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REVIEW ARTICLE

Anti-Aging Effect of Metformin: A Molecular and Therapeutical Perspective

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Abstract: Aging is a time-dependent inevitable process, in which cellular homeostasis is affected, which has an impact on tissue function. This represents a risk factor for the development of numerous non-transmissible diseases. In consequence, the scientific community continues to search for therapeutic measures capable of improving quality of life and delaying cellular aging. At the center of this research is metformin, a widely used drug in Type 2 Diabetes Mellitus treatment that has a reduced adverse effects profile. Furthermore, there is evidence that this drug has beneficial health effects that go beyond its anti-hyperglycemic properties. Among these effects, its geronto-protection capability stands out. There is growing evidence that points out to an increased life expectancy as well as the quality of life in model organisms treated with metformin. Therefore, there is an abundance of research centered on elucidating the mechanism through which metformin has its anti-aging effects. Among these, the AMPK, mTORC1, SIRT1, FOXO, NF.kB, and DICER1 pathways can be mentioned. Furthermore, studies have highlighted the possibility of a role for the gut microbiome in these processes. The next step is the design of clinical essays that have as a goal evaluating the efficacy and safety of metformin as an anti-aging drug in humans to create a paradigm in the medical horizon. The question being if metformin is, in fact, the new anti-aging therapy in humans?

Keywords: Metformin, Ageing, Anti-ageing drugs, Type 2 diabetes mellitus, geronto-protective properties.

1. INTRODUCTION

A progressive population ageing around the world has evolved in a slow, constant, and apparently irreversible fashion. It has been forecasted that by 2050, 22% of the humanity will be shaped by adults over 60 years of age, the equivalent of 2.1 billion people, representing a two-fold increase in the estimates from 2017 [1]. While age is not an indicator of health or illness, it does represent a risk factor for the development of non-transmissible diseases because of tissue, organ and cellular deterioration. The most common of these diseases are cancer, cardiovascular diseases and neurodegenerative diseases, among other entities capable of harming the quality of life (QoL) and the productivity of elderly people. Therefore, this represents a public health issue [2,3].

Ageing is an inevitable and time-dependent process in which subjacent molecular mechanisms affect cellular homeostasis and, therefore, tissue functions [4]. Lopez-Otin *et al*. classified ageing in stages, proposing nine distinctive markers during this process. The increase or decrease of these markers shows the possibility of accelerating or delaying the process. Therefore, life expectation is modified in function of the compensatory mechanisms with the goal of maintaining homeostasis [5]. However, when these mechanisms are overwhelmed, cellular death takes place, affecting the normal tissue functions [6].

Despite a worldwide increase in life expectancy, several research groups worldwide continue to search for both pharmacological and non-pharmacological measures to delay cellular ageing. In search of this goal, experimental models have been developed including parabiosis [7,8], and less caloric restriction, intermittent fasting, and protein restriction, as well as knock-out animal models [9-11]. Furthermore, genetic and pharmacological interventions focused on nutrients signalling pathways have been proposed, and drugs such as rapamycin [12,13] and, more recently, metformin, have become protagonists in this arena [14-16]. The aim of this review is to describe the molecular mechanisms involved in the geronto-protective effects of metformin according to the recent epidemiological, clinical and pre-clinical evidence.

2. METFORMIN: EXPLORING NEW THERAPEUTICAL USES

Metformin is a biguanide, which is a synthetic molecule whose origins can be traced to the herbaceous plant *Galega officinalis* (Galega, goat's-rue, French lilac, Italian fitch, professor-weed). This plant contains Galegine, a toxic guanidine derivative [17] used during the medieval era to treat thirst and polyuria, which are now understood as a cardinal symptom and sign of diabetes [18]. However, the antihyperglycemic role of guanidine was not reported until 1918 [19], when its discovery led to the development of biguanides, including metformin [20]. As the use of this drug was widely accepted, it became the most commonly prescribed drug for the treatment of Type-2 Diabetes (T2DM) due to its unique anti-hyperglycemic properties and reduced adverse effects profile [18]. Despite this, to date, the mechanism of action of this drug is not fully understood. A pleiad of research evidence has shown that metformin does not increase insulin secretion. However, metformin reduces fasting liver glucose production through gluconeogenesis inhibition and increases both glucose transport across the plasmatic

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membrane and oxidation in peripheral tissues [21–24]. Metformin also has a role in plasma and liver lipid composition [25]. These actions are achieved through the phosphorylation and activation of the 5' adenosine monophosphate-activated protein kinase (AMP) (AMPK) [26,27]. Importantly, AMPK knockout studies have found that Metformin has AMPK-independent mechanisms action since the effects of this drug can not be totally blunted in these experimental models [28]. In this vein, oral metformin has a better anti-hyperglycemic effect compared to intravenous metformin. Therefore, researchers have suggested that the gut could be another site of action of this drug, and more specifically, by gut microbiome restoration [29–31]. Interestingly, gut microbiome dysregulation has been associated with the development of multiple illnesses, including T2DM [32–35].

There is increasing evidence regarding the role of metformin in numerous pathological entities [36]. Pre-clinical studies have been successfully performed, evaluating the pleiotropic effects of metformin in animal models with diseases such as Alzheimer's disease [37,38], metabolic syndrome [39], obesity [40], autoimmune diseases [41,42], and, more recently, its possible anti-aging effect [14,43].

3. ANTI-AGEING EFFECT OF METFORMIN IN ANIMAL MODELS

A great number of animal models performed to study a relationship between metformin and anti-ageing has been done in mice (Table **1**). A study conducted by Martin-Montalvo *et al*. found an extension in life expectancy and overall health improvement in middle-aged male mice after the administration of non-toxic doses of metformin. They proposed that these effects were due to a caloric restriction mimic-effect of metformin [44]. On the other hand, Anisimov *et al*. hypothesized that neonatal treatment with metformin in mice could delay the ageing process. This study found that after the administration of 100 mg/kg of metformin on days 3, 5, and 7 after the birth of 129/Sv mice (in both sexes), a significant increase of +20% was registered in life expectancy in male mice when compared to the control group, reaching a survival rate of 71.8% at 800 days of age [45]. Similar results were reported by the same study group in transgenic mice [46] and SHR female mice [47,48]. Likewise, other studies in mice have demonstrated that metformin administration is associated with a delay in the ageing process or improvements in physical and cognitive functions, both usually affected negatively by age [49–51].

The nematode *Caenorhabditis elegans* is another useful experimental model for the evaluation anti-ageing effect of drugs (Table **1**). A study performed by Onken *et al*. reported that metformin decreased the speed of lipofuscin accumulation, and an increase in both the motor capacity and life expectancy of this invertebrate [52]. Similarly, Chen *et al*. by a design of an *in vitro* reconstitution system to study metformin mechanisms of action in *C. elegans* found the increased life expectancy in this nematode, which was mediated by the lysosomic V-ATPase-regulator pathway [53]. Besides, Cabreiro *et al*. reported that the administration of metformin at different doses achieved an ageing delay and, in consequence, an increase in life expectancy of *C. elegans* in a culture shared with E. coli. This effect was mediated by folate and methionine metabolism changes in this enterobacteria [54].

Conversely, some contradictory findings have been reported in studies conducted in *Drosophila melanogaster* (Table **1**). Na *et al*. evaluated the effects of metformin on age-related changes in gut stem cells from *D. melanogaster*. They reported that metformin administration reduced both DNA damage and hyperproliferation of stem cell growth induced by oxidative stress and ageing [55]. In a subsequent study, the same group reported inhibition of centrosome amplification associated with gut stem cells in *D. melanogaster* after metformin administration [56]. However, Slack *et al*. reported that there was no change in life expectancy of *D. melanogaster* after caloric restriction mimetic-effect of metformin, despite a significant AMPK-pathway activation associated with the anti-aging effects [57]. In summary, the well-documented geronto-protective impact of metformin in a variety of organisms has opened a new era of clinical essays in humans to evaluate the efficacy and safety of metformin as an anti-ageing drug [58].

4. MOLECULAR BASIS OF THE ANTI-AGING PROPERTIES OF METFORMIN

Numerous theories attempt to explain the underlying cause of ageing. From a classic point of view, it is a physiological process associated with age span; however, it still a matter of debate within the scientific community [59]. Recently, distinctive hallmarks of ageing have been described related to molecular and cellular hallmarks present in aged organisms: genomic instability, telomere deterioration, epigenetic alterations, loss of proteostasis, nutrient dysregulation, mitochondrial dysfunction, cellular senescence, stem cells exhaustion, and altered intercellular signalling [5,60]. In consequence, it may be proposed different therapeutical targets to delay ageing and to increase a healthy life expectancy [61,62].

One of the most well-documented and successful interventions related to a longevity increase in experimental models as well as in humans is caloric restriction (CR). CR is defined as a decrease in caloric ingestion without reaching starvation. Results have shown a decreased risk of experienced age-associated diseases as well as improvements in healthy ageing markers [63,64]. The underlying mechanisms responsible for these effects are related to the insulin/IGF1 nutrient-sensing pathway, a very conserved metabolic route implied as a canonical pathway responsible for the ageing process [65]. It has been found that mutations reducing the signal activity of this pathway can increase the life expectancy of mammals [66–68]. However, CR has numerous limitations, especially in regard to its practical implementation in humans [69,70]. Therefore, the study of CR mimetics (CRM), drugs that imitate CR effects without decreasing caloric ingestion, is an active field for research presently. Among these, metformin stands out as a promising drug (Fig. **1**) [71].

4.1. AMPK Pathway

The canonical pathway through metformin mimics CR, and in consequence, displays its anti-ageing effects is by AMPK activation. This pathway regulates a significant number of other metabolic pathways, inhibiting anabolic routes and activating the overall catabolism [72]. AMPK is a heterotrimeric protein composed of an α-subunit with catalytic function, a β-subunit and a γ-subunit. The γ-subunit is capable of binding with adenosyl nucleotides. When this subunit couples to ATP, its activity is inhibited, but when it combines to AMP, it is, in turn, activated [73]. Therefore, AMPK is activated when the AMP:ATP ratio increases, especially in situations like metabolic stress, hypoxia, or nutrient deficiency like hypoglycemia [74]. AMPK is capable of responding to small AMP changes, acting as a positive allosteric modulator [75]. In this sense, metformin increase the AMP: ATP ratio by selective inhibition of the complex I in the electron transport chain (C1) at the proton transfer domains [76,77] in the internal mitochondrial membrane [78]. Similarly, metformin action on C1 can reduce ROS production since this chain is a well-known ROS producer [79,80]. In contrast, there is evidence demonstrating that in rat models fed with a diet rich in fatty acids, the therapeutic dose of metformin significantly increases the activity of the mitochondrial respiratory chain in the hepatocytes, including C1 and the cellular levels of ATP [81]. Likewise, an *in vivo* study performed by Larsen *et al*. in human subjects showed that therapeutic doses of metformin do not inhibit C1 and that this inhibition takes place *in vitro* only with higher doses [82]. Therefore, it is necessary to evaluate the factors or conditions under which metformin would activate or inhibit C1 to be able to propose this drug as a possible anti-aging mechanism.

Table 1. Summary of key preclinical evidence regarding antiaging effect of metformin.

Animal model	Author	Methodology	Results	Refs.
Mice	Martin-Montalvo *et al*. (2013)	C57BL/6 middle-aged male mice received a diet supplemented with metformin at 0.1% (w/w) or at 1% and standard diet (control) during the rest of their lives. The Gehan-Breslow survival test (GBSt) was used to compare the survival curve of both cohorts.	Mice with diet supplemented with 0.1% of metformin showed an extension of mean life expectancy of 5.83% (X2 = 5.46 and p = 0.02) on the GBSt. Conversely, a higher metformin concentration (1% w/w) had toxic effects and significantly decreased life expectancy by 14.4% in C57BL/6 mice (X2= 51.70 y p= 0.001).	[44]
	Anisimov *et al*. (2015)	Female and male 129/Sv mice received 100mg/kg of subcutaneous metformin 3, 5, and 7 days after birth.	Male mice treated with metformin showed a significant increase in mean life higher than 20% ($p<0.05$) compared to the control group. At 800 days of life, male mice neonatally exposed to metformin had a survival rate of 71.8% vs 45% of the control group ($p<0.03$).	[45]
Nematode *(Caenorhabditis elegans)*	Onken *et al.* (2010)	*C. elegans* were raised at 20 °C in growth mediums without metformin or with metformin concentrations of 1 mM, 10 mM or 50 mM, respectively monitoring their survival rate. Motor capacity (swimming rate) was monitored as well, registering the number of body curves/30 seconds 5, 10, and 15 days after birth.	Animals raised with 50nM of metformin showed an increase in survival of up to 40% ($P<0.0001$). Swimming rates of these subjects were significantly higher than the swimming rate of the control group on days 10 and 15 (p= 0.0058 y p = 0.0346, respectively).	[52]
	Cabreiro *et al.* (2013)	Metformin was administered at 25, 50 and 100 nM, respectively in a co-culture of *C. elegans* and *E.* coli.	Life expectancy of *C. elegans* increased by 18%, 36%, and 3% with the administration of 25, 50, ad 100 nM of metformin, respectively. The co-culture of this nematode with *E. coli* pretreated with metformin markedly prolonged its life expectancy (+33%, $p < 0.001$).	[54]
Fruit Fly *(Drosophila melanogaster)*	Na *et al.* (2013)	Different models with *Drosophila melanogaster* were done to analyze the effects related to medium intestinal stem cells (ISCs) of these flies. They were kept at 22-25°C with standard nutrition, adding different concentrations of metformin. The number of positive γH2AvD cells was quantified.	The treatment with metformin in wild flies of 40 days of age reduced the number of γH2AvD positive ISC by 36%, while in mutant *Catn1* flies the decrease was 39%. These findings suggest that metformin inhibits the accumulation of γH2AvD spots associated with oxidative stress and aging in the ISC.	[55]
	Slack *et al.* (2012)	*Drosophila melanogaster* wild flies received increasing metformin concentration orally during 7 days. A survival curve was built to evaluate life expectancy.	The administration of metformin at 1 mM, 2,5 mM, 5 mM, 10 mM, concentrations showed no effect on survival rates of flies from both sexes. Higher metformin concentrations were associated with a dose-dependent decrease in life expectancy ($p<0.05$)	[57]

Abbreviations: GBSt: The Gehan-Breslow survival test; ISCs: medium intestinal stem cells.

In addition, Wang *et al*. observed that in rats, the antihyperglycemic action of metformin depended greatly on the catalytic AMPKα1 subunit. Furthermore, it has the capacity to improve mitochondrial fission and cell respiration [81]. In the context of aging, the promotion of mitochondrial fission mediated by AMPK activation [83] is very important as mitochondrial functions deteriorate with age, with a consequent energy decrease and a dysregulation of the control mechanisms pertaining to mitochondrial quality [84,85]. This idea is reinforced by the findings of the Rana *et al*. team, who showed that an increase in mitochondrial fission increases lifespan in *Drosophila melanogaster* models [86].

More recently, evidence suggests that metformin can activate AMPK by acting on lysosomes too [87] because metformin activates lysosomic V-ATPase, a proton pump present on intracellular membranes, that produces a proton gradient by ATP hydrolysis energy [88]; promoting the translocation of the bridge protein

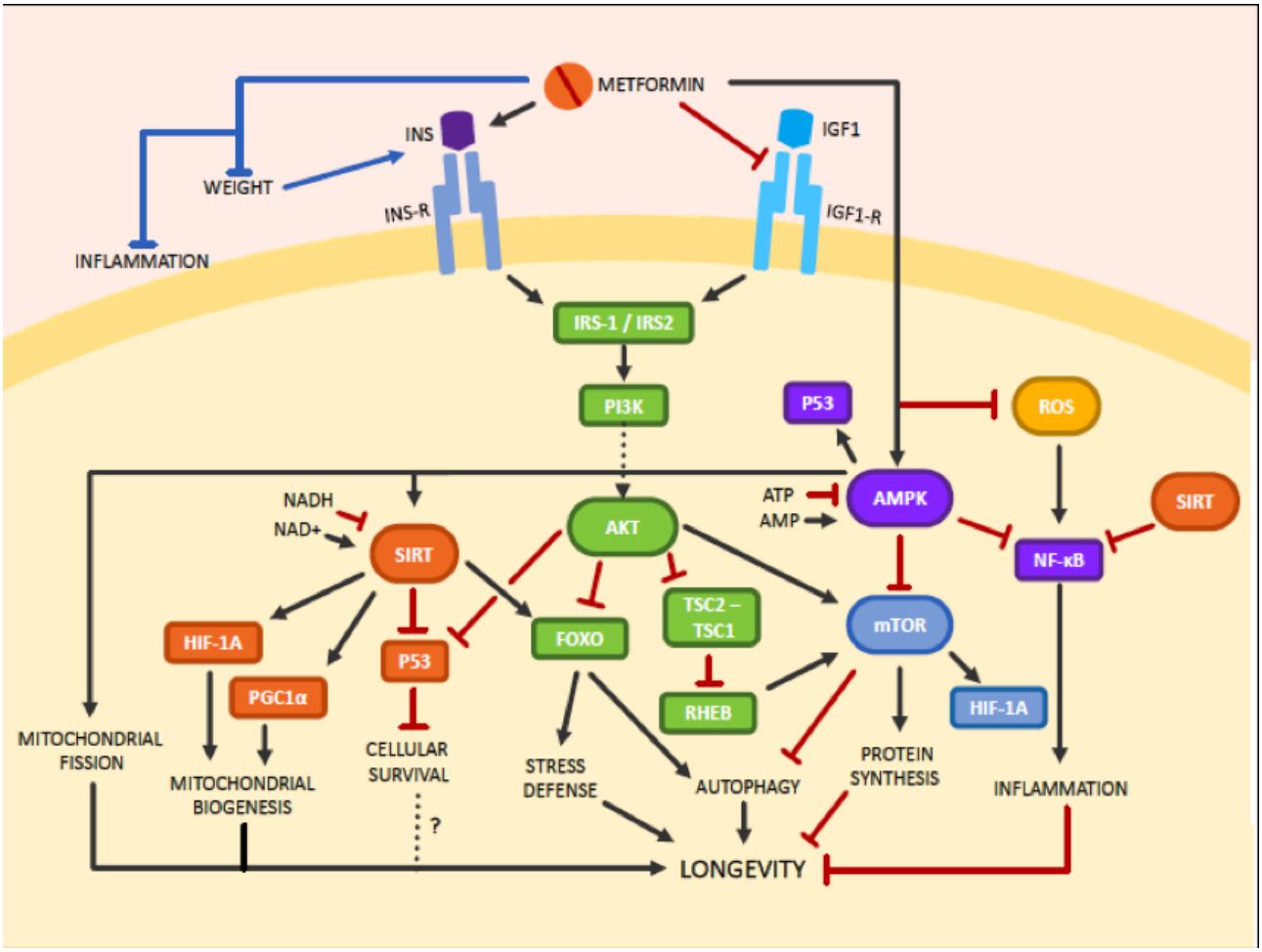

Fig. (1). Molecular mechanism of metformin in aging. There are numerous mechanisms through which metformin has its anti-aging action. One of the main ones is the nutrient detection signaling pathway, IGF1, through the activation of AMPK and the inactivation of mTORC1. This leads to a decrease in protein synthesis and an increase in autophagy. AMPK activates SIRT1, which through PGC-1α and HIF-1α improves mitochondrial biogenesis. SIRT1 also activates FOXO subgroups, which favor autophagy and antioxidant defense. Furthermore, metformin has anti-inflammatory effects by inhibiting translocation of the transcription factor NF-kB to the nucleus and reducing ROS production. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

Abbreviations: INS: Insulin; INS-R: Insulin receptor; IGF1: Insulin-like growth factor-1; IGF1-R: Insulin-like growth factor-1 receptor; IRS-1: Insulin receptor substrate 1; IRS-2: Insulin receptor substrate 2; PI3K: Phosphatidylinositol 3-kinase; AKT: Protein kinase B; FOXO: Forkhead box transcription factors; TSC1: Tuberous sclerosis 1 protein; TSC2: Tuberous sclerosis 2 protein; RHEB: Ras homolog enriched in brain; AMPK: Adenosine monophosphate-activated protein kinase; P53: Gene P53; ROS: Reactive oxygen species; NF-kB: Nuclear factor-kB; SIRT: Sirtuin; mTORC1: Mammalian target of rapamycin C1; HIF-1α: Hypoxia-inducible factor 1-alpha; ATP: Adenosine triphosphate; AMP: Adenosine monophosphate; NADH: Nicotinamide adenine dinucleotide reduced; NAD+: Nicotinamide adenine dinucleotide; PGC1α: Peroxisome proliferator-activated receptor γ co-activator 1α.

AXIN and the liver kinase B1 (LKB1) on the surface of the lysosome. These proteins form an oligomeric complex, the V-ATPase-Ragulator-AXIN/LKB1-AMPK complex, which in turn, activates AMPK by phosphorylation [53,89,90].

Another possible metformin mechanism of action could be related to its affinity with metal ions. Cu2+ is the most crucial metal present in the mitochondria, and it has been hypothesized that it might be involved in AMPK activation by direct inhibition of the electron transport chain [91,92]. Similarly, metformin has anti-inflammatory effects through its interaction with lysosomic Zn2+, which increases the inhibitory Zn2+ effect on cysteinyl proteases [93]. Moreover, metformin could directly increase glutathione peroxidase-7 activity, an antioxidant enzyme located in the endoplasmic reticulum [94].

4.2. mTORC1 Pathway

Protein complex mTORC1 is one of the downstream targets of AMPK. The essential components of this pathway include the mammalian target of rapamycin (mTOR) and the regulatory-associated protein of mTOR, Raptor [91,95]. mTORC1 activates highly conserved processes like protein synthesis and lipogenesis and inhibits autophagy. During autophagy, deteriorated cytoplasmatic organelles, proteins, and macromolecules are kidnapped in vesicles called autophagosomes, which fuse to lysosomes conducting the degradation and recycling of these organelles and biomolecules [96]. This process has a crucial role in cellular homeostasis because it protects the nutrient flux and the correct function of cellular organelles [72]. Furthermore, a disorder in this pathway leads to diseases associated with age, such as diabetes and neurodegeneration. [14]. AMPK indirectly inhibits mTORC1 through the activation of tumoral suppression genes belonging to the tuberous sclerosis complex 1 and 2 (TSC1 and TSC2) [97], which inhibit the Ras homolog enriched in brain (Rheb) a protein required for the activation of mTORC1 [98] and by direct action by phosphorylation of S772 and S792 on Raptor [27,99]. Likewise, metformin can inhibit MTORC independently from AMPK [100].

The accumulated evidence to date suggests that mTOR inhibition is an effective way to prolong life in experimental models [27,99,101,102]. mTORC1 inhibition mediates changes in protein metabolism characterized by a decrease in protein biosynthesis [103], the quality control mechanisms such as the unfolded protein response (UPR) [103-105], and protein degradation through the protein ubiquitin-proteasome system and lysosomic autophagy [106]. These processes occur more slowly in aged tissues [107]. This action contributes to proteostasis maintenance, decreasing the accumulation of unfolded proteins, which in turn reduces endoplasmic reticulum stress, and in consequence, decreases apoptosis or cellular senescence [108]. The accumulation of unfolded proteins is the base os the pathophysiology of neurodegenerative diseases like Alzheimer's and Parkinson's diseases [109-111].

4.3. SIRT1 Pathway

Another feature of the ageing process is mitochondrial dysfunction, with deficient ATP synthesis and an increase in ROS production [112]. These changes lead to oxidative stress defined as the unbalance between ROS production and the scavenger capacity of the cell to eliminate these harmful molecules [113]. Oxidative stress is an important cause of DNA damage, which contributes to genomic instability in aged organisms [114]. The role of mitochondrial dysfunction in ageing is well-understood thanks to the development of animal models with dysfunctional mitochondria, which present accelerated ageing [115]. Furthermore, the presence of mitochondrial dysfunction has been confirmed in diseases such as Hutchinson-Gilford progeria, a syndrome characterized by accelerated ageing [116].

Metformin AMPK activation drives to NAD-dependent deacetylase sirtuin-1 (SIRT1) phosphorylation, a key step, which causes epigenetic histones modification, specifically, disinhibition through the mTORC1 pathway. This is also achieved by increasing NAD+ [117]. Likewise, metformin activates SIRT1, possibly through the reduction of the Michaelis-Menten (KM) constant for NAD + [118]. SIRT 1 can improve mitochondrial biogenesis through transcriptional factors such as the coactivator of the γ receptor, which is activated by the peroxisome proliferator-activated receptor-gamma coactivator-1α (PGC-1α), and the hypoxia-inducible factor 1α (HIF-1α) [119].

4.4. FOXO Pathway

SIRT1 activation is one of the mechanisms implied in the antioxidative metformin properties by activating the O subgroup of the Forkhead family of transcription factors (FOXO). FOXO regulates a group of genes involved in cellular death and oxidative stress response by allosteric modulation activation of antioxidant enzymes such as superoxide dismutase and catalase. In consequence, their expression increases (as well as autophagy) dysfunctional mitochondria degradation, maintaining proteostasis [26,120–122]. Similarly, FoxO1 specifically also regulates genes implicated in the cell cycle arrest (p21), DNA repair (Gadd45a), apoptosis (BIM), and response to stress (MnSOD), which could also be responsible for the anti-ageing effects [121].

4.5. DICER1 Pathway

Moreover, there is evidence that metformin decreases cellular senescence by a DICER1-dependent pathway, which is a type III cytoplasmic endo-ribonuclease. DICER I play a significant role in the maturation of different classes of small non-coding ARN. Therefore, it is necessary for microARN-mediated silencing [123], decreasing p16 and p21 protein levels and inflammatory cytokine and oncogene abundance, which is characteristic of the secretory phenotype associated with ageing [124].

4.6. Microbiota, Metformin and Ageing

The term microbiota refers to a complex ecosystem of billions of microbes living in the human body. They exert systemic effects in the organism, which are mediated by diverse microbe metabolites. Therefore, an association between the alteration of this ecosystem might be related to different diseases, including cancer, diabetes, and obesity [125]. Furthermore, it has been demonstrated in experimental models that microbiota modulation can lead to life extension expectancy. Therefore, the results of recent studies are promising, mainly those showing the effects of metformin on gut microbiota. Among these effects, the increase of *Akkermansia muciniphila,* which degrades mucin has been reported as well as the increase of some short-chain fatty acid-producing bacteria [126]. These changes can contribute to weight loss and the suppression of inflammation induced by metformin in individuals with T2DM. However, the mechanisms through which metformin alters gut microbiota and the impact of this process in human ageing are not clear. In consequence, more research is necessary [91].

4.7. Inflamm-aging and Metformin

Inflammation is a protective mechanism of the organism against injurious agents, with the goal of eliminating the damage, repairing tissues, and promoting homeostasis, in which the participation of the innate immune response to the initial injury and the consequent adaptive response takes the main role in achieving healthy resolution. However, this response becomes more frequent and imperfect as the body ages, with quantitative and qualitative alterations of the immune system generating immunosenescence. This leads to a state of low-grade chronic inflammation associated with age, also called "Inflamm-aging" [127,128]. This process involves many factors and it is mainly a product of the dysregulation of cytokine production, with an increase in proinflammatory cytokines such as TNF-α, IL-6, IL-1 IFN-γ, and IFN-β. These are mainly secreted by T CD8+ and CD4+ lymphocytes as well as macrophages through the activation of NF-κB, which is one of the largest inflammatory pathways, mediating the inflammatory cascade [129]. It is understood that metformin is a potent anti-inflammatory that can act on the immune system response, favoring the inhibition of the NF-κB pathway [130]. Metformin inhibits the translocation of the NF-kB transcription factor to the nucleus, and it avoids IκB and IKKα/β phosphorylation, which is necessary for the activation of the pathway 1 of NF-κB [131]. Furthermore, metformin promotes the decrease of the proinflammatory M1 macrophage phenotypes, as well as allowing lymphocyte differentiation, promoting the differentiation of T CD8+ memory cells through the activation of the TNF receptor-associated factor 6 (TRAF-6). This is dependent on the induction of fatty acid metabolism, which indicates that it improves the functions of adaptive immunity [132]. Alternatively, the chronic inflammatory environment generated by these cytokines involves telomere and mitochondrial DNA damage, leading to mistakes in DNA replication and translocation, resulting in cellular senescence [133]. As a consequence, the increase of pro-inflammatory cytokines as well as the activation of transcription factors such as NF-κB, TOR, Retinoic-acid-inducible gene-I (RIG-I), and JAK/STAT block the cell cycle and induce and maintain the senescence-associated secretory phenotype (SASP), which generates more of these cytokines. This leads to intracellular self-perpetuation and the inhibition of the regenerative capacity of stem cells, transforming into a major vicious cycle [134]. Metformin inhibits SASP-related proinflammatory senescence in senescent cells [135]. Similarly, immunosenescence produces mitochondrial and autophagy decrease, which are key mechanisms that favor Inflamm-aging and that control the activation of the innate and adaptive immune systems. This leads to an increase in ROS and, in consequence, in oxidative stress, where lysosomes altered by the ROS increase, activate the Nod-like receptor 3 (NLRP3). This is one of the largest inflammasomes of intracellular stress, which is capable of activating Nf-kB [136]. Likewise, oxidative stress can activate IKK, and metformin can normalize IKK expression through previously described mechanisms [131].

5. METFORMIN AS ANTI-AGING THERAPY IN HUMANS

The prior sections provided evidence from numerous both *in vitro studies* and animal models studies showing the beneficial effects of metformin as well as the molecular pathways involved in its effects. Moreover, researchers have tried to take one-step further and confirm the existence of a geronto-protective effect of this drug in humans. The goal is to create a new paradigm and treat the aging itself. Will metformin be the new anti-ageing therapy in humans? [137].

5.1. Effects of Metformin in Ageing-associated Diseases in People with Diabetes

Clinical and epidemiological studies (Table **2**) have shown that metformin is not only an anti-hyperglycemic drug. It also has protective effects, such as decreasing the risk of suffering some chronic and degenerative diseases [138]. For example, in a case-control study performed by Bannister *et al*. in 2014, an observational retrospective data from the "UK Clinical Practice Research Datalink" in 2000, people with T2DM were analyzed. These patients were assigned to metformin or sulfonylureas as monotherapy by at least 180 days. They were compared with control subjects without T2DM and paired by sex and age. It was observed that those treated with metformin had survival rates similar or even better than control subjects, despite the fact of suffering diabetes, obesity and other comorbidities. Furthermore, those treated with sulfonylureas had a lower survival rate than those treated with metformin, showing the protective role of this drug [139].

Likewise, NG *et al*. performed a longitudinal study with 365 diabetic subjects older than 55 years old in treatment with metformin. They were compared with diabetic patients not treated with metformin. This cohort was part of the "Singapore Longitudinal Aging Study". The results of the study showed that metformin administration in diabetic individuals decreased neurodegeneration with a risk reduction of cognitive deterioration and dementia, especially in those who had been treated for six years or more with metformin [140].

In regards to cardiovascular diseases, the Metformin in Prediabetes on Atherosclerotic Cardiovascular Outcomes study (VA-IMPACT), a multi-centre, prospective, randomized, and double-blind clinical assay in phase IV (NCT02915198) was started in 2019 with a 7,868 individuals cohort over 18 years old, and both sexes with pre-existing conditions like pre-diabetes and diagnosed atherosclerotic cardiovascular disease (CVD). This study will be a follow-up period of 4,5 years, and the goal is to assess if metformin treatment decreases cardiovascular morbidity and mortality. The estimated date for the end of the study is August 21, 2024 [141].

There is evidence that metformin can correct metabolic and cellular processes associated with the development of conditions associated with age such as inflammation, oxidative damage, cellular senescence, as well as apoptosis. This led researchers of the Metformin in Longevity Study (MILES), to perform a double-blind, placebo-controlled clinical assay that evaluates the effect of metformin treatment in the biology of age. The goal of this study was to determine if metformin would allow for the restoration of beneficial gene expression in adult individuals with glucose intolerance. The study had a small sample, with 16 participants older than 60 years, who received a dose of 1,700 mg a day of metformin. Years later, the study yields its first results, which suggest that metformin modulates the expression of genes to a profile corresponding to young subjects. This was observed in the RNA-sequencing of muscle and adipose tissue biopsies [142].

Muscle mass and strength are an essential quality of life markers and indicators of functional independence in the late stages of life. Therefore, resistance training is considered the most effective intervention, which allows avoiding muscle atrophy associated with age (sarcopenia). Therefore, Long *et al*. performed a double blind, placebo-controlled clinical assay in adults older than 65 years with risk factors for T2DM. Participants received 1,700 mg of metformin a day or placebo for two weeks before starting a 14-week training regime. The objective of this study was to determine how metformin improves muscle response to resistance training and altering the inflammatory environment of the muscle tissue. This research assessed these goals by muscle tissue biopsies, observing that both muscle mass and strength had increased, the levels of pro-inflammatory cytokines, oxidative metabolism, structural integrity, and resistance to muscle damage improved due to AMPK activation. However, muscle response to the training program did not always show encouraging results due to some individuals lost muscle mass despite training and pharmacotherapy. Therefore, response to treatment needed to be optimized and personalized for each individual [143].

On the other hand, the association between cancer and diabetes has been widely documented, because diabetes presents with hyperinsulinemia and hyperglycemia states, as well as pro-inflammatory cytokine production, which promotes carcinogenic mechanisms [144]. Gandini *et al*. performed a systematic review and meta-analysis in 2014 in which 71 studies were included. They considered confusion variables that could have taken place in the selected studies, and they evaluated the association between metformin use and cancer. The results show that patients with diabetes treated with metformin had a significantly 31% lower cancer incidence. Similar results were seen in cancer mortality, with a 34% decrease in comparison to non-diabetic people or those who were treated with another hypoglycemic drug [145].

In this sense, similar results were found in a meta-analysis performed by Yin *et al*. in 2013 on 20 studies and 13,008 subjects with T2DM and cancer treated with metformin and other anti-diabetic drugs. They evaluated the association between metformin and both all-type and specific cancers survival. They found a relative survival benefit associated with metformin treatment compared with the other glucose-lowering medications in both overall survival as well as cancer-specific survival, with hazard ratio (HR) 0.66; 95% confidence interval (CI): 0.55– 0.79 and HR 0.62; 95% CI: 0.46–0.84, respectively) [144].

Finally, a meta-analysis conducted by Campbell *et al*. in 53 studies evaluates all-cause mortality in T2DM patients treated with metformin and compared with non-diabetic patients. The results showed that patients treated with metformin had lower mortality rates when compared to other groups, suggesting a metformin action related to an anti-ageing effect, which contributes to improving quality of life [146].

5.2. Metformin Effect in Ageing in Non-diabetic Patients

Although the studies mentioned above show the beneficial effects of the drug, there is no certainty that these effects are due to a slow down in the ageing process. All the studies have a critical limitation to have been conducted on diabetic patients, which could produce confusing results such as the benefits seen by blood glucose and another metabolic improvement and not by its anti-ageing effects [147]. Therefore, researchers have moved forward from animal models to human studies looking to demonstrate metformin gen-suppressant effects in preventing chronic illnesses [148]. This is the source of the first Food and Drugs Administration (FDA)-approved multi-centre clinical assay, Targeting Aging with Metformin (TAME) performed by Barzilai *et al*., and financed by the American Federation for Aging Research [149]. This assay began in 2019, and it has as the goal of to analyse metformin efficacy in decreasing risk or preventing the appearance of chronic diseases. It also examines if metformin has an effect in an extension in healthy life expectancy in a sample of 3,000 non-diabetic subjects between 65 and 79 years old without age-associated pathologies. The patients will be treated with a dose of 1,500 mg of metformin a day for six years, and there will be a follow-up period of 3.5 years. The

Table 2. Summary of key clinical and epidemiologic evidence regarding antiaging effect of metformin.

Authors	Type of study	Methodology	Results	Refs.
Bannister *et al.* (2014)	Case- control	Mortality causes in diabetic patients treated with metformin and other hypoglycemic drugs during at least 180 days were compared with patients without T2DM. 2000 subjects with T2DM from the data source "UK Clinical Practice Research Datalink" (CPRD) were included.	Patients with T2DM treated with monotherapy of metformin had longer survival than patients treated with other hypoglycemic drugs or non-diabetic patients.	[139]
NG *et al.* (2014)	Longitudinal study	365 subjects older than 55 years from the "Singapore Longitudinal Aging Study" were included. They were distributed in two groups; those treated for six years and those treated for more than six years.	Metformin treatment for over six years is associated with a lower risk of cognitive deterioration in adult diabetic patients.	[140]
Schwartz *et al.* (2019)	Clinical Assay	16 participants 60 years of age or older were administered one dose of metformin of 1700 mg a day for six weeks. The goal was to determine if the drug can restore youth gene expression in people of advanced age with glucose intolerance.	Metformin modulates the expression of genes in older individuals with glucose intolerance to that of young healthy subjects according to the RNA-sequencing findings in muscle and adipose tissue biopsies.	[142]
Crandall *et al.* (2014)	Clinical Assay	Multicenter study with 7,868 participants of both sexes, older than 18 years of age with a 4.5-year follow-up. The subjects were pre-diabetic and had atherosclerotic CVD.	Phase 4 study. Estimated date for results, August 21, 2024.	[141]
Long *et al*. (2017)	Clinical Assay	Adults older than 65 years of age with risk factors for T2DM treated with 1,700 mg of metformin a day for two weeks. Afterward, they started a resistance training program. The goal was to determine if metformin can increase strength and muscle mass.	After the training program, subjects improved muscle strength as well as muscle mass. It was determined through muscle tissue biopsy that structural integrity and muscle damage resistance improved due to AMPK activation.	[143]
Barzilai *et al*. (2016)	Clinical Assay	Multi-center study with 3,000 participants between 65 and 79 years old with no aging-associated pathologies. They will be treated with one dose of 1,500 mg of metformin a day for 6 years. The goal is to evaluate if metformin decreases the incidence of aging-associated pathologies.	Complete results are expected for 2022.	[149]
Gandini *et al.* (2014)	Meta-analysis	47 studies were included to compare cancer incidence in diabetic patients treated with metformin. The sample included diabetic patients treated with metformin and diabetic patients treated with other antidiabetic drugs.	Diabetic patients treated with metformin showed a cancer incidence decrease of 31%.	[145]
Yin *et al.* (2013)	Systematic revision and meta-analysis	20 studies with a total of 13,008 participants were included. The goal was to compare survival among all types of cancer as well as to specific cancers in diabetic patients treated with metformin and other hypoglycemic drugs.	There is a relative survival benefit associated with metformin treatment in diabetic patients with cancer compared to diabetic patients treated with another drug.	[144]
Campbell *et al.* (2017)	Systematic revision and meta-analysis	53 studies were included with samples that included healthy individuals, patients treated with metformin or other hypoglycemic drugs, or with a mean age of 50 years old. The goal was to evaluate all-cause mortality in T2DM patients.	Patients treated with metformin had lower all-cause mortality rates than non-diabetic and diabetic patients treated with other antidiabetic drugs. Likewise, patients treated with metformin had decreased cancer rates and cardiovascular disease rates compared to non-diabetic patients.	[146]

Abbreviations: CPRD: UK Clinical Practice Research Datalink; T2DM: Type 2 diabetes mellitus; CVD: Cardiovascular disease; AMPK: 5' AMP-activated protein kinase.

results of this study could set a change in current medicine, which would allow for the transition of pathology treatment to ageing treatment. The question is, will TAME be able to demonstrate that metformin can decrease the inevitability of human ageing? [91,150].

6. DOSE AND ADVERSE EFFECTS OF METFORMIN

Metformin, as an anti-ageing therapy in humans, is not free of risks. However, it is important to state that this biguanide has few adverse effects, which in its majority, falls on intestinal issues. These effects disappear with time or after a dose reduction. Despite this, there is evidence that in patients with abnormal kidney function, metformin administration increased the risk of lactic acidosis. However, this is an infrequent complication [151]. Moreover, a higher risk of vitamin B12 and B6 deficiency has been found as well, which has caused specialists to suggest that the drug is administered with vitaminic supplements. However, like with lactic acidosis, this has taken place in a small number of patients [91].

In regards to ageing, there is the question of which dose of metformin has geronto-suppressant effects and if these effects can be achieved in the entire population. It is necessary to consider that the existence of a small percentage of users that have no response to metformin has been identified. This means that there are individuals who may or may not respond to the effects of this drug [152]. This variability can be explained by genetic alterations in the metformin organic cation transporter (OCT1) [153]. Similarly, a critical issue to consider is high doses of metformin used in ageing studies both *in vitro* and *in vivo,* which are not comparable with the therapeutic doses in humans. Therefore, metformin dosing could represent a limitation to observe a strong anti-ageing effect in humans [154].

CONCLUSION

For decades, scientific evidence has led to discovering that metformin has pleiotropic effects beyond blood glucose control that involve different molecular pathways associated with ageing processes. These pathways are the target of metformin geronto-protective effects, which has been verified experimentally in non-human models. These results show that metformin can decrease the risk of cardiometabolic, neurodegenerative disorders, cancer, sarcopenia, as well as prolonging life expectancy. Furthermore, treatment with this biguanide is well-tolerated with few adverse effects. However, the dose necessary to observe geronto-protective effects is a limitation to overcome. Therefore, more studies are needed to determine optimal plasma levels of metformin as an anti-ageing therapy.

LIST OF ABBREVIATIONS

T2DM	=	Type 2 Diabetes Mellitus (T2DM)
AMPK	=	5' adenosine monophosphate-activated protein kinase
DNA	=	Deoxyribonucleic acid
CR	=	caloric restriction
CRM	=	CR mimetics
ROS	=	reactive oxygen species
LKB1	=	Liver kinase B1
C1	=	Complex I of the electron transport chain
mTOR	=	Mammalian target of rapamycin
Raptor	=	Regulatory-associated protein of mTOR
TSC1	=	Tuberous sclerosis 1 protein
TSC2	=	Tuberous sclerosis 2 protein
NF-kB	=	Nuclear factor-kB
TRAF-6	=	TNF receptor associated factor 6
RIG-I	=	Retinoic-acid-inducible gene-I
JAK/STAT	=	Janus kinase/signal transducers and activators of transcription
SASP	=	Senescence-associated secretory phenotype
NLRP3	=	Nod-like receptor 3
IKK	=	inhibitor of nuclear factor-κB kinase
ATP	=	Adenosine triphosphate
AMP	=	Adenosine monophosphate
NAD+	=	Nicotinamide adenine dinucleotide
UPR	=	Unfolded protein response
SIRT1	=	Sirtuin-1
PGC-1α	=	Peroxisome proliferator-activated receptor gamma coactivator-1α
HIF-1α	=	Hypoxia inducible factor 1α
FOXO	=	O subgroup of the Forkhead family of transcription factors
VA-IMPACT	=	Investigation of Metformin in Pre-diabetes on Atherosclerotic Cardiovascular Outcomes
CVD	=	Cardiovascular disease
MILES	=	Metformin in Longevity Study
TAME	=	Targeting Aging with Metformin
OCT1	=	Metformin organic cation transporter

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CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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