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Metformin: A Hopeful Promise in Aging Research

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Even though the inevitable process of aging by itself cannot be considered a disease, it is directly linked to life span and is the driving force behind all age-related diseases. It is an undisputable fact that age-associated diseases are among the leading causes of death in the world, primarily in industrialized countries. During the last several years, an intensive search of antiaging treatments has led to the discovery of a variety of drugs that promote health span and/or life extension. The biguanide compound metformin is widely used for treating people with type 2 diabetes and appears to show protection against cancer, inflammation, and age-related pathologies. Here, we summarize the recent developments about metformin use in translational aging research and discuss its role as a potential geroprotector.

Over the past two decades, metformin has emerged as the first-line treatment for people with type 2 diabetes (T2DM) and is the most widely prescribed antidiabetic drug in the world (American Diabetes Association 2014). In addition to its use in T2DM, metformin is being prescribed for the treatment of polycystic ovary syndrome, diabetic nephropathy, and gestational diabetes, and has shown early promise as a treatment for cancer. Historically, despite its well-accepted antidiabetic properties in the 1950s, and use for hyperglycemia treatment in England in 1958, metformin remained contraindicated largely because of concerns about lactic acidosis and it was not approved by the U.S. Food and Drug Administration until 1994 (Bailey and Turner 1996; Mahmood et al. 2013). We now know that the rare event of lactic acidosis occurs in >0.01 to 0.08 cases (average, 0.03) per 1000 patient-years caused by an insufficient metformin clearance by the kidneys (Bailey and Turner 1996). Therefore, the risk of side effects is relatively low in comparison to the multiple benefits of metformin.

The exact molecular mechanisms of metformin's therapeutic action still remain unknown. Metformin is a biguanide compound originally derived from a guanidine derivative found in the plant *Galega officinalis*. It acts as an insulin sensitizer and exerts its principal metabolic action on the liver. In addition to its glucoregulatory action, metformin has gained attention for its pleiotropic effects and activity in a variety of tissues, such as muscles, adipose tissue, ovary,

Editors: S. Jay Olshansky, George M. Martin, and James L. Kirkland
Additional Perspectives on Aging available at www.perspectivesinmedicine.org

 doi: 10.1101/cshperspect.a025932
Cite this article as *Cold Spring Harb Perspect Med* 2016;6:a025932

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endothelium, and brain (Diamanti-Kandarakis et al. 2010; Foretz et al. 2014). Food intake (Adeyemo et al. 2014; Pernicova and Korbonits 2014) and body weight (Glueck et al. 2001) are decreased as a result of a direct action of metformin on the hypothalamic centers regulating satiety and feeding (Stevanovic et al. 2012); it may also influence metabolic and cellular processes associated with the development of chronic conditions of aging, including inflammation, fatty liver, oxidative damage, protein glycation, cellular senescence, diminished autophagy, apoptosis, and development of several types of cancer (Isoda et al. 2006; Kita et al. 2012; Hirsch et al. 2013; Woo et al. 2014). A number of recent studies support the role of metformin in improving health span and life span in different animal models (Anisimov et al. 2011; Cabreiro et al. 2013; Martin-Montalvo et al. 2013; Anisimov 2014; De Haes et al. 2014). The possibility exists, therefore, for similar beneficial actions of metformin in human health and longevity.

The purpose of this article is to review the role of metformin as a possible geroprotector drug. We will try to summarize recent evidence for the antiaging properties of metformin, the molecular mechanisms implicated in this role, and, finally, discuss new (research opportunities) directions to better understand the translational potential of metformin.

HOW DOES METFORMIN WORK?

Metformin is excreted intact in the urine, without being metabolized by the liver or kidney. About 50%–60% of an oral dose is absorbed into the systemic circulation and distributed in most tissues at similar concentrations, although higher concentrations are found in gastrointestinal tract, liver, and kidney (Bailey and Turner 1996; Gong et al. 2012; Pawlyk et al. 2014). Age, gender, nutritional status, lifestyle, and genetic variations represent some of the factors that influence metformin's susceptibility and distribution to target tissues. For instance, membrane transporter polymorphism is a key determinant in the pharmacokinetic properties of this drug (Chen et al. 2013; Pawlyk et al. 2014). Metformin exerts its therapeutic effects, through a number of mechanisms and physiological pathways that resemble those generated by caloric restriction (CR), an experimental model known to extend life span and health span in various organisms. Indeed, microarray analyses have shown that metformin induces the same gene expression profile as CR (Dhahbi et al. 2005; Spindler 2006; Martin-Montalvo et al. 2013), despite no reduction in food intake (Mercken et al. 2012; de Cabo et al. 2014).

The inhibition of hepatic gluconeogenesis and lipogenesis by metformin occurs via alterations in cellular energetics. The decrease in cellular respiration that results from metformin's inhibition of mitochondrial complex I activity (El-Mir et al. 2000; Owen et al. 2000) yields lower ATP levels. Although the interaction with mitochondrial copper ion appears essential for the metabolic effects of metformin (Logie et al. 2012), more still needs to be learned about whether the drug inhibits respiration through direct or indirect action (Fontaine 2014). The nonclassical effects of metformin on the expression of glucose transporters and glycolytic enzymes (up-regulation) result indirectly from mitochondrial respiratory chain inhibition (Owen et al. 2000). This inhibition of the electron transport chain (Batandier et al. 2006; Guarente 2008) combined with the induction of antioxidant gene expression by the SKN-1/Nrf2 transcription pathway (Onken and Driscoll 2010) provides mechanistic insights into metformin's role in lowering the production of reactive oxygen species (ROS). AMP-activated protein kinase (AMPK) is a key sensor of energy status that regulates metabolic energy balance at whole-body level (Hardie et al. 2012). The increase in AMP/ATP and ADP/ATP ratios stimulates AMPK (Stephenne et al. 2011); however, metformin can activate AMPK without eliciting detectable changes in AMP, ADP, and ATP levels (Hawley et al. 2002). It was later determined that the tumor suppressor protein LKB1 (alternatively termed SK11) was responsible for the activating phosphorylation of AMPK in response to metformin (Shaw et al. 2005). Indeed, the LKB1–AMPK pathway controls the expression of key hepatic gluconeo-

genic genes by regulating the transcriptional coactivator cAMP-response element-binding protein (CREB)-regulated transcription coactivator 2 (CRTC2, also known as TORC2) (Shaw et al. 2005), a key regulator of fasting glucose metabolism (Koo et al. 2005). The role of LKB1–AMPK as mediator of metformin's action on hepatic gluconeogenesis and lipogenesis (Zhou et al. 2001; Zou et al. 2004; Shaw et al. 2005) was put to test in studies using conditional *Ampk* knockout mice (Foretz et al. 2010). The observed inhibition of glucononeogenesis, independent of LKB1–AMPK signaling, was accompanied by a decrease in hepatic energy state in response to concentrations of metformin that were far higher than those reached in hepatic portal vein after standard treatment (Foretz et al. 2010). When therapeutic concentrations of metformin were tested, hepatic gluconeogenesis was suppressed via AMPK activation (Cao et al. 2014) and formation of AMPK αβγ complexes (Meng et al. 2014). The ability of AMPK to improve lipid metabolism helps explain the reduction in hepatic steatosis by metformin (Woo et al. 2014), which requires the inhibitory phosphorylation of acetyl-CoA carboxylase (ACC) by AMPK, an essential step toward the lipid-lowering and insulin-sensitizing effects of metformin (Fullerton et al. 2013). Moreover, metformin treatment decreases the levels of sterol regulatory element-binding protein 1 (SREBP-1), a key lipogenic transcription factor, via direct phosphorylation by AMPK (Zhou et al. 2001; Li et al. 2011). The regulation of lipid metabolism by metformin also takes place by enhancing the fatty acid β-oxidation pathway (Collier et al. 2006). New molecular mechanisms by which metformin inhibits hepatic gluconeogenesis have been proposed and include the ability of the drug to inhibit adenylate cyclase through AMP accumulation, thereby blocking the glucagon-signaling pathway (Miller et al. 2013), and direct inhibition of mitochondrial glycerophosphate dehydrogenase (mGPD) (Madiraju et al. 2014). In the latter study, metformin-mediated mGPD inhibition was accompanied by lower mitochondrial NADH/NAD^+ ratios, a result inconsistent with prior reports showing that complex I inhibition by metformin increased this ratio (Owen et al. 2000). The different doses and route of administration of metformin between the two studies might explain these discrepancies (Baur and Birnbaum 2014).

Another potential mechanism through which metformin inhibits hepatic gluconeogenesis is the down-modulated expression of genes encoding for the gluconeogenic enzymes, phosphoenolpyruvate carboxykinase (PEPCK), and glucose-6-phosphatase (G6Pase), a molecular mechanism that requires AMPK-mediated up-regulation of orphan nuclear receptor short heterodimer partner (SHP) expression (Kim et al. 2008). Additionally, metformin improves glucose homeostasis by promoting an increase in insulin-independent phosphorylation of insulin receptor and insulin receptor substrates (IRS)-1 and (IRS)-2, and subsequent translocation of glucose transporters GLUT4 to the plasma membrane (Gunton et al. 2003; Yuan et al. 2003). The regulation of the incretin hormone (e.g., glucagon-like peptide 1) and insulin secretory responses with metformin treatment has been reported (Cho and Kieffer 2011; Maida et al. 2011; Kim et al. 2014).

Metformin also acts as an inhibitor of mechanistic target of rapamycin complex 1 (mTORC1) through AMPK-dependent and -independent mechanisms. AMPK activation by metformin inhibits the protein kinase mTOR, thus preventing the phosphorylation of downstream targets, including S6K, rpS6, and 4E-BP1 (Dowling et al. 2007). Inhibition of the Ras-related GTP binding (Rag) GTPases (Kalender et al. 2010) and up-regulation of REDD1, a hypoxia-inducible factor 1 (HIF-1) target (Shoshani et al. 2002; Ben Sahra et al. 2011), are among the AMPK-independent mechanisms by which metformin inhibits mTORC1 signaling. Because of the many faces of mTOR in life span and metabolism, it is intriguing that metformin may act as a potential therapeutic drug for the treatment of aging and age-related diseases, such as cancer and metabolic syndrome (Johnson et al. 2013).

METFORMIN AS AN ANTI-AGING DRUG

Recent reviews have reported the geroprotective effects of biguanides, mainly metformin,

because of its superior safety profile (Bulterijs 2011; Berstein 2012; Miles et al. 2014). As indicated earlier, metformin treatment enhances insulin sensitivity, induces glycolysis, and suppresses hepatic gluconeogenesis. There is some evidence that metformin may also have cardioprotective effects (Eurich et al. 2013; Hong et al. 2013) and contribute to the prevention of some forms of human cancer (Cazzaniga et al. 2013; Anisimov 2014; Laskov et al. 2014). This therapeutic profile of metformin supports its use for age-related diseases and longevity. Of significance, many studies have confirmed the positive effect of metformin on life span of worms, flies, mice, and rats. Moreover, diabetic and cardiovascular disease patients who are prescribed metformin have increased rates of survival (Scarpello 2003; Yin et al. 2013), and it was recently proposed that metformin might promote longevity by preventing frailty in older adults with T2DM (Wang et al. 2014). Chronic treatment with metformin among patients with diabetes might reduce the risk of cognitive decline and dementia (Ng et al. 2014; Patrone et al. 2014) and improve survival in several types of cancer (Greenhill 2015; Ko et al. 2015; Lin et al. 2015; Rego et al. 2015).

STUDIES IN INVERTEBRATE MODELS

Many molecular mechanisms implicated in aging and age-related diseases have been elucidated in *Caenorhabditis elegans*, an experimental model widely used for the identification of new pharmacological agents capable of delaying the aging process (Olsen et al. 2006; Lapierre and Hansen 2012). Metformin supplementation (50 mM dose) was found to increase the mean life span of *C. elegans* by about 40% without maximum life span extension. This increase in health span had CR-like features that involved activation of the LKB1–AMPK–SKN1 pathway both in wild-type worms and in mutant animals with disrupted insulin pathway (Onken and Driscoll 2010). The increase in carbohydrate levels in metformin-treated worms provides a good source of ATP to better survive 2 to 3 d of anoxia exposure through a mechanism that depends on specific AMPK subunits (LaRue and Padilla 2011). Active bacterial metabolism is a critical nutritional requirement for *C. elegans* life span (Lenaerts et al. 2008; Cabreiro and Gems 2013). Biguanide-treated worms lived longer (~30% increase compared with their normal life span) only when cultured with a *Escherichia coli* strain sensitive to the drug, which contrasts with the pathogenic effects of drug-resistant bacteria on nematode health and aging. An alteration in microbial folate and methionine metabolism helps explain the extended longevity, which is consistent with the notion that metformin is a CR-mimetic drug. Metformin is primarily used for the management of hyperglycemia in T2DM, which led Cabreiro and colleagues to test whether high glucose adversely affected bacterial growth inhibition by metformin and, consequently, *C. elegans* longevity. The reduction in metformin-induced life span extension in response to glucose supplementation led the investigators to suggest that altering gut microbiota might represent a new therapeutic approach for delaying aging and the treatment of age-related diseases (Cabreiro et al. 2013). Conversely, glucose restriction extends *C. elegans* life span by inducing mitohormesis, a physiological process based on mitochondrial oxidative stress (Schulz et al. 2007; Zarse et al. 2012). Of significance, metformin-treated nematodes showed increased respiration and higher ROS production, consistent with the generation of a mitohormetic signal (De Haes et al. 2014). These investigators established that the mitohormetic signal was propagated by the hydrogen peroxide scavenger peroxiredoxin PRDX-2, whose expression was up-regulated after metformin treatment, and deletion of the *prdx2* gene led to decreased overall life expectancy. *C. elegans* treated with metformin also had a youthful morphology for a longer time, which contributed to their improved health span (De Haes et al. 2014).

The beneficial effects of metformin on the life span of nematodes do not appear to be evolutionarily conserved in *Drosophila*. AMPK activation increases life span in *Drosophila* (Tohyama and Yamaguchi 2010; Stenesen et al. 2013), and metformin treatment reduces lipid storage via robust activation of AMPK without

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promoting longevity in either male or female flies. Perturbations in intestinal homeostasis may be responsible for metformin toxicity in flies when taken in high enough doses (Slack et al. 2012) and when different antidiabetic compounds were tested for their potential antiaging properties (Jafari et al. 2007). In the latter study, metformin given in doses of 0.4, 0.8, and 1.6 mg/ml did not decrease the mortality rate in flies (Jafari et al. 2007). Even though the prolongevity effects of metformin have yet to be found, this drug can inhibit age- and oxidative-stress-induced DNA damage and delay stem cell aging in *Drosphilia* (Na et al. 2013).

STUDIES IN RODENT MODELS

C57BL/6J mice and Fisher 344 (F344) rats are the preferred strains of rodents for use in gerontological studies (Anisimov et al. 2012). The physiology of these animals, mainly at the cellular level, is very similar to humans, which allows the study of various compounds for their life extending properties and the extrapolation of these findings to human aging. The sole publication on the impact of metformin in rat longevity indicated a lack of effect of the biguanine on mean life span and mean of the last surviving 10% male F344 rats, compelling the investigators to question the claims about metformin acting as a CR-mimetic drug (Smith et al. 2010). However, this strain of rats is resistant to the health benefits of CR and, thus, may provide a partial explanation for the lack of prolongevity effects of metformin in F344 rats (Smith et al. 2010). This study was rather inconclusive and new approaches will be required to determine whether metformin can prolong life span in rats.

More studies were performed in different mouse strains using male and female animals. In general, female mice responded better to metformin vis-à-vis mean life span extension, as compared with male mice and rats. Metformin treatment (100 mg/kg in drinking water for 5 consecutive days every month) significantly increased mean (+8%) and maximal life span (+9%) of short-lived, cancer-prone female HER-2/neu transgenic mice (strain FVB/N carrying a HER-2/neu oncogene) with significant reduction in the mean size and accumulation of mammary adenocarcinoma (Anisimov et al. 2005a,b). When combined with melatonin, metformin inhibited the growth of a HER2 mammary tumor and Ehrlich tumor growth in mice, whereas metformin treatment alone was shown to slow down the development of spontaneous mammary tumors and increase mean life span in female HER-2/neu transgenic mice (Anisimov et al. 2010a).

The geroprotective effects of metformin and its ability to suppress spontaneous tumorigenesis were also observed in other mouse strains. Long-term treatment with metformin significantly increased mean (+37.9%) and maximum life span (+10.3%) of female outbred SHR mice, and slowed down the age-associated disturbances in the estrous function without impacting on body weight or food intake (Anisimov et al. 2008). However, metformin treatment did not alter the incidence or mean latency of tumors, an unexpected finding that was attributed to the inherent genetic makeup of the SHR mouse strain. Nevertheless, this result emphasizes the fact that metformin can prolong life independently of its ability to suppress cancer (Blagosklonny and Campisi 2008). It is interesting to note that the responsiveness of female SHR mice to the prolongevity effects of metformin was dependent of the age of the animals at the onset of treatment. An increase in the mean life span was observed when metformin treatment was started at the age of 3 or 9 mo (+14.1% and +6.1%, respectively), but not at 15 mo of age (Anisimov et al. 2011). Focusing the analysis on tumor-free mice only, there was a significant increase (20.7% and 7.1%) but significant reduction (−12.8%) in mean life span when metformin administration was initiated in 3-, 9-, and 15-mo-old animals, respectively.

The possibility that metformin can improve the outcomes of two neurological disorders was investigated both in male and female mice. In the first study, different metformin doses (0, 2, or 5 mg/ml) were given in the drinking water of 5-wk-old transgenic mice with Huntington's disease (the R6/2 line with ~150 glutamine

repeats) (Ma et al. 2007). The investigators observed that metformin, only at 2 mg/ml, significantly increased mean life span (+20.1%) and decreased the duration of hind limb clasping, a phenotypic marker of motor defect, in male but not female animals. In the second study, three doses of metformin (0.5, 2, and 5 mg/ml) were given in the drinking water of male and female $SOD1^{G93A}$ mice (transgenic model of amyotrophic lateral sclerosis [ALS]) from 35 d of age (Kaneb et al. 2011). Metformin treatment had no effect on disease onset, progression or survival in male $SOD1^{G93A}$ mice at any dose while eliciting a dose-dependent negative neurological response in females owing to metformin's ability to inhibit estrogen production (Rice et al. 2009). Inhibition of estrogen can accelerate ALS progression and reduce life span in female $SOD1^{G93A}$ mice (Choi et al. 2008). All treatment groups appeared to weigh less and displayed no significant differences in their life span, as compared with control mice. However, a tendency toward increased survival was observed with reduction in the dose of metformin (Kaneb et al. 2011).

The notion that the prolongevity effects of metformin depend on the developmental stage of the animal at the onset of treatment was further explored in inbred male and female 129/Sv mice (Anisimov et al. 2010b). Addition of metformin (100 mg/kg) in drinking water of 3-mo-old male animals elicited a significant decrease in mean life span (−13.4%) without affecting maximum life span. A higher incidence of chromosome aberrations was also noted in metformin-treated male mice. In females, metformin did not influence maximum life span, but it slightly increased mean and median life span by 4.4% and 7.8%, respectively, with a significant reduction in the total incidence of malignant tumors. However, an increase of benign angiogenic tumors was observed in metformin-treated female mice (Anisimov et al. 2010b). The reasons for these gender-specific differences on metformin responses are still under study and may be attributed to the fact that males and females have different mechanisms of aging. Potential gender-related variability in outcomes are exemplified by the next series of reports. Deletion of ribosomal S6 protein kinase 1 (S6K1), a component of the mTOR pathway, significantly increased the life span of female C57BL/6 mice (+20.4%) without changes in that of male animals (Selman et al. 2009). Subcutaneous administration of metformin (100 mg/kg body weight) in 3-, 5-, and 7-d-old 129/Sv mouse pups caused an inversion of the gender response to the prolongevity effects of the drug (Anisimov et al. 2015). These investigators reported an increase in mean life span (+20%) and a slight maximum life span extension (+3.5%) in males who received metformin neonatally, while a decrease in mean and median life span (−9.1% and −13.8%, respectively) without significant differences in maximum life span was observed when female mouse pups were treated with metformin, as compared with control animals. The neonatal period is critical for the development of the hypothalamic circuits that control energy homeostasis (Contreras et al. 2013) and it has been suggested that reprogramming of these circuits, especially the mTOR-signaling pathway, may be part of the aging process (Blagosklonny 2013). Many aspects of aging are controlled by the hypothalamus, and alteration of hypothalamic pathways might allow the manifestations of aging to be modified (Zhang et al. 2013).

The long-term effects of metformin supplementation (0.1% and 1% w/w) in the food was performed in male C57BL/6 mice, starting from the age of 54 wk for the remainder of their lives (Martin-Montalvo et al. 2013). The mean life span of mice supplemented with 0.1% metformin increased by 5.83%, while that of mice on 1% metformin was significantly reduced (−14.4%), likely caused by renal failure. Diet supplementation with 0.1% metformin tended to preserve body weight with advancing age, a condition known to increase longevity in mice (Pearson et al. 2008). There were no significant differences in the number of pathologies in mice on 0.1% metformin; however, liver cancer incidence was significantly reduced with 1% metformin supplementation (3.3% vs. 26.5% in metformin- and vehicle-treated mice, respectively) (Martin-Montalvo et al. 2013). An improvement in physical performance and glucose

homeostasis combined with increased insulin sensitivity, and a reduction in low-density lipoprotein and cholesterol levels occurred in 0.1% metformin-fed mice without a decrease in caloric intake. By preserving overall health span in mice, metformin may prevent the development of metabolic syndrome through significant reduction in oxidative stress and chronic inflammation (Martin-Montalvo et al. 2013). These investigators reported similar gene expression patterns in the liver (and skeletal muscle) of mice fed 40% CR and 0.1% metformin, reinforcing the role of metformin as a CR mimetic (Mercken et al. 2012; de Cabo et al. 2014). Of significance, the prolongevity effect of metformin was observed also in a second strain of male mice (hybrid B6C3F1), with a 4.15% increase in mean life span in response to 0.1% metformin supplementation in the diet (Martin-Montalvo et al. 2013).

CONCLUSIONS AND PERSPECTIVES

According to recent published data in different animal models, metformin appears to be a promising candidate as a life-extending drug (Fig. 1). This compound is generally well tolerated and its long history of clinical use makes it an even more attractive candidate. Besides, metformin is more beneficial than any other antidiabetic drug in reducing age-related diseases and improving survival in diabetic patients. Although the initial results are very hopeful, more work is needed to elucidate several aspects that still remain unclear. Many of these positive results have been obtained using doses of metformin that exceed therapeutic levels in humans (Martin-Castillo et al. 2010; Aldea et al. 2014). Moreover, the modes of administration varied among research teams, with the addition of metformin either in drinking water or to the

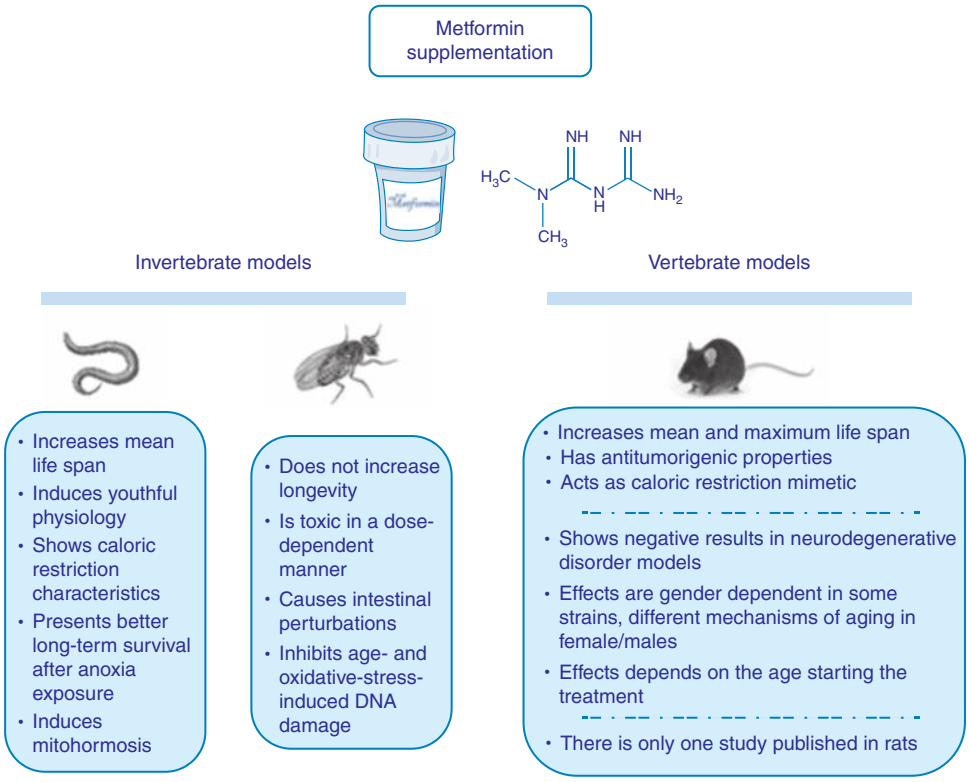

Figure 1. Summary of the effects of metformin supplementation in invertebrate (*Caenorhabditis elegans* and *Drosophila melanogaster*) and vertebrate models (rodents, mainly mice).

diet. Although female mice were initially found to show a better response to metformin supplementation, recent results from our laboratory indicated no gender or stain differences in the actions of metformin (Martin-Montalvo et al. 2013). Therefore, to establish the molecular mechanisms and pathways of aging, it is imperative to investigate potential hormone-metformin interactions in male and female animals of varying ages, as the age of starting metformin treatment determines whether an increase in mean and maximum life span occurs (Menendez et al. 2011; Anisimov et al. 2015). There are not enough studies to conclude whether there are epigenetic/genetic differences in metformin effect on aging, life span, and tumorigenesis. Because not all organisms studied seem to respond positively to metformin supplementation (e.g., flies and rats), new approaches with different protocols and experimental designs would be crucial to understanding how metformin might be a good geroprotector throughout phylogeny, including in humans.

A new interesting functional interplay has emerged during the last years that might explain some of the molecular mechanisms through which metformin could improve health and life span. There is some evidence that the anticancer protection conferred by metformin treatment may involve the modulation of miRNAs (Pulito et al. 2014). These small noncoding RNAs regulate gene expression at the posttranscriptional level and metformin modulates miRNAs that regulate apoptosis and inhibit proliferation (Li et al. 2012).

Despite these advances, it is the hope that better coordination among basic and clinical researchers and use of more sophisticated approaches will facilitate the development of new interventions aimed at improving human health and life span.

ACKNOWLEDGMENTS

This work is supported by the Intramural Research Program of the National Institute on Aging, National Institutes of Health. CIBER de Fisiopatología de la Obesidad y Nutrición is an initiative of ISCIII. The funding agency had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

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Cold Spring Harb Perspect Med 2016; doi: 10.1101/cshperspect.a025932