 12–13 months- 1 mg dry powder/g bodyweight of rat- anti-oxidative marker GSH level declined	Adipokine Panel- metabolic assay kit	Anti-diabetic, Anti-oxidative, anti-cancer, anti-obesity, hepatoprotec-Tive, neuroprotective, anti-allergic, and immunomod-Ulatory activities	184
Procyanidin C1	to inhibit SASP(senescence-associated secretory phenotype) formation, kills senescent cells at higher concentrations,	Mice PSC27 cell −600 μM or higher-exerted apoptotic effects within 12 h	one-way ANOVA with Tukey's post hoc comparison and Dunnett's test	Geroprotective Agent, and anti-aging	185
Congea chinensis Moldenke	maintenance of cellular senescence is mainly due to the initiation of the DNA damage response (DDR) pathway	Mice-pGL4-p53-GFP cells−10 μg/mL -systemic delay in aging	fluorescence microscopy	Antitumor and longevity-promoting activities	186

(*continued on next page*)

Table 1 (*continued*)

Drug	Targeting Pathway	In-vivo studies/In-vitro studies	Evaluation Parameters	Biochemical activity or therapeutic potential	References
25-hydroxyvitamin D	modulating cellular processes to delay aging, including reducing oxidative stress, enhancing autophagy	cohort study- 18,738 participants from the NHANES (National Health and Nutrition Examination Survey) 2007–2010 & 2015–2018, a survey conducted by the National Center for Health Statistics (NCHS) more than 80.4 nmol/l - showed increased lifespan	Rao-Scott Chi-square	Calcitropic activity	[187]

species-specific differences in metabolism, tissue complexity, and aging trajectories. Recent clinical studies provide critical insights into metformin's potential for mitigating age-related muscle decline in humans, though outcomes are context-dependent and sometimes paradoxical.

In older adults, metformin shows promise in reducing disuse-induced muscle fibrosis and cellular senescence. A randomized trial in healthy older adults (60 + years) revealed that metformin pretreatment during 5 days of bed rest attenuated type I myofiber atrophy, lowered collagen deposition, and reduced markers of senescence and the senescence-associated secretory phenotype (SASP) in muscle tissue during recovery. This was linked to improved proteostasis and reduced inflammation, aligning with Drosophila findings on metformin's role in clearing protein aggregates via autophagy[213]. Conversely, the MET-PREVENT trial found no improvement in 4-m walk speed or physical function in frail older adults with sarcopenia after 4 months of metformin, highlighting tolerability issues and suggesting limited efficacy in advanced frailty[214].

Metformin's impact on mitochondrial function and oxidative stress in humans partially mirrors Drosophila outcomes but exhibits nuances. Short-term metformin use (2 weeks) in glucose-tolerant older adults increased mitochondrial H_2O_2 emission a sign of mitohormesis which correlated with satellite cell activation, suggesting a role in muscle repair[215]. However, metformin blunted muscle hypertrophy and AMPK/mTOR-related adaptations after resistance training in older adults, indicating potential interference with anabolic pathways[216]. This contrasts with Drosophila, where metformin consistently promotes muscle integrity. Beyond muscle, metformin may protect against age-related cognitive decline. In men with T2D, metformin altered the gut microbiome (increasing Akkermansia muciniphila) and plasma metabolome (enriching arginine/proline pathways), which correlated with improved memory scores[217]. Epigenetic age reversal in monocytes of older people with HIV further supports its geroprotective potential[218].

Large-scale clinical trials are currently exploring the broader anti-aging effects of metformin. One such initiative, the TAME trial, aims to evaluate the drug's impact on age-related diseases in non-diabetic older adults. The study plans to enroll approximately 3000 participants, aged 65–79, across about 14 centers throughout the United States. Rather than examining metformin's effects on individual conditions, the trial will focus on the time to first occurrence of a composite outcome including cardiovascular events, cancer, dementia, and all-cause mortality. In addition, TAME will assess critical functional and geriatric endpoints. If successful, the TAME trial could represent a paradigm shift in medical research and practice, moving from disease-specific treatments toward targeting the aging process itself[219]. This approach has the potential to accelerate the development of more effective pharmacological strategies and significantly reduce healthcare costs associated with aging. Meanwhile, the ANTHEM study is probing how metabolic health status influences metformin's efficacy on insulin sensitivity and mitochondrial function[220]. Notably, metformin did not reduce cancer incidence over 21 years in high-risk adults in the DPP trial, underscoring the need for biomarker-guided patient stratification[221].

Metformin's mechanisms, elucidated in Drosophila, provide a robust framework for exploring its therapeutic potential in human muscle aging. Clinical evidence supports its role in reducing fibrosis and senescence in specific contexts but cautions against uniform application in frailty or alongside intense exercise. Future research should prioritize personalized dosing, combinatorial approaches, and biomarkers like epigenetic clocks or IgG glycosylation patterns to identify responders. Bridging invertebrate models to human trials will be essential to harness metformin's full potential in promoting healthy aging.

Limitations and challenges in the study of metformin's anti-aging effects in Drosophila

The study of metformin's anti-aging effects in *Drosophila melanogaster* presents several limitations, as highlighted in Table 3[222–230]. These include species-specific metabolic differences that may not translate directly to humans, challenges in determining appropriate dosage, and the lack of clarity regarding healthspan versus lifespan benefits. Experimental

Table 2
Molecular pathways involved in metformin-mediated muscle aging suppression in drosophila.

Pathway/Components	Primary Function	Metformin's Action	Molecular Target	Effect on Muscle Aging	Experimental Evidence in Drosophila	Genetic Manipulation	References
Akt (Protein kinase B)	Promotes cell survival and growth by inhibiting FOXO and promoting mTORC1 activity.	Metformin inhibits Akt via suppression of mTORC2 and ↑ AMPK activity.	Inhibition of Akt leads to FOXO activation, ↑ autophagy and stress resistance.	Suppresses mTORC1 activity, promotes autophagy, delays muscle aging.	Metformin ↓ Akt phosphorylation, ↑ FOXO activity and ↑ autophagy in muscle.	Muscle-specific Akt inhibition mimics metformin's effects on aging and autophagy.	188–190
AMPK (AMP-activated protein kinase)	Energy sensor that shifts metabolism from anabolic to catabolic pathways under low energy conditions.	Metformin activates AMPK by ↑ AMP/ATP ratio, promoting autophagy and inhibiting protein synthesis.	Phosphorylation of AMPKα at Thr184; promotes catabolic processes (autophagy) while inhibiting anabolic pathways.	Extends lifespan, ↓ protein aggregation, ↑ proteostasis in aging muscle.	Overexpression of constitutively active AMPK delays muscle aging. AMPK knockdown nullifies metformin's effects.	Muscle-specific overexpression of AMPKαT184D (constitutively active); RNAi-mediated depletion.	191–194
Atg1 (Autophagy-related gene 1)	Key initiator of autophagy, activated by mTOR inhibition and AMPK signaling.	Metformin promotes Atg1 activation via mTORC1 inhibition, initiating autophagy.	AMPK-mediated activation of Atg1 promotes formation of autophagosomes.	↑ autophagy, ↑ proteostasis, delays muscle aging.	Metformin induces Atg1-dependent autophagy, ↓ protein aggregation in Drosophila muscle.	Overexpression of Atg1 ↑ lifespan, while depletion prevents metformin-induced autophagy.	193,195,196
Atg8 (Autophagy-related gene 8)	Autophagosome component essential for autophagy progression and protein degradation.	Metformin ↑ Atg8 activity, promoting autophagosome formation and ↑ autophagic flux.	Promotes autophagosome maturation and degradation of protein aggregates.	Clears damaged proteins, maintains muscle integrity, delays age-related muscle decline.	Metformin upregulates Atg8 expression in Drosophila muscle, improving autophagic flux.	Atg8 overexpression mimics metformin's anti-aging effects; Atg8 depletion negates them.	197,198
Autophagy	Cellular degradation and recycling process essential for proteostasis.	Metformin induces autophagy via AMPK activation and mTORC1 inhibition.	AMPK-mediated activation of ULK1 initiates autophagy, while mTORC1 inhibition ↑ autophagic flux.	↑ clearance of damaged proteins, ↓ protein aggregates, delays muscle degeneration.	Metformin promotes autophagy (Atg8-positive autophagosomes) in Drosophila muscle, improving proteostasis.	Atg6 RNAi in muscle nullifies metformin's effects; overexpression of Atg8 mimics metformin's effects.	199
eIF−4E (Eukaryotic Initiation Factor 4E)	Initiates translation by binding to the 5′ cap of mRNAs.	Metformin ↑ 4E-BP activity, inhibiting eIF−4E and ↓ cap-dependent translation.	Inhibits eIF−4E through 4E-BP binding, which blocks its interaction with the mRNA cap.	Suppresses translation, preventing protein aggregate accumulation, ↑ proteostasis.	Overexpression of eIF−4E abolishes metformin's anti-aging effects, indicating its role in protein synthesis regulation.	Muscle-specific overexpression of eIF−4E nullifies metformin effects; 4E-BP overexpression mimics metformin effects.	200
FOXO (Forkhead box O transcription factors)	Regulates genes involved in longevity, autophagy, and stress resistance.	Metformin activates FOXO via AMPK, ↑ transcription of autophagy-related genes like Atg and 4E-BP.	Promotes expression of genes ↑ autophagy and stress resistance, including 4E-BP.	Extends lifespan, ↑ stress resistance, ↑ proteostasis, delays muscle aging.	FOXO activation is necessary for metformin-mediated lifespan extension and 4E-BP upregulation.	FOXO activation through genetic overexpression mimics metformin effects; FOXO RNAi nullifies metformin's benefits.	201–203
mTOR (mechanistic Target of Rapamycin)	Regulates cell growth, proliferation, and protein synthesis.	Metformin inhibits mTORC1 activity via AMPK activation, ↓ translation and ↑ autophagy.	Downregulates mTORC1 by phosphorylating TSC2, thereby ↓ S6K activity and protein synthesis.	↓ protein synthesis, delays age-related muscle decline, ↑ autophagy.	Suppression of mTORC1 mimics metformin's anti-aging effect; overexpression of mTOR negates metformin's benefit.	Overexpression of TorTED (dominant-negative) replicates metformin effects; TorWT does not prevent aging.	193,204,205
mTORC2 (mechanistic Target of Rapamycin Complex 2)	Regulates cytoskeletal organization and cell survival, activated by growth factors.	Indirectly inhibited by metformin via suppression of insulin/Akt signaling pathway.	Inhibition of mTORC2 downregulates Akt activity, promoting FOXO nuclear translocation and autophagy.	Promotes stress resistance, ↑ autophagy, delays muscle degeneration.	mTORC2 inhibition, along with AMPK activation, ↑ autophagy and lifespan in Drosophila muscle.	mTORC2 depletion ↑ lifespan and autophagy, mimicking metformin effects.	206,207
Ribosomal S6 Kinase (S6K)	Promotes protein synthesis by phosphorylating the S6 ribosomal protein.	Metformin inhibits S6K by suppressing mTORC1, ↓ ribosome biogenesis and protein synthesis.	Inhibits mTOR-mediated activation of S6K, ↓ phosphorylation of ribosomal S6.	↓ protein synthesis, prevents age-related accumulation of damaged proteins.	Overexpression of S6KCA cancels metformin's effects on muscle aging, highlighting S6K's role.	S6KCA overexpression negates metformin's anti-aging effects; inhibition mimics metformin.	193,208
SOD1 (Superoxide dismutase 1)	↓ oxidative stress by catalyzing the dismutation of superoxide into oxygen and hydrogen peroxide.	Indirectly modulated by metformin via autophagy activation, ↓ oxidative stress.	SOD1 interacts with AMPK to ↑ autophagy and ROS clearance.	↓ oxidative stress, preventing ROS-induced damage and muscle atrophy.	Muscle-specific depletion of SOD1 accelerates aging; metformin protects against oxidative stress in SOD1-depleted flies.	SOD1 RNAi leads to muscle aging, but metformin counteracts these effects through AMPK activation.	209,210
TORC1 Inhibition	Regulates cell growth and autophagy by sensing nutrient and energy levels.	Metformin inhibits TORC1 via AMPK-mediated TSC2 phosphorylation, promoting autophagy.	Direct inhibition of TORC1 ↓ protein synthesis and promotes autophagy through Atg1/ULK1 activation.	Delays muscle aging by ↓ protein synthesis and ↑ autophagy.	Inhibition of TORC1 mimics metformin's effect on lifespan and muscle aging in Drosophila.	Overexpression of TorTED (dominant-negative TOR) reproduces metformin's benefits on muscle aging.	193,211,212

Table 3
Limitations of metformin's anti-aging effects in Drosophila.

Limitation	Description	References
Species-Specific Responses	-Divergent insulin signaling: *Drosophila* has fewer insulin-like peptides (Dilps) and lacks the complex hormonal regulation seen in mammals. -Tissue heterogeneity: Mammalian muscle aging involves satellite cell decline and systemic inflammation ("inflammaging"), not fully recapitulated in flies. -Autophagy regulation: Key regulators exhibit structural and functional divergence.	222
Dosage and Duration of Treatment	Metformin concentrations effective in *Drosophila* vastly exceed human therapeutic doses, raising concerns about: - Off-target effects at high doses (e.g., mitochondrial toxicity). - Uncertain long-term metabolic consequences (e.g., altered gut microbiota, vitamin B12 deficiency).	223
Lifespan vs. Healthspan	While metformin extends longevity in flies, its impact on functional aging is inadequately characterized: - Limited data on age-related declines in mobility, resilience to stress, or reproductive fitness. - Risk of prolonged lifespan with increased frailty (e.g., reduced climbing ability late in life).	224,225
Genetic Background & Experimental Variability	- Genetic background alters metformin sensitivity. - Diet (sugar-yeast vs. high-sugar), temperature, and microbiota composition confound reproducibility.	226
Lack of Long-Term Mechanistic Insights	- Sustained AMPK activation/mTOR inhibition may impair muscle regeneration by suppressing satellite cell activity. - Excessive autophagy could deplete essential organelles/proteins, accelerating aging.	227
Broader Metabolic Effects of Metformin	Metformin's systemic actions (e.g., glucose lowering, mitochondrial inhibition) may inadvertently accelerate aging in mammals by: - Disrupting nutrient-sensing cross-talk. - Altering NAD^+ sirtuin pathways.	228,229
Conflicting Evidence in Mammalian Systems	Paradoxical effects on muscle: While metformin reduces protein aggregation in flies, mammalian studies report: -Blunted hypertrophy: Metformin inhibits resistance training-induced muscle growth in older adults by suppressing mTORC1-driven protein synthesis. -Exacerbated sarcopenia risk: Chronic mTORC1 inhibition may impair myofiber repair, worsening age-related muscle loss.	216,230

variability, genetic background effects, and the broader metabolic impact of metformin also complicate the interpretation of its long-term effects on aging. Additionally, while short-term mechanisms such as AMPK and mTOR regulation are well-documented, long-term consequences of sustained pathway modulation, including potential detriments to tissue regeneration and immune function, remain unclear. The influence of genetic diversity and environmental factors, such as diet and stress, is often overlooked, even though these variables significantly impact metformin's efficacy. The potential for synergistic effects with other anti-aging therapies, such as rapamycin or dietary interventions, also remains underexplored, raising questions about how combination treatments might enhance or hinder its benefit. Another limitation lies in the absence of consistent biomarkers to reliably measure muscle aging and the therapeutic impact of metformin. This makes it difficult to standardize findings across different studies. Addressing these limitations is crucial for advancing the clinical relevance of metformin as an anti-aging therapy.

Future directions

Metformin has shown promise in delaying muscle aging, but further research is needed to fully understand its mechanisms and optimize its applications. A key area of focus is determining the dose-dependent effects and exploring how metformin interacts with dietary and environmental factors, such as caloric restriction or ketogenic diets, to enhance its efficacy. Additionally, investigating its impact on autophagy and proteostasis using advanced tools like transcriptomics could uncover critical insights into its molecular pathways.

While much attention has been given to AMPK activation, future studies should explore AMPK-independent mechanisms, such as mTOR inhibition and mitochondrial biogenesis, to provide a more comprehensive view of metformin's role in muscle aging. Longitudinal studies tracking muscle strength, endurance, and structural changes over the lifespan of *Drosophila melanogaster* are essential to assess its long-term benefits. Genetic and epigenetic research, including studies on histone modifications, could further elucidate how metformin influences aging processes across diverse genetic backgrounds.

Combination therapies with other geroprotective agents, like rapamycin or NAD + precursors, present an exciting avenue for amplifying metformin's effects. Translational studies bridging findings from *Drosophila* to mammals and humans are crucial for clinical applications. Moreover, the development of advanced biomarkers for oxidative stress and mitochondrial health, along with non-invasive imaging methods, could improve the evaluation of metformin's efficacy. Finally, studying how chronological age and sex influence its effects will help tailor interventions to combat age-related muscle decline effectively. These efforts will enhance our understanding of metformin's potential and contribute to innovative strategies for managing muscle aging.

Conclusion

Metformin exerts its anti-aging effects in *Drosophila* muscle through a multifaceted mechanism involving AMPK activation, mTOR inhibition, reduced protein synthesis, and enhanced autophagy. Metformin delays muscle aging and extends lifespan in Drosophila by targeting these pathways. The molecular mechanisms by which metformin exerts its effects, including the activation of AMPK, inhibition of mTOR, and promotion of autophagy, all of which are critical for maintaining protein homeostasis and muscle integrity during aging. The suppression of excessive protein synthesis through pathways like eIF-4E and ribosomal S6 kinase, coupled with enhanced autophagic clearance of damaged proteins, collectively contribute to preserving muscle function and delaying age-related decline. Experimental evidence from Drosophila models has demonstrated that genetic manipulation of these pathways can either replicate or negate metformin's anti-aging effects, further underscoring the importance of these signaling cascades. Given the high conservation of these pathways across species, the findings in Drosophila provide valuable insights into potential therapeutic interventions for age-related muscle decline in humans. Future research should focus on translating these discoveries into clinical applications, exploring metformin's broader potential as a geroprotective agent.

Declarations

Not applicable.

Authors' contributions

Milind Umekar: Conceptualization, Supervision, Writing - Review & Editing; **Mohammad Qutub:** Conceptualization, Methodology, Data Curation, Writing - Original Draft, Visualization; **Tanvi Premchandani:**

Investigation, Visualization, Writing - Review & Editing; **Amol Tatode:** Supervision, Project Administration, Methodology, Writing - Review & Editing; **Jayshree Taksande:** Writing - Review & Editing; **Priyanka Singanwad:** Visualization; **Mayur Kale:** Investigation, Formal Analysis; **Mithun Maniyar:** Writing - Review & Editing; **Ujban Md Hussain:** Writing - Review & Editing.

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