

Review

Metformin as Anti-Aging Therapy: Is It for Everyone?

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Metformin is the most widely prescribed oral hypoglycemic medication for type 2 diabetes worldwide. Metformin also retards aging in model organisms and reduces the incidence of aging-related diseases such as neurodegenerative disease and cancer in humans. In spite of its widespread use, the mechanisms by which metformin exerts favorable effects on aging remain largely unknown. Further, not all individuals prescribed metformin derive the same benefit and some develop side effects. Before metformin finds its way to mainstay therapy for anti-aging, a more granular understanding of the effects of the drug in humans is needed. This review provides an overview of recent findings from metformin studies in aging and longevity and discusses the use of metformin to combat aging and aging-related diseases.

Metformin Usage Beyond Type 2 Diabetes (T2D): Aging and Aging-Related Disease

The history of the antidiabetic drug metformin dates to the 17th century, where extracts of the leaves of the French lilac *Galega officinalis*, which contain metformin-like guanidine compounds, were used to treat plague, fever, snake bites, and other ailments. The antihyperglycemic property of *G. officinalis* was first described in *Culpeper's Complete Herbal* in 1653 [1]. Although guanidine-containing compounds are responsible for the plant's antihyperglycemic effect in animals, these agents proved too toxic for use in humans. In 1922, synthesis of metformin and related biguanide compounds phenformin and buformin was achieved by Werner and Bell [2], paving the way for metformin to attain widespread use in humans as first-line therapy for T2D worldwide [3] (Figure 1, Key Figure). Metformin also has proven roles in prevention of diabetes [4], in treatment of polycystic ovary syndrome (PCOS) [5], and in helping individuals with diabetes prevent weight gain or even lose weight [6].

The first milestone step for the use of metformin to treat diabetes was taken by the French physician Jean Sterne in 1957, who achieved approval for metformin use under the brand name Glucophage [7]. Metformin was slow to gain approval in the USA due to concerns over lactic acidosis that were far greater with sister compounds buformin and phenformin (which are no longer in clinical use). Metformin went into use in the USA in 1995, boosting its use and stimulating research targeted at elucidating its mechanism of action.

Emerging evidence indicates that metformin has favorable effects on health beyond those associated with improvement in glycemia. Observational studies suggest that diabetic individuals treated with metformin manifest a survival benefit even when compared with nondiabetic controls [8,9]. Metformin not only reduces cardiovascular disease incidence in patients with T2D [10], it similarly reduces atherosclerotic burden in nondiabetic individuals at risk for the disease [11]. Observational data in humans further support a role for metformin in prevention of aging-related decline and cancer [9,12], an area of immense clinical interest. Molecular analyses of septuagenarians treated with metformin indicate that the drug elicits metabolic and nonmetabolic effects consistent with multiple effects on aging [13]. In this article, recent progress on our understanding of metformin actions in aging are reviewed and explored with a concluding proposal that precision medicine approaches may be needed to apply metformin broadly as an anti-aging therapy in humans.

Recognition of Aging as a Disease

Aging is often referred to as a risk factor for age-related diseases and is sometimes described as the 'sum of age-related diseases' [14]. Although it has been a long time coming, the World Health Organization now formally recognizes aging as a disease in the latest version of the International

Highlights

With continuous improvement in living conditions, interest and investment in anti-aging therapies are vastly growing. The antidiabetic drug metformin has garnered tremendous interest owing to its position as first-line therapy for type 2 diabetes treatment and exhibition of anti-aging properties in model organisms.

In spite of its widespread use, the mode of metformin action is not fully understood. Multiple targets and distinct mechanisms have been proposed by which its anti-aging effects are mediated.

Many uncertainties exist in metformin mechanisms and side effects that may prevent its widespread use in aging in otherwise healthy individuals.

Studies on metformin's antidiabetic effects demonstrate that metformin does not affect all users in the same fashion. Thus, precision metformin therapy may be needed to fully realize the benefit of metformin to combat aging-related diseases in humans.

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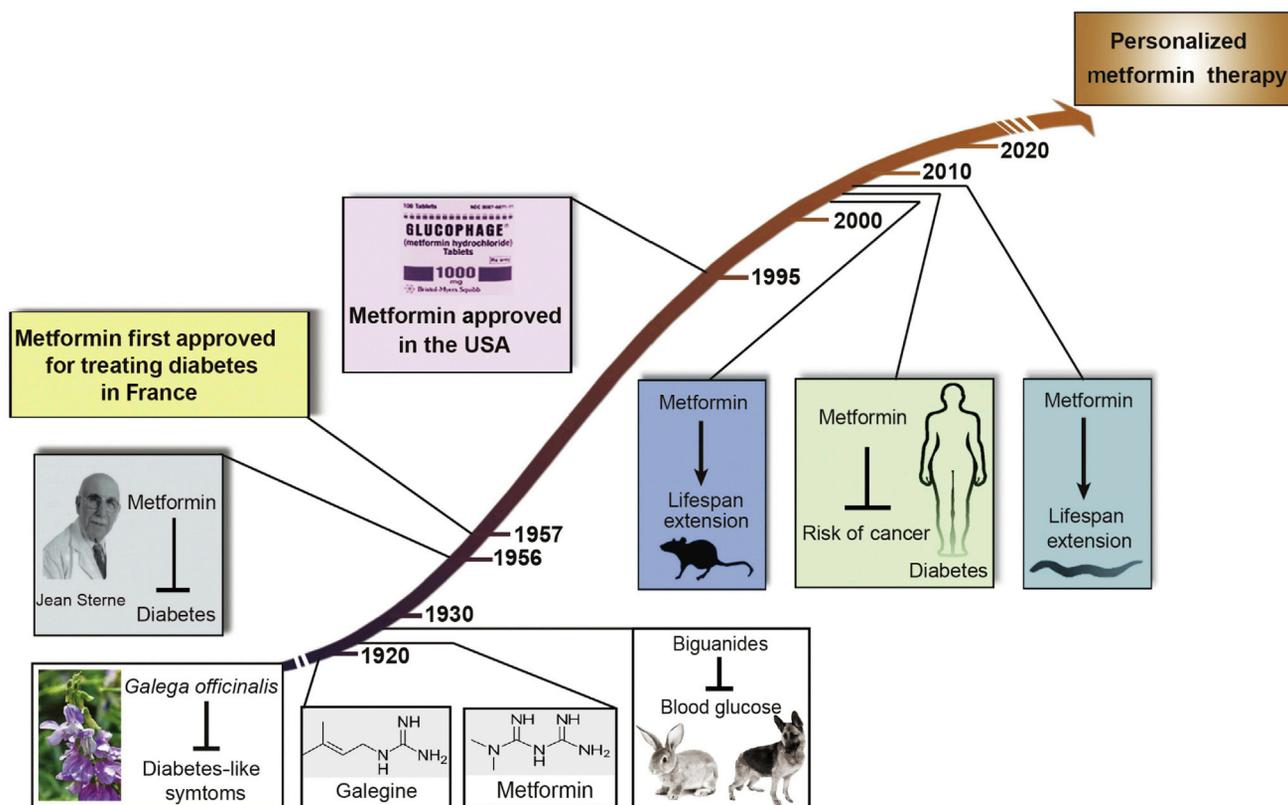
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Key Figure

Significant Events in Metformin Use in Diabetes and Aging-Related Diseases



Trends in Endocrinology & Metabolism

Figure 1. Metformin-like compounds such as galegine are the active compounds in the French lilac *Galega officinalis* that has been used since medieval times to treat diabetes-like symptoms. Metformin, phenformin, and buformin were synthesized by Werner and Bell in 1922, and studies determined that biguanides lowered blood glucose in laboratory animals in the mid-1920s. Owing to studies in humans by the French physician-scientist Dr Jean Sterne, metformin went into use in Europe in the 1950s and was later approved in the US in 1995. Its US approval was delayed due to concerns over lactic acidosis, far more likely with its sister drugs phenformin and buformin. In the early 2000s, studies at the National Institutes of Health determined that metformin extends lifespan and healthspan in laboratory mice, and shortly thereafter metformin was found in observational studies to reduce morbidity and mortality from aging-associated diseases such as cancer in humans. Metformin extends the lifespan of the roundworm *Caenorhabditis elegans* by up to 50%, a discovery that has enabled genetic dissection of the pathways necessary for metformin longevity effects. We predict that the future of metformin use to combat aging in humans will involve the use of personalized medicine approaches.

Classification of Diseases (ICD-11, code 'Ageing-related' XT9T). The formal recognition of aging as a disease is meaningful for the development of future therapeutic interventions or strategies targeting aging and aging-related diseases [15]. It is also likely to raise interest in repurposing drugs to treat aging, such as metformin. Metformin has been explored as an anti-aging agent in model organisms and humans [16,17], given its excellent safety record for over six decades in the clinic, well-documented beneficial properties in cardioprotection, and potential value in cancer prevention and treatment [18,19].

Metformin Prolongs Lifespan and Healthspan in the Invertebrate *Caenorhabditis elegans*

C. elegans is a powerful model organism for mechanistic study of longevity, having aided in identification of more than 200 longevity-affecting genes and regimens [20]. The lifespan prolonging effects of metformin in *C. elegans* were first reported in 2010 [21]. This study demonstrated that metformin also prolonged healthspan, the portion of the lifespan where animals are active, suggesting that metformin promotes both lifespan and healthy aging (Figure 1).

Metformin-mediated lifespan extension in *C. elegans* is genetically dependent upon the cellular energy sensor adenosine monophosphate-activated protein kinase (AMPK) and its upstream activating kinase liver kinase B1 (Lkb1, *par-4* in the worm), as well as the stress-induced transcription factor *skn-1*/nuclear factor erythroid 2-related factor 2 (Nrf2). This is in contrast to effects on glycemia and cell growth, suggesting that the glycemic and anti-aging effects of the drug have distinct mechanisms of action. Subsequent work confirmed these observations but indicated that the effects of metformin on lifespan are far from straightforward [22–25]. Our studies indicate a requirement for the nuclear pore complex (NPC) and acyl-CoA dehydrogenase family member 10 (ACAD10) in lifespan extension, a pathway that is activated by direct action of metformin on *C. elegans* [24]. Other work suggests that metformin prolongs lifespan in *C. elegans* through direct action on lysosomes [25]. And yet another study suggests that metformin slows aging of *C. elegans* through metabolic modulation of the *Escherichia coli* food source [22]. The potential mechanisms by which metformin exerts its anti-aging effects are discussed in detail below.

Metformin Extends Lifespan and Healthspan in Mice

In the early 2000s, studies at the National Institutes of Health and elsewhere determined that metformin extends the lifespan and healthspan of genetically outbred and inbred laboratory mice [26–30] (Figure 1). Some, but not all these studies indicate a sexual dimorphism suggestive of a greater benefit for female mice.

In contrast to observations in *C. elegans*, mice, and observational studies in humans, lifespan extension is not evident with metformin treatment in the fruit fly *Drosophila* [31] or rats [32], although AMPK activation in flies and body weight loss in rats was detected. The exact explanation for these disparate effects of metformin in different organisms remains elusive. Numerous individual factors affect aging, such as nutrient availability and the intensity of exercise [33]. Thus, factors such as activity may explain why metformin promotes lifespan of certain organisms (worm, caged mice) but not others (fruit fly). Further, metformin may not further extend longevity of already long-lived species such as the F344 rat strain [32]. Finally, dosing regimens that are not optimized for each organism may also explain failure to achieve lifespan extension in some cases.

Current Knowledge of Metformin Targets and Its Mode of Action

Metformin has been used to treat T2D for more than 60 years, and yet even its antihyperglycemic mode of action remains incompletely characterized. Recent advances have revealed multiple cellular effects of metformin that may be relevant for its effects both on metabolism and aging. Under different circumstances, effects of metformin may be mediated by molecular targets as disparate as mitochondrial complex I [34], mitochondrial glycerol-3-phosphate dehydrogenase (mGPDH) [35], and the H3K27me3 demethylase KDM6A/UTX [36]. It should be noted that very few studies attempt to discriminate between direct and indirect metformin response pathways. Here, we will make an effort to illuminate direct versus indirect metformin targets with available evidence.

Metformin Targets the Mitochondrial Respiratory Chain

It is widely accepted that the mitochondrion is a primary target of metformin, responsible for its antihyperglycemic effect [34,35,37,38]. In line with early studies indicating a primary effect of metformin on complex I of the mitochondrial electron transport chain, recent work also provides strong genetic evidence that metformin inhibits cancer cell growth through its actions on complex I [34,37,39–41].

Our own work also shows that rotenone (a complex I inhibitor) and metformin both activate the same signaling cascade in the same manner in *C. elegans* and mammals [24]. Ectopic expression of the metformin-resistant *Saccharomyces cerevisiae* NADH dehydrogenase ND11 in place of complex I renders HCT 116 p53^{-/-} colon cancer cells resistant to killing by metformin *in vitro* and in tumor allografts *in vivo* [34]. However, mitochondria continue to be challenged as a primary target of metformin, mainly because experimentally discernible inhibition of mitochondrial function by metformin can require millimolar levels of drug. It remains an unanswered question as to whether levels of drug that are achievable in humans also mediate metformin's benefit on aging and prevention of aging-related diseases through modest effects on mitochondria. It is little appreciated that metformin inhibits production of reactive oxygen at far lower doses than those required to affect respiratory capacity [42]. Thus, it remains extremely plausible that mitochondrial effects of metformin dominate even at the micromolar levels obtainable in humans *in vivo* [43].

By targeting complex I, metformin lowers the relative energy charge of the cell, raising AMP levels relative to ATP [41]. Among other effects, the rise in AMP allosterically primes activation of the energy sensor AMPK [44], the significance of which in metformin's antihyperglycemic, prolongevity, and anticancer effects remains unclear (discussed below). An additional immediate consequence of the rise in AMP levels is inhibition of the gluconeogenic enzyme fructose-1,6-bisphosphatase (FBP1) [45]. Recent elegant genetic work in mice demonstrates that metformin lowers glucose levels through allosteric inhibition of FBP1 [45]. Thus, while there is some debate on the relevance of the action of metformin on mitochondrial energetics, the data on FBP1 provide serious credence to the idea that metformin at attainable levels *in vivo* manifests important effects via modulation of cellular energy charge.

Metformin and Mechanistic Target of Rapamycin Complex 1 (mTORC1)

The heteromultimeric protein kinase mTORC1 plays a central role in regulating cell growth, proliferation, and survival in response to nutrient and energy availability [46–48]. Metformin inhibits mTORC1 activity in cells in culture independently of AMPK [49,50]. From a longevity standpoint, metformin effects on mTORC1 and longevity are in line with the well-known ability of genetic and pharmacologic inhibition of mTORC1 to extend lifespan across multiple model systems [51–57]. In support of the idea that metformin treatment modulates certain downstream cellular effects by blocking mTORC1, both metformin and canonical mTOR inhibitors have similar molecular effects, decreasing translation of mRNA's encoding cell-cycle and growth regulators [58].

Cellular mTORC1 signaling is regulated by several, distinct pathways, including the TSC-Rheb pathway [47,59] and Ras-related GTP-binding protein (Rag) GTPase-mediated amino acid signaling [60,61]. Our work builds upon the observation that metformin inhibits mTORC1 via the Rag GTPases [50], as we have identified the molecular mechanism by which this occurs: (i) metformin action at mitochondria leads to restricted transport through the NPC; (ii) reduced NPC transport restricts the entrance of small GTPase RagC into the nucleus, thus preventing its full activation; and (iii) as RagC activation is critical for normal mTORC1 activity, inactivation of RagC leads to inhibition of mTORC1 [24].

Metformin and mGPDH

mGPDH is localized on the outer layer of the inner mitochondrial membrane, where metformin has been found to bind directly to the enzyme and inhibit its function, converting glycerol-3-phosphate to dihydroxyacetone phosphate [35]. mGPDH plays a role in the glycerol-phosphate shuttle, which is responsible in part for shuttling NADH reducing equivalents into the mitochondrial matrix, in the process regenerating cytosolic NAD⁺ [62]. The inhibition of mGPDH by metformin decreases the cytoplasmic NAD/NADH ratio and reduces hepatic glucose production in mice. Downregulation of GPD2 (the gene encoding mGPDH) mimics the antihyperglycemic effects of metformin, while metformin does not lower glucose in mice genetically lacking GPD2. More recent work demonstrates that metformin at therapeutic concentrations impedes gluconeogenesis by inhibiting mGPDH activity in a redox-dependent manner [63,64]. Metformin's ability to inhibit mGPDH may also contribute to

the drug's anticancer effects by altering cellular redox potential [65]. Whether mGPDH mediates other anti-aging properties of metformin requires further exploration.

Metformin-Mediated Activation of AMPK

Mitochondrial inhibition by metformin results in depletion of ATP and elevation of cellular AMP [34,39,40], activating the master cellular energy sensor, AMPK [44]. However, multiple studies demonstrate that AMPK is dispensable for the beneficial effects of metformin, particularly in lowering of blood glucose [63,66,67]. Although mice genetically deficient in AMPK in liver respond normally to the antihyperglycemic effects of metformin, some work suggests that AMPK activation that occurs at therapeutically attainable metformin levels may be important for certain effects of biguanides [68,69].

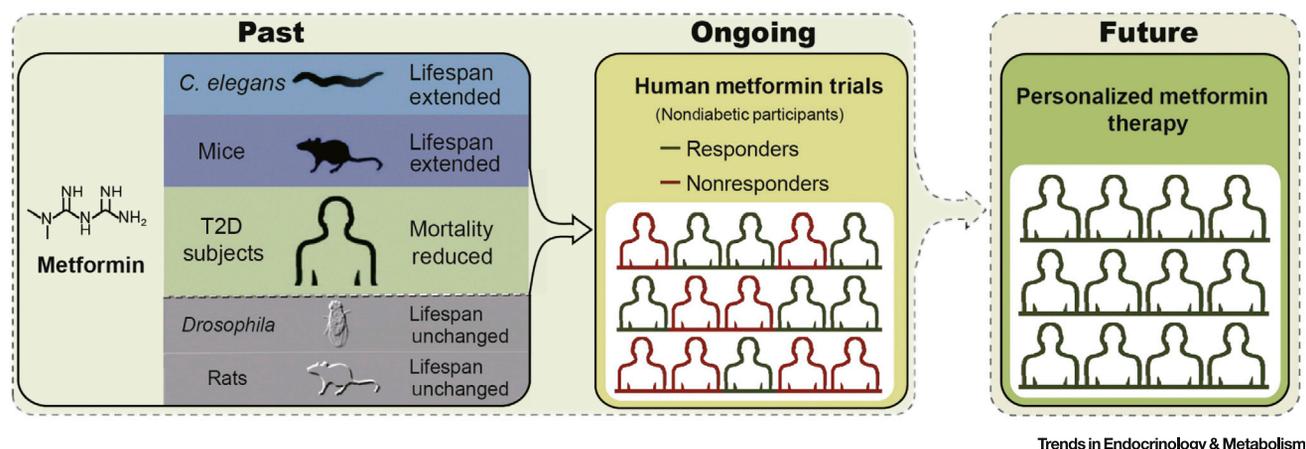
An overwhelming amount of data support the conclusion that metformin blocks cancer growth in a manner dependent upon inhibition of mitochondrial complex I, but independently of AMPK [34]. Metformin inhibits mTORC1 in a manner dependent upon the Rag GTPases, independently of AMPK [50]. Further, AMPK and its upstream activating kinase LKB1 are dispensable for metformin to inhibit cancer growth, albeit in the limited numbers of cancer cell types investigated [67,70]. Our work shows that AMPK is completely dispensable for metformin effects on *C. elegans* growth, and that the pathway defined by metformin-NPC-RagC-mTORC1-ACAD10 is not affected by AMPK in *C. elegans* or in human cancer cell lines [24]. Thus, AMPK is unlikely to be a major effector of metformin action in cancer.

These observations of metformin action in cancer contrast sharply with metformin action in aging. Seemingly paradoxically, at least in *C. elegans*, AMPK is genetically required for the longevity effects of metformin, an effect that has been reproduced by multiple research groups [21,22,25]. Thus, metformin response pathways, while they may begin at the mitochondrion, are complex and branching. More work is needed to determine how the disparate effects of metformin are mediated by common versus distinct effector mechanisms.

Metformin and Lysosomes

The lysosome, an acidified, membrane-bound cellular organelle that participates in nutrient sensing and recycling, is a central hub in control of cell signaling and metabolism. Alterations in AMPK and mTORC1 signaling in response to metformin both require biochemical events that converge on the lysosome [25,71]. AMPK phosphorylation and activation by LKB1 occurs on the surface of lysosomes in response to starvation [35]. Metformin activates AMPK via a similar mechanism on lysosomes, possibly through the lysosomal V-ATPase [71,72]. Early work in isolated lysosomes also suggests that metformin may act to coordinate AMPK activation and mTORC1 inhibition via direct effects [25]. However, the exact link between metformin action and lysosomes remains elusive.

Lysosomes and lysosome-related organelles, which play important roles in modulation of aging and longevity [73], also house cellular stores of metal ions such as copper, zinc, and iron [74]. Curiously, metformin has metal binding properties [75,76]. Accordingly, metformin can affect cellular copper homeostasis, particularly in mitochondria [77,78]. These findings suggest that metformin, by virtue of its concentration in mitochondria, could set up a copper competition between mitochondria and lysosomes, which could connect metformin action at mitochondria to lysosomal regulation on AMPK and mTORC1. Earlier work also suggests that metformin's zinc-binding activity might target lysosomal zinc stores, thereby promoting the drug's known anti-inflammatory activity [79]. Most recently, relying on an assay called homomer dynamics protein-fragment complementation assay (hdPCA), Stynen *et al.* uncovered that metformin induces an iron deficiency-like state in cells [80]. Together, it appears that metformin has the potential to modulate effects on mitochondrial function, lysosomal function, cellular signaling, and inflammation on the basis of alterations in lysosomal metal homeostasis. Further investigation is also needed to fully reveal whether metformin action on lysosomal ions contributes to the longevity and health-promoting effects of the drug.



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Figure 2. Metformin Effects on Longevity in Model Organisms and in Humans.

Metformin has been shown to have pro-longevity and healthspan-extending properties in the roundworm *Caenorhabditis elegans*, mice, and humans. In other model organisms such as *Drosophila* (fruit fly) and rats, similar benefit has not been identified. Although data from prospective clinical trials in humans on metformin in aging are only just planned or beginning to emerge, widespread use of the drug in aging in otherwise healthy individuals requires far more granular understanding of its effects and the genetic and environmental determinants of its success in promoting aging versus potential detrimental effects. T2D, type 2 diabetes.

Epigenetic Modulation by Metformin

Advances in machine learning have enabled next-generation studies of drug–target interactions [36,81]. A recent study examined 300 000 chemical compounds and more than 9000 protein binding cavities, yielding up to 41 putative metformin-binding targets. Among these potential metformin targets, the H3K27me3 demethylase KDM6A/UTX contains an experimentally validated unique metformin direct-binding motif.

A second computational modeling study provided evidence that SIRT1, a NAD⁺-dependent histone deacetylase, may also be a direct target of metformin [82]. SIRT1 plays a vital role in growth regulation, stress response, and aging modulation. It is plausible that metformin extends lifespan by activating SIRT1. Metformin may also impact the epigenome indirectly by modulation of metabolite levels known to alter the activity of histone and DNA-modulating enzymes. Metformin is known to affect cellular NAD⁺, ATP, and tricarboxylic acid intermediate levels as well as AMPK, all of which impact the activity of epigenome-modifying enzymes [83]. Thus, it remains a compelling, but largely untested, possibility that metformin may exert some of its health-promoting effects through epigenomic alterations.

Metformin and the Microbiome

An increasing amount of evidence suggests that epigenetics and environmental factors (including diet and the gut microbiota) may trump genetics as the major determinant of longevity (Figure 2). The microbiota has been reported to have strong associations with many age-related disorders, such as T2D, obesity, and cancer [84,85]. In certain organisms, metformin may impede aging and age-related disorders by modulating the microbiome [22,86,87]. We and others propose microbiome-independent mechanisms for the anti-aging effect of metformin in *C. elegans* [24], but microbiome-dependent effects remain a possibility. Cabreiro *et al.* first reported that metformin promotes *C. elegans* lifespan by changing microbial folate and methionine metabolism [22]. Studies in rodents have focused on whether metformin modulation of the microbiota affects metabolism rather than aging *per se* [86,88]. Studies in humans have yielded important findings on metformin's microbial effects: (i) metformin increases the population of bacteria good at producing short-chain fatty acids that contribute to weight loss and inflammation suppression in T2D individuals [87], and (ii) microbial shifts following metformin exposure may account for the antihyperglycemic effect of metformin and

its accompanied side effects in people with T2D [89]. However, the precise mechanisms by which metformin modulates the microbiome is still largely unknown.

Metformin and Extension of Human Longevity

In addition to the lifespan-promoting activity of metformin in various model organisms, metformin has the capability to reduce the mortality rate of diabetic patients from all causes independent of its effect on diabetes control [9] (Figures 1 and 2). These findings have prompted great interest among researchers and physicians in setting up human trials with nondiabetic participants to evaluate metformin as an agent to extend human longevity.

The Metformin in Longevity Study (MILES) is a double-blind, placebo-controlled crossover clinical trial with 14 human participants launched in 2014, to determine whether taking metformin 1700 mg/day can restore more youthful gene expression in elderly people with impaired glucose tolerance (<https://clinicaltrials.gov/ct2/show/NCT02432287>). Recent publication of gene expression profiling of skeletal muscle and adipose tissue from the MILES study provided the first direct evidence that metformin modulates metabolic and nonmetabolic gene expression linked to aging [13]. The far larger double-blind, placebo-controlled multicenter trial Targeting Aging with Metformin (TAME), plans to enroll 3000 individuals aged 65–79 years, with a primary endpoint of the time until presence of any aging-related morbidity (including coronary heart disease, stroke, congestive heart failure, peripheral arterial disease, cancer, T2D, cognitive impairment, and mortality) (Figure 2). Subjects will take 1500 mg of metformin daily for 6 years, with a mean follow-up time of more than 3.5 years [16]. Results from the TAME will provide a widely expected answer to the question whether metformin reduces aging-associated disease and disability in nondiabetic individuals. Further, the trial will set the stage as a paradigm of investigating anti-aging therapies, using disease and biomarkers as surrogates of the aging process [90].

An extremely important consideration is that the doses of metformin used in preclinical studies of aging *in vitro* and *in vivo*, are, in most cases, not comparable with doses achievable in humans. Levels of the drug used *in vitro* to elicit molecular effects discussed below are 10–100-fold higher than maximal serum levels of metformin achieved in clinical studies in humans (reviewed in [43]). It remains a distinct possibility that the aging-related benefits of metformin in humans are manifest at lower doses via chronic, low-level effects on pathways affected at higher concentrations in cells in culture. Early evidence in support of this possibility is suggested by gene expression changes manifest in human muscle and adipose tissue from metformin-treated individuals [13]. None the less, further testing is required to determine the optimal levels of metformin required to maximize benefits in aging, and at that dose, which molecular effects predominate.

In spite of the lack of randomized prospective trial data on aging in humans, many in Silicon Valley have embraced metformin in attempts to live longer and healthier (<https://www.cnbc.com/2019/03/23/metformin-for-cancer-prevention-longevity-popular-in-silicon-valley.html>). While it is likely based upon data on diabetics that the drug is safe and generally well tolerated, it is unclear whether healthy individuals will manifest a net benefit on aging in the same way that diabetic subjects do. Below, we highlight the possible issues with widespread metformin use to promote healthy aging in humans.

Uncertainty in the Widespread Use of Metformin

Metformin and Vitamin B12 Deficiency

Evidence indicates that long-term use of metformin can cause vitamin B12 deficiency in T2D patients [11,91,92]. The mechanism underlying this vitamin deficiency and its clinical consequences are still unclear. Unlike other, more severe forms of vitamin B12 deficiency [93], the vitamin B12 deficiency associated with metformin use is typically less severe and generally not accompanied by neuropathy or anemia [11]. However, whether metformin-induced B12 deficiency could be more clinically significant if the drug is taken by a larger group of people for a more substantial period of the lifespan remains unknown.

Metformin and Increased Risk of Lactic Acidosis

Metformin increases levels of lactate in mice [35] and humans [94]. While this is generally not clinically significant, in the setting of abnormal kidney function, the body is not able to eliminate metformin, leading to accumulation of the drug and risk for lactic acidosis. Generally metformin-associated lactic acidosis is extremely rare, even in individuals with substantial renal dysfunction [95], but, when evident, carries a 50% mortality rate [96]. Phenformin, a member from the same biguanide family as metformin, was withdrawn from clinical use in the 1970s due to a tenfold higher rate of severe lactic acidosis versus metformin [97,98]. It is not known whether the incidence of clinically important lactic acidosis will increase if the drug is taken on a more widespread basis.

Uncertainty Surrounding Metformin and Its Cellular Targets

In addition to multiple targets discussed above for metformin action, Stynen *et al.* also identified 745 proteins that are altered by metformin treatment [80]. There is still uncertainty on whether those proteins represent beneficial or potentially detrimental off-target effects when metformin is taken across the lifespan. Strong consideration should be given to additional possible targets of the drug before it attains widespread use for anti-aging in humans.

Viability of Metformin as an Anti-Aging Therapy in Humans

Beyond the uncertainty surrounding metformin mechanisms of action in aging, additional uncertainty exists in potential side effects of metformin. Typically, gastrointestinal side effects, including diarrhea, nausea, flatulence, indigestion, vomiting, and abdominal discomfort, dominate in individuals taking metformin. In most patients these effects are not evident or disappear over time, and only a minority have to reduce the dose or stop the drug altogether (<5% of people) [99]. Generally, the effects are minimized by starting metformin with food, at a low dose and increasing gradually.

A second major area of concern is whether metformin will have efficacy across the population for aging. Even though metformin is first-line therapy for T2D treatment, with regard to glycemic effects of the drug, there are responders and nonresponders [100]. Metformin is effective in restoring ovulation to a much greater extent in PCOS patients with overweight and impaired glucose tolerance versus patients with a lean body habitus [101]. The mechanisms underlying differential responses to metformin in humans remain largely unknown, although some variability may be explained by genetic variations in the metformin transporter organic cation transporter (OCT1) [102]. A recently published clinical trial suggests that metformin may negate some of the benefits of exercise on muscle, though this study was small in size and results were highly variable between individuals [103]. Further, we do not know, at the present time, whether individuals without diabetes will manifest longevity and reduction in aging-associated disease in response to metformin as individuals with or at risk for T2D. Given the uncertainty surrounding the full spectrum of metformin effects, whether the drug will benefit all who take it for aging, and the possibility of negative pleiotropies, we suggest that precision metformin therapy may be needed to apply metformin as an anti-aging drug in humans (Figure 2).

Concluding Remarks and Future Perspectives

Metformin has over six decades of use in diabetes with an outstanding safety record in treating human T2D. Mounting evidence in preclinical models and in humans suggests beneficial effects in reducing the risk of aging-related diseases, such as neurodegeneration and cancer. These properties of metformin have attracted an enormous amount of attention from research and industry to develop indications for metformin as an anti-aging therapeutics in humans. Although on the surface this would appear to be justified based upon the safety and tolerability of the drug, there is still much we do not know on the mode of metformin action, especially in aging. Aging is a heterogeneous phenomenon, and different individuals in the same population also respond to metformin differently. Therefore, large-scale, multicenter, randomized, placebo-controlled trials are necessary to further elucidate the anti-aging effects of metformin. We also suggest that individualized,

Outstanding Questions

Does metformin have a sole and direct target for all effects on aging in model organisms and human? Alternatively, does it manifest favorable effects on metabolism and aging through distinct mechanistic targets? What targets or pathways would support its use in human to combat aging?

How exactly does metformin work at the organellar level, such as at the mitochondrion and lysosome?

How does metformin change the epigenetic landscape and are these changes responsible for the effects of metformin on aging? Are the epigenetic effects heritable?

Why does metformin promote the lifespan of certain but not all organisms?

What are the human determinants of metformin action in aging and metabolism? Are the major determinants genetic or environmental (microbiota, diet)? What biomarkers can be leveraged to achieve precision metformin therapy in aging in humans?

What is the fate of metformin in the ecosystem? Is metformin or its derivatives harmful to the future of the planet?

precision approaches may be needed to implement metformin in aging. These could be developed once we have better biomarkers of metformin effects in humans that correlate with favorable effects on healthspan and lifespan. Last but not least, if hundreds of millions of humans take metformin, we will need to understand the consequences of unmanaged discharge into ecosystem, as metformin is eliminated from the human body unchanged and has already been detected in the environment and surface water.

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