

Metformin and Aging: A Review

Hartmut H. Glossmann^a Oliver M.D. Lutz^b^aInstitute for Biochemical Pharmacology, Medical University of Innsbruck, Innsbruck, Austria;^bAustrian Drug Screening Institute GmbH, Innsbruck, Austria

Keywords

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Abstract

Metformin is sometimes proposed to be an “anti-aging” drug, based on preclinical experiments with lower-order organisms and numerous retrospective data on beneficial health outcomes for type 2 diabetics. Large prospective, placebo-controlled trials are planned, in pilot stage or running, to find a new use (or indication) for an aging population. As one of the metformin trials has “frailty” as its endpoint, similar to a trial with a plant-derived senolytic, the latter class of novel anti-aging drugs is briefly discussed. Concerns exist not only for vitamin B₁₂ and B₆ deficiencies, but also about whether there are adverse effects of metformin on individuals who try to remain healthy by maintaining cardiovascular fitness via exercise.

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Introduction

Confronted with the task to write a review on “metformin and aging,” it was decided to extract information from the most recent literature relevant to humans, focusing on planned or ongoing clinical trials. Mechanisms of action are briefly discussed. A detailed analysis of the evidence for metformin to extend the life of model organisms follows. Anti-aging research is a hot – yet not unproblematic – topic, as will be exemplified.

Anti-Aging Drugs Are Big Business: The Three Promises – Longevity, Rejuvenation, and Health Span (Fig. 1)

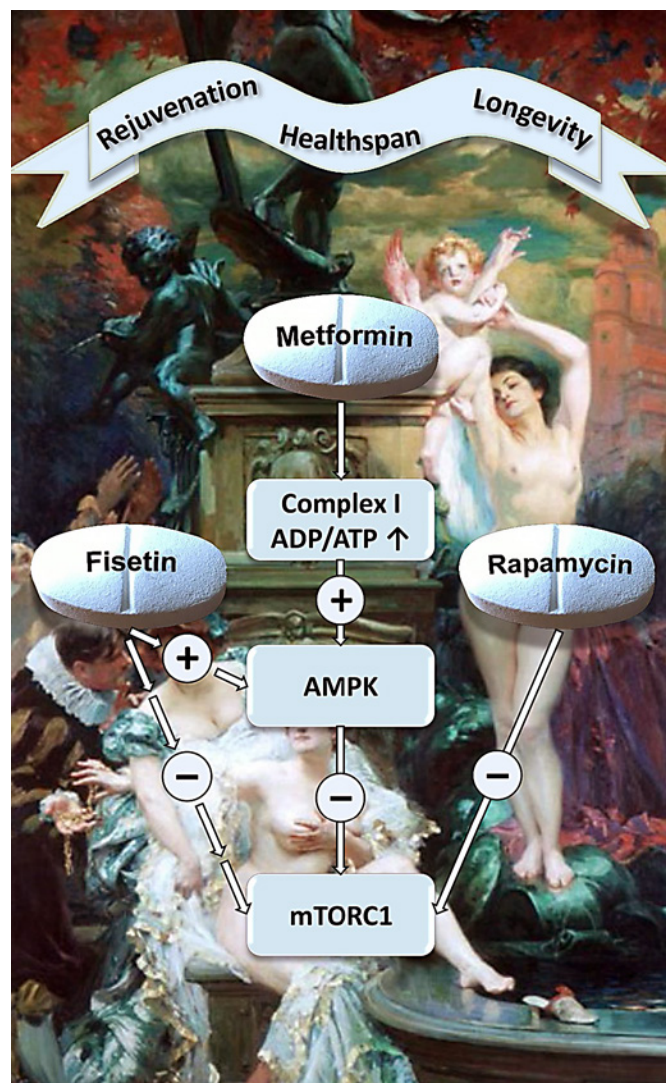
A 2017 review [1] lists 16 biotech companies which develop drugs to fight aging and/or age-related diseases, 5 “big data” anti-aging enterprises, and 7 stem cell/regeneration companies. Among the 5 big data companies is Calico Life Sciences LLC, “one of Google’s moonshot projects.” The unique features of the ground-dwelling naked mole rat, which lives about 10 times longer than a mouse, develops almost no cancer, and defies the Gompertz law (see online suppl. Fig. S1; for all online suppl. material, see www.karger.com/doi/10.1159/000502257), seems to be of interest to Calico. Rochelle Buffenstein is now a senior staff member of the company [2] (Box 1 [3–5]).

Longevity and Lifespan

Life extension – as a simple read-out of slowing aging by manipulation of model organisms with either drugs or, e.g., dietary restrictions – is enjoying a prominent place in aging research. This approach was stimulated by observations with the mTORC1 (mechanistic target of rapamycin complex 1) inhibitor rapamycin in 2009 [6], when the drug was given more or less accidentally [7] to 600-day-old mice. Life was extended by 28 and 38% for

Dedicated to em. O. Univ. Prof. Dr. med. Georg Wick on occasion of his 80th birthday.

Fig. 1. The three promises of anti-aging research. The painting “La Fontaine de Jouvence” by Paul-Jean-Louis Gervais shows an example of an artist’s impression of the “Fountain of Youth” with the three promises: “healthspan,” “rejuvenation,” and “longevity.” Drugs (fisetin, rapamycin, and metformin) which are tested in clinical trials or under development for age-related diseases, and are propagated to fulfil one or even all of the promises, are shown for illustrative purposes. Mechanisms relevant to anti-aging are displayed by solid arrows with a plus or a minus sign. Interrupted arrows indicate activities reported in the preclinical literature without identification of a primary molecular drug receptor. The “classic” molecular target for metformin is complex I of the mitochondrial respiratory chain (see main text). An increased cellular ADP/ATP ratio upon metformin enrichment in the mitochondrial matrix leads to activation of AMPK. Active AMPK inhibits mTORC1 directly by phosphorylation of RAPTOR and indirectly by activating TSC2, one subunit of TSC. Whereas several other metformin targets in addition to complex I are discussed in the literature, rapamycin, by forming a complex with FKBP12, is exquisitely selective of mTORC1; there are no “off-target” binding sites. In contrast, fisetin, similar to other plant polyphenols, has multiple cellular actions (antioxidant, kinase inhibition, etc.; see main text and Schubert et al. [21]). Preclinical assays with laboratory mice, sometimes genetically modified, use extension of lifespan and prolonged time periods of being free from age-related symptoms as important read-outs. See online suppl. Fig. S1 for the theoretically ultimate result of any anti-aging research; it exemplifies an “exceptional model for biogerontology” [101], the naked mole rat. These small rodents are not treated with drugs. Possible exceptions are compounds, produced by their intestinal microbiome, as the animals practice coprophagy. Naked mole rats stay “forever young and healthy” and survive their rat and mice counterparts treated with senolytics or any other drug by at least an order of magnitude. Painting: Capitole Toulouse, Salle des Illustres, Paul Gervais, 1908 (public domain).



males and females, respectively. Analogs of rapamycin are in early clinical studies for age-related diseases [8], and the parent, off-patent drug was recently proposed for prevention of Alzheimer’s disease [9].

Rejuvenation by Elimination of Senescent Cells

The term “rejuvenation” was used for senolytics by Chang et al. [10]. One of the hallmarks of aging is an increased number of “senescent” cells [11]. These irreversibly growth-arrested cells are resistant to apoptosis, and many age-related diseases are claimed to be linked to cellular senescence [12, 13]. Senescent cells secrete a variety of bioactive factors (senescence-associated secretory phenotype [SASP]). The SASP may be harmful to neighboring cells and even induce senescence [14].

Compounds that selectively eliminate senescent cells in cell culture or in rodents are classified as senolytics; compounds that suppress the SASP are termed senomorphics [15]. The first molecules, claimed to be discovered by “bioinformatics” [16] but more likely by “phenotypic screening” [17], were dasatinib (a Federal Drug Administration [FDA]-approved kinase inhibitor) and quercetin. When the (so-called) Koch-like criteria in preclinical testing were fulfilled [18], dasatinib and quercetin, as a fixed-dose combination, entered the first clinical trial in an open-label pilot study (NCT02874989) for patients with idiopathic pulmonary fibrosis [19].

Fisetin is another senolytic, tested in a clinical trial for age-related disorders (NCT03675724; AFFIRM-LITE). Fisetin also met the Koch-like criteria [20] and was pat-

Box 1. Anti-aging research is a hot topic

“Viviremos 135 años!” (“We will live for 135 years”) was on the title page of the Spanish newspaper “El Mundo” on October 15, 2015. Lauded were Spanish scientists who published a paper in *Nature Cell Biology* [3]. Therein, the role of nuclear factor kappa-light-chain-enhancer of activated B cells (NF- κ B) and its downstream targets, such as disruptor of telomeric silencing 1-like (DOT1L), which is a histone H3 (H3K79) methyltransferase, were propagated as targets for “rejuvenation.” Three years earlier, the authors had reported that sodium salicylate (200 mg/kg by daily i.p. injection) greatly prolonged life in progeria mouse models [4]. It took 4 years until the 2015 paper [3] was retracted by *Nature Cell Biology* in March 2019 [5]. In January 2019, another 8 publications from the same group were retracted from the *Journal of Biological Chemistry*. This illustrates that in the rather young scientific discipline of anti-aging research, competition is fierce and reproducibility by other, independent research groups is most important. The retractions finally occurred because of numerous comments and complaints, especially about “photoshopping” (see <https://pubpeer.com/>).

ented for improving memory (US7897637B2). Fisetin is one of several other compounds postulated to act as “geroneuroprotectors” [21]. Fisetin and its major metabolite geraldol apparently cross the blood-brain barrier when given orally to mice [22]. Typical for plant xenobiotics and similar to quercetin, fisetin undergoes multiple biotransformations. More than 50 metabolites were identified in rodents [23]. Fisetin is marketed as a nutraceutical (e.g., under the trade name Novusetin).

The interested public apparently screens ClinicalTrials.gov for ongoing anti-aging studies and immediately started consuming fisetin, often together with quercetin and reduced nicotinamide adenine dinucleotide (NADH) boosters, either daily or twice a week (as in the clinical trial), and report their self-experimental results on a website [24].

Health Span

Matt Kaerberlein recently proposed to refrain from using the term health span in the scientific literature as health is not a binary trait with only two states (good or bad) [25]. However, it is used in numerous preclinical publications to emphasize that an intervention (with a drug or otherwise) prolongs not only the lifespan but also the percentage of time without any health deficits, as defined by the authors.

In contrast to rapamycin, where data from all preclinical trials on mouse models and other lower-order organisms prove that the drug increases the lifespan (“longevity”), there is little such evidence for metformin (see be-

low). Rapamycin also increases the health span or even reverses age-related diseases (“rejuvenation”) in worms, flies, mice, rats, and dogs [9].

Metformin’s Mechanism of Action

Metformin Stimulates Hormone Secretion from the Gut

Metformin is only active in type 2 diabetes (T2D)/prediabetes when applied orally. The usual dose of an immediate-release formulation is around 2 g per day (12 mmol). About 6 mmol are excreted daily via the kidneys (active secretion). Kidney cells as well as epithelia in the urinary tract are exposed to concentrations higher than 4 mM. The other half of the drug is lost in the feces [26]. The colon is exposed to concentrations of up to 40 mM. Concentrations of metformin in human post-dose jejunal biopsies are 4,000 μ mol per kg [27], and in colon biopsies (steady state) they are between 26 and 1,820 μ mol per kg [28].

It is not known in which cellular compartment (cytosol, mitochondrion, endoplasmic reticulum, or endo-/lysosomes) the drug is sequestered. Previously, it has been postulated that the liver is the primary target organ of the drug. This view has changed, as the intestine (uptake into enterocytes and enteroendocrine cells is mediated by specific carriers) is now recognized to be responsible for most of the antidiabetic (and preventive) activity [29]. The evidence is based on the efficacy of a delayed-release formulation which spares the upper part of the gut (duodenum and jejunum) with lower systemic availability as well as liver exposure [30], and on clinical studies with antagonists which block the gut hormone glucagon-like peptide-1 (GLP-1) [31].

In addition to GLP-1, plasma levels of two other hormones, peptide YY (PYY) and growth/differentiation factor 15 (GDF15), are increased significantly on metformin therapy. The latter, produced mainly in the intestine via the “integrated stress response” [32], acts exclusively on neurons of the hindbrain (area postrema) to control appetite, food intake, and body weight, and plasma levels correlate with benefits in prediabetes [33].

Metformin Increases the Activity of Adenosine Monophosphate-Activated Protein Kinase

Adenosine monophosphate-activated protein kinase (AMPK) activity is increased upon chronic metformin dosing in human skeletal muscle [34] and adipose tissue [35]. Activation of AMPK is also suggested to participate in the acute release of the gut hormones GLP-1 and PYY

from human mucosal preparations, as metformin stimulation is blocked by an inhibitor of the kinase [36]. AMPK can be activated by the “canonical” pathway (increase in adenosine diphosphate/adenosine triphosphate [ADP/ATP] ratio and phosphorylation by upstream liver kinase B1 [LKB1]) or by AMP-independent mechanisms [37] including a lysosomal pathway triggered by a decrease in fructose 1,6-bisphosphate [38].

Mitochondrial Complex I Inhibition, Uncoupling, or Blocking of Lysosomal Vacuolar ATPase?

The classic “receptor” for “antidiabetic” biguanides (metformin < buformin < phenformin) is mitochondrial complex I [39]. One other postulated mitochondrial target (mitochondrial glycerol phosphate dehydrogenase) was proven to be an experimental artifact [40]. Purified complex I can be inhibited by the antidiabetic as well as the antimalarial biguanides cycloguanil and proguanil. The two latter biguanides do not inhibit complex I in intact mitochondria, nor do they increase AMPK in intact cells. This has led to the proposals that (a) a specific carrier exists in the inner mitochondrial membrane and (b) the negative membrane potential (−180 mV) enriches the antidiabetic drugs from micromolar cytosolic to high millimolar concentrations in the matrix. As a consequence, so the “inhibitory” hypothesis, the ADP/ATP ratio increases. Metformin is also suggested to act as a “mild uncoupler” [41]. Metformin and phenformin are indeed potent inhibitors (1 μM) in isolated mitochondria, blocking retrograde electron transport and peroxide production, similar to an uncoupler [42].

As an alternative to the mitochondrial pathway (complex I “inhibition” and/or “uncoupling”), direct activation of AMPK and inhibition of mTORC1 via blockage of lysosomal vacuolar ATPase (V-ATPase) was postulated [43]. As of April 2019, neither a structure-activity relationship for guanidines, as for mitochondria and complex I, nor dose responses have been reported. Likewise, the subunit of the V-ATPase postulated to bind the drug has not been published.

Prospective Metformin Megatrials for Age-Related Diseases: TAME, VA-IMPACT, and GLINT

On June 24, 2015, Nir Barzilai (Albert Einstein College of Medicine, New York, NY, USA) met with regulators from the FDA to discuss the now famous phase III multi-site TAME (Targeting Aging with Metformin) trial [44]. The acronym chosen and the intention behind it – name-

ly, that aging is a “disorder” that can be treated like any other disease – was a clear provocation. The FDA’s mandate is to regulate medications and devices to cure diseases or aid in their diagnosis, but aging is not (yet) an indication. The current International Statistical Classification of Diseases and Related Health Problems 10 (ICD-10) does not list “aging” as a disease. Efforts are undertaken [45] to include it in ICD-11, a final version of which is expected in January 2022.

It should be noted that metformin’s efficacy for diabetes prevention and weight loss in obese patients has been proven in clinical trials. These are not among the FDA-listed indications. Nevertheless, metformin’s “off-label” use alone or in combination with other antidiabetics enjoys popularity for effective prevention of diabetes [46], as well as for treatment of metabolic syndrome, obesity, or polycystic ovarian disease [47, 48]. TAME, if metformin’s efficacy is proven, seeks approval for the novel indication to allow for healthcare providers to pay for such therapy [49]. The investigators want to “repurpose” [50] metformin for diseases which increase logarithmically, e.g., in mice and humans with advancing age [51, 52] (online suppl. Fig. S1). TAME’s primary (composite) endpoint is the incidence of any one of several age-related diseases: myocardial infarction, congestive heart failure, stroke, most cancers, dementia, and death, but not diabetes.

Discussions about TAME with the FDA have been ongoing for almost 4 years. The American Federation for Aging Research as a sponsor has already guaranteed USD 35 million. A grant proposal for further funding (USD 40 million) included a grant from the NIH which was up for review in October 2018. Barzilai hoped that the trial would be running by the end of 2018 [53], but the trial was not yet listed in ClinicalTrials.gov as of April 2019 and the study protocol is not in the public domain.

Interestingly, frailty is missing from the proposed composite outcome. Other ongoing trials (e.g., NCT02570672) with metformin provide arguments that frailty may be an important endpoint [54]. It will be interesting to compare the results with the ongoing fisetin trial (NCT03675724). A megatrial, sponsored by the Veterans Administration (NCT02915198; VA-IMPACT), started on February 19, 2019. This trial plans to study 7,868 subjects with prediabetes and established atherosclerotic disease for 4.5 years in a double-blind fashion with metformin extended release versus placebo for a combined primary endpoint. Time-to-events for oncology-related diseases and diabetes are secondary endpoints. TAME instead plans to randomize 3,000 older persons (65–79 years) without diabetes, but it will include indi-

viduals already “burdened with a chronic disease” [55] and most likely only subjects with impaired glucose tolerance/fasting hyperglycemia. A recent double-blind, randomized feasibility trial (GLINT) with metformin and placebo in elderly obese patients at a high risk of cardiovascular diseases (CVD) and nondiabetic hyperglycemia came to the conclusion that 20,000 subjects are required to obtain significant results for only CVD [56], hence the combined TAME or VA-IMPACT endpoints. The TAME biomarker workgroup selected blood-based biomarkers for geroscience-guided trials, which included interleukin-6, C-reactive protein, insulin-like growth factor 1, insulin, cystatin C, N-terminal prohormone of brain natriuretic peptide, HbA_{1c}, and GDF15, but not GLP-1 [57]. Barzilai and coworkers [58] performed a small, double-blind, placebo-controlled crossover trial (Metformin in Longevity Study [MILES]) in which they investigated differentially expressed genes in muscular and adipose tissue biopsies after 6-week administration of placebo or 1.5 g per day of metformin. They reported results for some of the markers selected by Justice et al. [57] but did not mention GDF15.

Important arguments from basic science for TAME and other trials – e.g., for cancer or neurological diseases (not specifically addressed here) – are that metformin increases the activity of AMPK and may stimulate autophagy [59–61] or modulates it in T2D white blood cells [62].

“Correlation Is Not Causation, but It’s the Way to Bet”

The title was taken from an informative article [63]. There are numerous meta-analyses and systematic reviews on metformin’s effects beyond control of T2D, termed “nonglycemic benefits,” e.g., on cancer, CVD events, mortality, and dementia. A few selected examples are presented below. The benefits are suggested to be mediated by “pleiotropic” activities of the biguanide, which may be simply summarized as effects “beyond control of blood sugar.” Metformin is routinely recommended/preferred for overweight/obese T2D and causes weight loss or less weight gain, depending on adherence [64]. Its use was restricted, until recently, to populations with normal kidney function, which can introduce an important selection bias. Other important confounding factors may exist, which must be adjusted for in case-control studies, as underlined by the recent analysis of metformin benefits for hepatocellular carcinoma [65].

Depending on whether only randomized clinical trials are included [66] or cohort and case-control studies are

added [67], the cancer risk with T2D is either not lowered or decreased significantly for patients taking metformin. With respect to CVD, an analysis from 2012 concluded that metformin had no effect on the risk of all-cause mortality or cardiovascular mortality [68]. A more recent meta-analysis of only randomized clinical trials favored metformin, but not significantly and not for stroke [69]. The clinical cohort of male veterans with T2D ($n = 41,204$) who were >65 years old included $n = 8,393$ metformin users. They were followed up for 9 years, and four distinct age-related comorbidity trajectory classes could be identified. In the so-termed “healthy class,” the odds ratio of mortality associated with metformin use was 0.53; in the high-cancer-risk class it was 0.72, in the high-CVD-risk class it was 0.58, and in the high-frailty class it was 0.39, indicating that the last group benefitted most from the drug with respect to mortality [70].

If observational studies (cohort, case-control, and cross-sectional studies) are included and the onset or prevalence of “diseases of aging” (cancer, CVD, kidney failure, fracture, or cognitive impairment) is measured, the results suggest that diabetics taking metformin had a lower rate of all-cause mortality and of developing any cancer even compared to the general nondiabetic control population [71, 72]. The better cancer survival of metformin users compared to nonusers is also observed from very large electronic health records [73], but will not be discussed here any further. Campbell et al. [71] concluded that “[the] apparent reductions in all-cause mortality and diseases of ageing associated with metformin use suggest that metformin could be extending life and healthspans by acting as a geroprotective agent.” Given that metformin has a record of safety and efficacy yet unsurpassed by any other T2D drug, the rationale behind the trials and the inclusion of a placebo arm is justified both ethically and scientifically.

In summary, TAME, GLINT, VA-IMPACT, and the frailty metformin trial are “making a bet” supported by association evidence, obtained from T2D patients, with an aging study population (impaired glucose tolerance/increased fasting blood glucose) already at risk. It will take several years until the results are known. In the diabetes prevention program (DPP) and the still ongoing DPP Outcomes Study (DPPOS), patients aged <60 years did not benefit from metformin after 15 years [74]. This explains why diabetes is not included in the composite endpoint of TAME, and only as a secondary endpoint in other planned or active trials. Alternatives exist: weight loss [75] and/or lifestyle interventions such as exercise and Mediterranean diet [76] are suggested to prevent diabetes or/and to protect against age-related diseases. The

widely cited Prevención con Dieta Mediterránea (PRE-DIMED) trial [77] has been retracted [78], and the published evidence for benefits of Mediterranean diet is in doubt.

Metformin as an “Anti-Aging” (Longevity) Compound in Model Organisms

As supportive evidence for the TAME trial, it was stated that “metformin modulates the biology of aging and health span in model organisms” [79]. To our dismay, we could not find much evidence, but even to the contrary, for the claim. An apparent exception is *Caenorhabditis elegans*, but the present data favor an indirect mechanism via live *E. coli* food.

C. elegans: Good Evidence that Metformin Acts Indirectly, but Conflicting Interpretations of the Mechanisms

Whereas *Drosophila melanogaster* as a model organism for aging research is more than 100 years old, the nematode can claim to be the “premier” model [80]. The first report on metformin characterized it as a “dietary restriction-like factor” and demonstrated the participation of AMPK and LKB1 [81]. For some time, the drug was praised as a prototypic “caloric restriction mimetic,” a view that is no longer shared today [82]. The average maximum lifespan of the wild-type (WT) worm, fed with live bacteria (*E. coli*), is 30 days; the mean lifespan is 20 days. The most extensively studied long-lived mutants (*daf-2*) have a mutation in the insulin/insulin-like growth factor 1 receptor, with doubling of the maximum lifespan to 60 days. The mortality curves of longevity mutants and the WT can be described by the Gompertz equation (online suppl. Fig. S1).

The lower Gompertz exponent of the longer-living mutants does not reveal any information on health changes, i.e., whether the increased lifespan was associated with better health for a longer time period not in absolute terms of days but as a percentage of the total lifespan. This has been studied in great detail for four longevity mutants (*daf-2*, *ife-2*, *clk-1*, and *eat-2*) [83]. The disturbing result was that none of the mutants gained anything: a longer life was always associated with increased time spent in frailty. It is explained in online supplementary Box S1 that the environmental temperature may be a strong confounding factor in mouse experiments. Surprisingly, light exposure is an important variable in *C. elegans* experiments. Light may add to oxidative stress (which is preventable by antioxidants), can dramatically change the lifespan, and could explain some of the variable results/

interpretations by different laboratories [84]. Three illustrative metformin lifespan studies are discussed below.

The Gut Bacteria-Based Microbial Folate and Methionine Mechanism

Metformin at 25 or 50 mM increased the mean lifespan of worms fed with live *E. coli* by 18 and 36%, respectively [85]. This effect was associated with a lower Gompertz exponent and could be imitated by pretreatment of live bacteria with metformin. Culturing the worms axenically (in the absence of live bacteria) increased the lifespan dramatically (to 75 days), but metformin at 50 mM was toxic. The authors observed a strong positive correlation between the ability of metformin to act bacteriostatically on different strains of *E. coli* and the lifespan ratio. They finally traced the origin of the life-extending metformin effect to inhibition of the folate metabolism of the bacteria and less delivery of methionine. Trimethoprim, an inhibitor of bacterial dihydrofolate reductase, mimicked the effects of metformin. It was concluded that metformin is intrinsically toxic (life-shortening), but that as long as live bacteria deliver less methionine, the nutrient deficiency resulting in decreased levels of SAM (S-adenosyl methionine) and decreased SAM/S-adenosyl-L-homocysteine ratios will balance out the direct toxic effect. In summary, this publication concluded that the lifespan extension with metformin that was observed by others [81] was indirect.

Mitohormesis and Reactive Oxygen Species

After it had been clarified that metformin acted via changing the composition of the nutrients derived from the *E. coli*, the question remained as to whether there were also *direct* effects on the worm. A comprehensive differential analysis of the proteomic data [86] indicated that several pathways were upregulated by metformin, especially degradation of branched-chain amino acids, the citrate cycle, glycolysis, and pyruvate metabolism. An increased production of reactive oxygen species (ROS) was observed, and antioxidants completely prevented the small effects of the drug on lifespan. As the missing link between ROS production and the increase in lifespan, the peroxiredoxin PRDX-2 was identified. Peroxiredoxins (EC1.11.1.15) are a family of peroxidases which also function as signaling molecules [87]. When the *prdx-2* gene was deleted, metformin no longer induced longer life but killed the worms.

The entire process was identified as the “mitohormetic” pathway. Experiments with isolated mitochondria and 25 mM metformin demonstrated complete inhibition

of complex I-driven O₂ flux and increased ROS production. As a consequence, one would expect low respiration rates and less heat production. Significantly lower respiration rates had been reported before with 50 mM metformin applied for just 30 min [88]. When worms (and their food) were drugged with 50 mM metformin for 48 h [86], increased respiration as well as heat production as a consequence of the “mitohormetic” response were observed.

In summary, the authors suggest that there are direct effects of metformin but surprisingly never measured metformin concentrations in the animals.

Metformin Acts through the Lysosomal Pathway

Whereas the primary (postulated) target of metformin in the worm for the mitohormetic mechanism [86] is mitochondrial complex I, another publication [89] favors lysosomes. Metformin (50 mM) prolonged life from 30 to 40 days and activated (phosphorylated) the orthologue of AMPK (AAK-2). A variety of mutants were studied, including heterozygotes of *daf-15* (regulatory-associated protein of mTOR [RAPTOR]) and four loss-of-function mutants (*vha-3* [subunit of the V-ATPase V₀ domain], *vha-12* [subunit of the V-ATPase V₁ domain], *lmtr-3* [LAMTOR 3 subunit of Ragulator], and *lmtr-2* [LAMTOR 2 subunit of Ragulator]). Heterozygotes of *daf-2* per se had a prolonged lifespan, which was further increased by metformin. All of the lysosomal mutants failed to increase their lifespan. Mutants lacking the orthologues of LKB1 (*par-4*) or axin (*axl-1*) were also unresponsive. The authors concluded that metformin induced lifespan extension via inhibiting mTORC1 and activating AMPK through the “lysosomal” pathway. It was noted that the metformin-treated worms looked healthier (fewer age pigments) and had an attenuated age-related decline in fitness (locomotion body bends). The metformin concentrations in the worms were not reported. The authors argued that they were “feeding” the animals with the drug, but the crucial question remains: does metformin prolong life in *C. elegans* by entering the animal or by changing the nutrients of their live feed? Controls with trimethoprim, bacteria pretreated with metformin, or dead *E. coli* are missing.

D. melanogaster: Metformin Decreases the Lifespan and Is Toxic

D. melanogaster is the Methuselah among the model organisms for aging research [90]. In contrast to its effect on worms, metformin did not increase the lifespan of either male or female flies [91]. The authors measured tissue levels of the drugged flies with mass spectrometry and

observed 0.3 millimoles/kg when the flies received 10 mM in the food. AMPK was stimulated in a dose-dependent manner, but the lifespan decreased with increased metformin concentrations and tissue levels. The toxic action was especially pronounced in post-reproductive female flies. The negative lifespan results were suggested [90] to be the results of optimized dietary conditions, but no explanation was given for the toxicity.

The lifespan and toxicity results have been replicated [88] and the target for toxicity identified as NHE3, the fly orthologue of mammalian NHE6 (SLC9A6), the Christianson syndrome protein [92], which is one member of endosomal/lysosomal localized Na⁺/H⁺ exchangers (eNHE). When mutant flies which have a P element insertion in the first NHE3 exon and have no detectable mRNA are tested, they are resistant to the toxic effects. Likewise, the *C. elegans* orthologue NHX5 is responsible for the metformin toxicity observed in the first larva stage upon starvation. The authors noted that metformin blocked the time-dependent increase in autophagosomes upon starving the larva and suggested that the drug inhibited autophagy. Although no direct measurement of effects of metformin on the activity of the exchanger was reported, a model was subsequently presented in which metformin inhibited V-ATPase of the late endosome/lysosome via eNHE [93].

Taken together, there is no evidence that metformin increases the lifespan of *Drosophila*, but it is toxic. The culprit in mediating toxicity was identified in both starving *C. elegans* larva and well-fed flies as an eNHE. These exchangers are most likely responsible for fine-tuning the internal pH of endo-/lysosomes. NHE6 is upregulated in mice upon calorie restriction and currently discussed as a target for various human pathologies [92].

Mus musculus: Little or No Evidence for an Increase in Lifespan

Although widely cited as evidence for the small effects of 0.1% metformin in the diet on the lifespan of older male inbred mice, the article by Martin-Montalvo et al. [94], as well as earlier results obtained by other researchers cited by Martin-Montalvo et al., should be dismissed: the National Institute on Aging Interventions Testing Program could not replicate the findings regarding an extension of the lifespan with 0.1% metformin. The negative results were obtained at three different locations using genetically heterogeneous female and male mice [95]. These negative results are rarely cited, likely since they do not fit authors' views. The article [94] found a significant lifespan extension only in male C57BL/6 mice, a strain

which is prone to obesity and likes alcohol and narcotics [96], but not in B6C3F1 mice.

Another, perhaps even more important, point to consider is the environmental temperature at which mice are kept (see online suppl. Box S1). This is underlined by numerous mouse experiments with disruption of growth hormone secretagogue signaling. At room temperature, one observes a remarkable extension of the lifespan and health span. However, the major metabolic differences and gene expression profiles almost completely disappear when WT and growth hormone secretagogue-deficient mice are housed in a thermoneutral environment [97].

Conclusions, Recommendations, and Perspectives

The rationale for the ongoing or planned metformin trials is almost exclusively based on observations (associations) of potential benefits in a diabetic (or prediabetic) population. Its efficacy even in an at-risk cohort of aged people has not yet been proven. Metformin is associated with a higher risk of vitamin B₁₂ and vitamin B₆ deficiency, which may result in an increased risk of cognitive dysfunction [98]. Supplementation is strongly recommended to metformin users.

Of greater concern are the results of small trials in which the effects of metformin on metabolic responses to exercise or on cardiorespiratory fitness were tested. In a placebo-controlled, double-blind, crossover trial with

healthy young subjects, metformin caused a small but significant decline in maximal aerobic capacity [99]. A double-blind, placebo-controlled landmark trial with older adults with one risk factor for T2D investigated the effects of metformin and 12 weeks of aerobic exercise [100]. Contrary to expectations – namely, that the effects of exercise and the drug would be additive – “metformin attenuated the increase in whole-body insulin sensitivity and abrogated the exercise-mediated increase in skeletal muscle mitochondrial respiration.” The results of the (re-purposing) MASTERS trial (NCT02308228; Metformin to Augment Strength Training Effective Response in Seniors) [100] will be instructive. MASTERS is testing the hypothesis that older individuals’ long-term treatment with metformin augments the effects of resistance exercise, especially in the “nonresponder” aging population.

Statement of Ethics

The authors have no ethical conflicts to disclose.

Disclosure Statement

The authors have no conflicts of interest to declare.

Author Contributions

Both authors discussed the topic and wrote the paper.

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