

We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,900

Open access books available

186,000

International authors and editors

200M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities



WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com



Metformin Modulates the Mechanisms of Ageing

*Adriana Florinela Cătoi, Andra Diana Andreicut,
Dan Cristian Vodnar, Katalin Szabo, Andreea Corina,
Andreea Arsene, Simona Diana Stefan,
Roxana Adriana Stoica and Manfredi Rizzo*

Abstract

Living in a time when population is continuously ageing, the challenge and demand for assessing the age-related pathways, potential diseases and longevity have become of major interest. The pharmaceutical industry possesses huge resources in this field, mainly due to the recent discoveries of novel mechanisms of action of old-established, classical drugs. Here we find metformin, a well-established antidiabetic medicine but with new potential benefits, as the most recent reports quote. We present the main pathways of the possible implications of metformin in the modulation of ageing processes, evolution and diseases, focussing on its ageing counteraction, based on the latest scientifically based biochemical reports.

Keywords: metformin, type 2 diabetes, mechanisms of ageing, anti-ageing

1. Introduction

At present, metformin is the preferred first-line drug used for the treatment of type 2 diabetes mellitus (T2DM) [1–4]. However, the journey of metformin (1,1-dimethylbiguanide hydrochloride) has not been a simple one. *Galega officinalis*, also termed as French lilac, Italian fitch, or Spanish sainfoin, the herb metformin derives from, has been known as a traditional medicine since the seventeenth century and was recommended for the treatment of thirst and frequent urination (symptoms of diabetes) by John Hill in 1772. The identification of guanidine and of its related compounds within *Galega officinalis*, which proved to be able to reduce blood glucose in animals, led to the synthesis of metformin (dimethylbiguanide) in 1922. However, it was only in the 1950s that more information on metformin's properties was published and when the name of Glucophage, meaning glucose eater, was suggested by Jean Sterne. Metformin was introduced as a treatment for T2DM in 1958 in the UK and in other European countries, whereas in the USA it was approved only in 1994 and started to be used beginning in 1995 [5]. A milestone multicentre trial, the United Kingdom Prospective Diabetes Study (UKPDS) in 1998, showed that the newly T2DM diagnosed patients receiving metformin for more than a decade displayed significant reduction of the cardiovascular events and of diabetes-related death and highlighted that these effects were independent of the glucose-lowering efficacy. Moreover, the potentially beneficial effects of metformin on the

macro- and microvasculature have also been revealed [5–8]. Finally, in a 10-year posttrial analysis, metformin continues to offer cardiovascular benefits [9]. Based on these evidence data, in 2009, the American Diabetes Association (ADA) and the European Association for the Study of Diabetes (EASD) indicated metformin as the first-line therapy for T2DM [10]. Furthermore, metformin holds a significant role in the delay/prevention of T2DM onset, as shown by the randomised trial conducted in the USA, i.e. the Diabetes Prevention Program (DPP). The study highlighted that metformin reduces the incidence of T2DM by 31% compared to placebo in adults at high risk for T2DM (obese and with impaired glucose tolerance) [11–14]. Hence, metformin is also recommended as a pharmacologic tool for the prevention of T2DM in subjects with prediabetes, mainly for those with a BMI ≥ 35 kg/m² [2], those aged <60 years, and in women with prior gestational diabetes mellitus [15–17].

Ageing continues to be an intruding topic and an area of great interest, constantly addressed by researchers worldwide. It encompasses a plethora of complex processes that have urged scientists to decipher its underlying mechanisms and to find the possible avenues to postpone its onset and that of its associated diseases [18]. Data from the literature have demonstrated a sustained ageing of the world's population, estimating a total of around 21.8% of subjects over 60 years old in 2050 and 32.2% in 2100 [19]. Installed as a result of the interaction between genetic, epigenetic, environmental and stochastic factors, ageing involves a progressive decline of the body functions as a consequence of the gradual cellular impairment due to a failure of the repair mechanisms [20–23]. Age is a major risk factor for the onset of metabolic, cardiovascular, neurodegenerative, immune and malignant diseases [24]. Ageing has been reported to be conditioned by the genetic factor in a proportion of 25–30%, while the remaining 70–75% is ruled by the environmental factor, making it a possible target for therapeutic tools among which metformin has been found [25, 26].

Beyond its blood glucose-lowering effect, metformin has been described as a drug used for preventing or delaying several conditions associated with ageing [27]. As such, metformin has been proven useful in overweight and obesity [28, 29], hypertension [30], atherosclerosis [31], coronary artery disease [32], dementia [33] and cancer [34]. Moreover, in terms of mortality [35], it has been shown that patients with T2DM under metformin monotherapy had a longer survival than the matched, nondiabetic controls. However, the precise beneficial mechanisms by which metformin performs its non-glycaemic work are yet to be analysed. Hence, given the complex mechanisms of action of metformin, there is a growing interest in approaching and studying the potential anti-ageing effect of this drug. With regard to this interest, some large randomised clinical trials have been recently set up in order to evaluate the potential role of metformin in reducing the burden of age-related diseases. The Investigation of Metformin in Pre-Diabetes on Atherosclerotic Cardiovascular outcomes (VA-IMPACT) trial is a placebo-controlled study started in February 2019 and aimed at shedding light on the potential role of metformin in reducing mortality and cardiovascular morbidity in patients with prediabetes and established atherosclerotic cardiovascular disease. More precisely, the primary outcomes include the time to death from any cause, nonfatal myocardial infarction, stroke, hospitalisation for unstable angina, or symptom-driven coronary revascularisation [27]. The other clinical trial, also a placebo-controlled trial, i.e. Targeting Ageing with Metformin (TAME), investigates subjects who have been diagnosed with one single age-associated disease and will provide insight on the ability of metformin to postpone and/or prevent the installation of a second pathology, such as cancer, CVD and dementia [13, 36]. Finally, more information is needed for a better understanding of the mechanistic targets and therapeutic implications of certain drugs (such as metformin) that might delay/alleviate the development of age-related diseases [37].

Herein, we revisit the mechanisms involved in ageing and the mechanistic target of metformin as a potential anti-ageing drug, and we review the available data on the clinical and experimental results showing the ability of metformin to promote healthspan and longevity.

2. Epidemiological data on the anti-ageing effect of metformin

A large body of evidence has demonstrated that metformin could be considered a geroprotective agent in humans [23]. As explained, the protective role of metformin in survival has been largely demonstrated by the UKPDS multicentre trial in terms of cardiac and all-cause mortality, as compared with usual care [8, 9]. However, given its main role, that is to reduce hyperglycaemia, and knowing that a good control of diabetes correlates with an extended lifespan, the question arises whether metformin could be accounted as a tool to prolong longevity in patients that do not display T2DM. In keeping with this question, a recent systematic review by Campbell et al. [23] summarised the studies in which the effects of metformin on all-cause mortality or diseases of ageing have been compared to the nondiabetic or general population or to diabetics controlling the disease through other means. Overall, the meta-analysis revealed that subjects with T2DM under metformin treatment have a lower rate of all-cause mortality and longer survival than people free of T2DM not using metformin and the general population, suggesting that this drug could be an effective instrument to extend the lifespan of those not affected by T2DM [23, 35, 38–40]. Moreover, the meta-analysis revealed that subjects with T2DM taking metformin had lower rates of all-cause mortality than those following other therapies, such as insulin or sulphonylurea [23]. Given these results, it may be argued that the outcome is attained by the geroprotective role of metformin resulting in delaying or preventing diseases of ageing, such as cancer or cardiovascular disturbances, which are the two most encountered ageing-related diseases [23, 41]. Firstly, in terms of malignancies, Campbell et al. [23] showed that people with T2DM taking metformin had a lower rate of developing any cancer compared with the general population. Moreover, the risk of developing colorectal, breast or lung cancer in individuals with T2DM on metformin treatment, as compared to those using other therapies, was lower. Secondly, subjects with T2DM following metformin therapy displayed a lower rate of any form of cardiovascular disease with respect to those managing their T2DM through any non-metformin therapy. In addition, although the incidence of stroke was also lower with metformin, for myocardial infarction the effect of the drug seems to be non-significant [23].

Finally, apart from the cardiovascular diseases and cancer, there are also other age-related pathologies that could be targeted by metformin, such as cognitive dysfunction. However, the evidence in patients with T2DM is conflicting with some studies showing a protective role of metformin against cognitive decline, whereas others are arguing that metformin treatment could induce neurodegeneration as well as Parkinson's and Alzheimer's disease. Nevertheless, the interpretation of the data is difficult given the possible presence of other concomitant conditions that may contribute to this cognitive decline [42].

3. Mechanisms involved in ageing

Ageing is a complex process that occurs at the molecular, cellular, organ and organismal level that everyone faces in time [43]. It involves the loss of the body's ability to overcome and respond to stress (homeostenosis) by repair and

regeneration, thus leading to various disturbances within the human body [24]. Overall, the ageing processes are of a heterogeneous and heterochronic nature. As a heterogeneous process, ageing can evolve at different rates in diverse organisms, while the heterochronic feature implies that cells and tissues within a single organism can age in an asynchronic manner, finally making chronological age different as compared to biological age [24, 43]. Growing body of evidence has shown that ageing involves multiple mechanisms that inter-relate with and modulate each other. In this respect, two elegant reviews have described nine hallmarks of ageing, which have been classified into primary hallmarks (genomic instability, telomere attrition, epigenetic alterations, and loss of proteostasis) as the main culprit of molecular damage, antagonistic hallmarks (deregulated nutrient sensing, mitochondrial dysfunction, and cellular senescence) with beneficial effects when at low levels, by protecting the human organism against damage, but with deleterious effects when at high levels, and finally, the integrative hallmarks (stem cell exhaustion and altered intercellular communication) that arise when the accumulating damage cannot be balanced by homeostatic mechanisms, thus ultimately inducing ageing [22, 36].

Genomic instability has been revealed to be a major stochastic mechanism of ageing [44, 45]. Broadly, deoxyribonucleic acid (DNA) damage can be induced by both exogenous genotoxic factors, such as ionising radiation and ultraviolet irradiation as well as endogenous genotoxic agents, i.e. products of normal metabolism that lead to the formation of reactive oxygen species (ROS) and subsequently to oxidative stress, that may finally result in deleterious effects on the cell. DNA lesions can cause mutations, block transcription and replication but can also trigger DNA damage response (DDR), which implies mechanisms that intervene and arrest cell cycle progression, resulting in the repair of almost all the alterations that occur within the genome. However, when DNA damage is extensive and prevails over repair, DDR effectors trigger cell death (apoptosis) or cell senescence, contributing to ageing and age-related diseases [46, 47]. In fact, in ageing, DNA damage overtakes DNA repair, leading to genomic instability, a fact sustained by studies showing accumulation of DNA alterations in old tissues [48]. On the other hand, genomic instability has been reported to be a driver of accelerated ageing, widely demonstrated by the presence of hypersensitivity to genotoxins and defects in genome maintenance in progeroid syndromes termed as diseases of accelerated ageing. Collectively, DNA damage as a culprit in ageing is highlighted by the accrual of sources of damage, i.e. oxidative stress (the oxidative stress theory of ageing) associated with the mitochondrial theory of ageing, as mitochondria is the primary source of ROS, increased activation of the DDR, mutations and presence of senescent cells along with a decreased capacity for DNA repair [47]. Among these factors oxidative stress is a well-known pathogenic mechanism and seems to be the most important one [49]. The overproduction of ROS along with a reduced antioxidant defence, i.e. oxidative stress, leads to DNA, protein and lipid damage [50, 51]. Also, ROS lead to age-related DNA lesions acting via nuclear factor kappa-light-chain-enhancer of activated B cells (NF- κ B) which controls cytokine and chemokine expression and regulates adhesion molecules [45, 52, 53].

Telomeres are chromosomal end structures that play important roles in the protection of DNA from degradation [54]. In each cell division, 20–200 base pairs are lost within the telomeres, and telomerase is in charge of repairing telomeres after cell division. However, when they reach a certain critical length, i.e. shortening or attrition, the cells stop replicating and die [43]. The shortening process, as the telomerase fails to replicate completely the terminal ends of the DNA molecules, has been reported in ageing [55, 56]. Moreover, in humans, damaged telomerase can cause degenerative defects associated with ageing [57, 58].

Epigenetics meaning “above the genes” is termed as the inheritance of changes in gene function with no modifications in the nucleotide sequence of DNA [43, 59]. Epigenetic changes that comprise alterations in DNA itself as DNA methylation and modifications of histones (acetylation and methylation) as well as of other chromatin-associated proteins and chromatin remodelling can also be involved in ageing [22]. Sirtuins, a family of NAD-dependent deacetylases that act on Lys16 of histone H4, are emerging as a link between cellular transformation and lifespan [59]. Of note, epigenetic alterations seem to be reversible, underpinning the anti-ageing interventions [60]. Moreover, Greer et al. [61] showed transgenerational epigenetic inheritance of longevity in *Caenorhabditis elegans* suggesting that manipulation of specific chromatin modifiers in parents can induce an epigenetic memory of longevity in descendants.

Proteostasis or protein stability is an important feature of the cells and involves a complex network that coordinates protein synthesis with polypeptide folding, conservation of protein conformation and protein degradation [62, 63]. When damaged, as a consequence of various external and endogenous stress factors, it leads to the accumulation of protein aggregates holding proteotoxic effects and becomes a contributor to ageing and to age-related diseases [63–65]. In fact, it has been demonstrated that with age, proteostasis becomes compromised, leading to proteotoxicity [43, 62, 66]. More precisely, intracellular damaged protein deposition has been described in age-related diseases such as Alzheimer’s and Parkinson’s [62, 63, 67]. Finally, evidence data have revealed a double-sense link between DNA damage and proteostasis, which jointly induce an increased cellular lesion [63].

Deregulated nutrient sensing represents another important hallmark of ageing [22, 68]. Nutrient sensing is mediated by specific molecular pathways, such as insulin and insulin-like growth factor 1 (IGF-1 informs the cells about the presence of glucose and has the same intracellular signalling pathway as insulin), termed as “insulin and/IGF1-signalling” pathway (IIS) as well as the mechanistic target of rapamycin (mTOR) that senses nutrients, whereas AMP-activated protein kinase (AMPK) and sirtuins detect the energy levels [22, 43]. All these systems named as “nutrient sensing” pathways regulate metabolism and influence ageing [43]. More precisely, current data show that anabolic signalling induces accelerated ageing, while decreased nutrient signalling (attained through caloric restriction) promotes a healthy span and extends longevity [69, 70].

The “insulin and/IGF1-signalling” pathway (IIS) operates on the forkhead box proteins or FOXO family of transcription factors and on the mTOR complexes and has been reported to be the most conserved ageing-controlling pathway. Indeed, mutations that reduce the functions of insulin and IGF-1 receptor or downregulate the intracellular effectors, i.e. AKT, mTOR and FOXO, result in increased lifespan [22, 69, 71].

The mTOR kinase is part of two complex proteins and is sensitive to high levels of amino acids controlling a wide range of cellular functions, mostly anabolic metabolism [72]. It is noteworthy that mTOR is a target of rapamycin (an mTOR inhibitor), an antibiotic that exerts anti-proliferative effects by acting through this specific pathway. Several studies have shown that mTOR manipulation by inducing downregulation is involved in extending longevity [22, 43].

Finally, the AMPK pathway and sirtuins that sense changes in energy levels, i.e. low levels of ATP, act in the opposite direction as compared to IIS and mTOR, their activation leading to increased energy production and decreased ATP utilisation [22, 43]. In fact, caloric restriction seems to activate the AMPK pathway [73]. Finally, upregulation of both AMPK and sirtuins favours healthy ageing [74].

Mitochondrial dysfunction is a feature of ageing that refers to reduced respiratory chain efficiency, resulting in electron leak and diminished ATP production [75]. The

consequence of mitochondrial dysfunction, installed across ageing, is the formation of ROS, and the theory of free radicals as a mechanism inducing ageing has been widely discussed [76]. However, this theory has been re-analysed and reconsidered as emerging data show that oxidative stress up to a specific threshold has, in fact, a beneficial effect in prolonging lifespan [77, 78]. More specifically, it seems that ROS in a certain amount may play a role as a trigger of compensatory homeostatic reactions as a response to the ongoing and increasing stress factors that come along with ageing, resulting in facing damage and maintaining survival [79]. Still, when over the specific threshold, ROS change their purpose and induce deleterious age-related effects [77, 80, 81].

Apart from the ROS theory, accumulating data have revealed that impaired mitochondrial function may contribute to ageing through other mechanisms, such as the increase of permeability in response to stress that triggers inflammatory reactions, the damaged interface between the outer mitochondrial membrane and the endoplasmic reticulum as well as reduced biogenesis of mitochondria [22]. Furthermore, it seems that both endurance training and alternate-day fasting have the ability to improve healthspan through mitochondrial degeneration avoidance [82, 83].

Finally, the mitochondrial dysfunction seems to be related to the hormesis which is deemed as an adaptive response of the organism to low doses of a toxic agent or physical condition, such as ROS, that induces the ability of the organism to tolerate higher doses of the same toxic agent [63]. Hence, although severe mitochondrial dysfunction is deleterious, mild respiratory damage may increase lifespan, possibly subsequently to a hormetic response [84]. In fact, data from the literature have shown that metformin could be considered a mild mitochondrial “toxic agent” as it induces a low energy state and activates AMPK [85]. In this respect, Anisimov et al. [74] showed that when administrated early in life, metformin treatment increases life span in mice.

Senescence is an age hallmark that stands out as a response triggered by genomic instability and telomere attrition resulting in growth arrest, thus limiting the proliferation of aged and damaged cells [22, 46, 47, 86]. A second important feature of senescent cells is the development of a peculiar secretome, termed as the senescence-associated secretory phenotype (SASP), which encompasses cytokines, chemokines and proteases, resulting in a pro-inflammatory state [87, 88]. Under normal conditions the SASP is involved in the recruitment of macrophages, neutrophils and natural killer (NK) cells, thus holding a beneficial effect in eliminating the senescent cells. However, across the ageing process, the senescence cells accumulate resulting in increased cytokine production and recruitment of more immune cells, which jointly contribute to the onset of the inflammageing state, a true driver of ageing [36, 87]. Moreover, a declined activity of the immune system, termed as immunosenescence, is installed in aged people, thus impairing the clearance of senescent cells and, in turn, increasing even more the chronic inflammation state. Collectively, senescence, inflammageing and immunosenescence promote ageing and operate together, rendering aged people more susceptible to age-related diseases [87, 89]. Finally, interestingly, mitochondrial dysfunction can also trigger cellular senescence, a process termed as “mitochondrial dysfunction-associated senescence” (MiDAS). MiDAS support the existence of a strong inter-relation between cellular senescence and metabolic dysfunction, highlighting that targeting metabolism may be a proper way to extend lifespan in humans [36].

Stem cell exhaustion, i.e. the progressive decline in the regenerative potential of the stem cells needed for tissue repair, is another characteristic of ageing. As explained, ageing is accompanied by immunosenescence, a condition that results from reduced haematopoiesis and that has several deleterious consequences [22].

Finally, apart from cellular damage, ageing also implies *altered intercellular communication*. Inflammation is an ageing-associated damage in intercellular communication termed as “inflammageing,” as previously described. Inflammageing may result from multiple causes, such as the accumulation of tissue damage, the reduced ability of the immune system to remove pathogens, the increase of senescent cells that produce pro-inflammatory cytokines, immunosenescence that fails to remove the senescent cells, the activation of the NFkB transcription factor, as well as the onset of a dysfunctional autophagic response [22]. Noteworthy, that inflammation is involved in the pathogenesis of obesity and T2DM, diseases that contribute to the onset of ageing [71]. Apart from inflammation, the intercellular communication has been revealed by the bystander effect referring to senescent cells inducing senescence in neighbouring cells via gap-junction-mediated cell–cell cross talk [90].

Given the aforementioned complex hallmarks of ageing, researchers worldwide have searched for proper tools to obtain the delay of ageing and the avoidance of age-related diseases. Here we find metformin, a drug that has been reported to be useful in modulating some of the age-related features. In fact, in cellular and animal models, metformin has been shown to influence and to hold beneficial effects on the following age related hallmarks [91]: (1) genomic instability [92, 93], (2) telomere attrition [94], (3) epigenetic changes [95], (4) proteostasis [96, 97], (5) nutrient-sensing pathways [98, 99], (6) mitochondrial function [100], (7) cellular senescence [101, 102], (8) stem cell function [103], and (9) low-grade inflammation [104].

4. Experimental evidence on the anti-ageing effect of metformin

Evidence-based data have revealed that metformin holds an important role in extending survival and delaying the onset of age-related diseases in nematode *Caenorhabditis elegans* [105, 106] and mice [107], but not in *Drosophila melanogaster* [108, 109]. In this respect, metformin supplementation was shown to increase mean lifespan and to prolong the healthspan of nematode *Caenorhabditis elegans* (an experimental model often used to study ageing and anti-ageing therapies) via AMPK [106]. Moreover, other authors have shown that metformin has the ability to retard ageing in *Caenorhabditis elegans* by metabolic alteration of its trophic microbial partner, *E. coli*. In brief, metformin disrupts the bacterial folate cycle, which reduces the levels of methionine in the worm. Finally, this results in postponing ageing by triggering a metabolic dietary restriction phenomenon and AMPK activation [105, 110]. Based on these results, we might argue another important role of metformin, that of modulating human microbiota, i.e. an increased abundance of *E. coli*, resulting in an increased production of short-chain fatty acids, such as butyrate and propionate, by which metformin might induce significant positive results in T2DM and might interfere with longevity [36, 111, 112].

In a very recent study, Song et al. [113] used the silkworm, a popular experimental model, to investigate the impact of metformin on lifespan and the underlying molecular pathways. They found that metformin prolonged lifespan without reducing body weight, which suggests that it can increase lifespan by remodelling the animal's energy distribution strategy. Also, metformin increased fasting tolerance and levels of the antioxidant glutathione and activated APMK. Finally, these results suggest that activity in this pathway may contribute to metformin-induced lifespan extension in silkworm by increasing stress resistance and anti-oxidative capacity, while reducing energy output for silk product [113].

Studies on ageing and lifespan have also been performed on mice, highlighting the potential anti-ageing effect of metformin, resulting in an extended

lifespan [114–116]. Anisimov et al. [116] demonstrated that chronic treatment of female mice with metformin significantly increased mean and maximum lifespan, even without cancer prevention in that model. In a further study, the authors showed that in female mice, metformin increased lifespan and postponed tumours when started at young and middle, but not at the old age [74]. Besides the increase of lifespan in mice, Martin-Montalvo et al. [107] pointed out that metformin seems to mimic some of the benefits of calorie restriction and leads to improved glucose-tolerance test, increased insulin sensitivity and reduced low-density lipoprotein and cholesterol levels without a decrease in the caloric intake. With respect to the mechanisms of action, metformin seems to increase the antioxidant activity, resulting in reductions in both oxidative stress and chronic inflammation [107].

Finally, as previously mentioned, not all experimental models confirm the anti-ageing role of metformin. It is the case of *Drosophila* fruit fly, another animal model where the authors showed that metformin induced a robust activation of AMPK and reduced lipid stores, but did not increase lifespan. Moreover, they found that when administered in high concentrations, metformin is toxic to flies. Finally, it seems that metformin appears to have evolutionarily conserved effects on metabolism but not on fecundity or lifespan [108].

5. Mechanisms of metformin action: A focus on molecular pathways that modulate ageing

The main universally accepted role of metformin is to alleviate hyperglycaemia. This outcome is obtained through the inhibition of hepatic gluconeogenesis [117, 118]. Metformin holds an insulin-sensitising action and insulin-induced suppression of endogenous glucose production [119]. Although other organs have been discussed as a target for metformin, such as the gut [120], liver remains the main ground of action, as reduced hepatic uptake of metformin prevents the lowering blood glucose effect [91]. There are several mechanisms by which metformin downregulates gluconeogenesis. Firstly, metformin induces alterations in cellular energetics [117], i.e. by decreasing cellular respiration through inhibition of the complex I mitochondrial respiratory chain [121, 122]. The result of this inhibition is the increase of the ADP:ATP and AMP:ATP ratios, which subsequently activate the cellular energy state sensor AMP-activated protein kinase (AMPK) [91, 110, 123], the key player of metformin. Once activated, AMPK leads to an increase in ATP production and a decrease in ATP consumption [42]. Noteworthy, AMPK is one of the molecular pathways that can modify the rate of ageing [43]. The importance of the activation of AMPK in obtaining the reduction in hepatic glucose production was investigated by Hawley et al. [85] who showed that an AMPK mutant does not respond to metformin treatment. On the other hand, Foretz et al. [124] showed that in AMPK knockout mice, the inhibition of gluconeogenesis is still present and associated with a reduction in energy state, but this happens in response to higher concentrations of metformin as compared to standard treatment. With regard to therapeutic concentrations of metformin, it seems that AMPK activation is mandatory for the suppression of gluconeogenesis [117, 125]. Finally, we have to mention that the activation of AMPK via inhibition of the complex I mitochondrial respiratory chain has been recently debated [126] as physiological/low concentration of metformin, which cannot induce AMP/ATP change, can still activate AMPK [125].

Another effect mediated by AMPK activation by metformin refers to the inhibitory phosphorylation of acetyl-CoA carboxylase (ACC), which leads to increased fatty acid uptake and β -oxidation and hence to improved lipid metabolism and subsequently to improved insulin sensitivity [127]. Furthermore, activated AMPK

decreases glucagon-stimulated cyclic AMP (cAMP) accumulation, cAMP-dependent protein kinase (PKA) activity and downstream PKA target phosphorylation and increases cyclic nucleotide phosphodiesterase 4B (PDE4B). The authors provided a new mechanism by which AMPK antagonises hepatic glucagon signalling via phosphorylation-induced PDE4B activation [128]. Moreover, the decreased PKA activity promotes glucose consumption and inhibits glucose output [129]. Finally, metformin inhibits hepatic gluconeogenesis through AMPK-dependent regulation of the orphan nuclear receptor small heterodimer partner (SHP) [130].

Secondly, AMPK-independent mechanisms by which metformin inhibits hepatic gluconeogenesis have been reported [117]. In this respect, Miller et al. [131] point towards the ability of the drug to inhibit adenylate cyclase, reduce levels of cAMP and PKA activity, abrogate phosphorylation of critical protein targets of PKA, and block glucagon-dependent glucose output from hepatocytes through accumulation of AMP and related nucleotides independently of AMPK [131]. In addition, metformin inhibits the mitochondrial glycerophosphate dehydrogenase, resulting in an altered hepatocellular redox state, reduced conversion of lactate and glycerol to glucose and hence decreased hepatic gluconeogenesis [132].

Taken together, given the important role of metformin in inhibiting hepatic gluconeogenesis and therefore in reducing hyperglycaemia and subsequently hyperinsulinemia, jointly, important accelerators of ageing, several studies regard metformin as a potential anti-ageing drug [42, 117]. Metformin works through complex mechanisms that have been demonstrated to be similar to those associated with caloric restriction, a well-known model that underpins extended lifespan and healthspan. More precisely, it seems that both metformin and caloric restriction induce the same gene expression profile [107, 117, 133].

Another important target involved in changing the rate of ageing is mTOR [117]. TOR responds to insulin, amino acids and hormones and is involved in controlling a wide range of cellular functions, such as glucose metabolism, lipid and protein synthesis, inflammation and mitochondrial function [72]. Metformin has been demonstrated to downregulate mTOR in both a AMPK-dependent and AMPK-independent manner [98, 134–136]. Through stimulation of AMPK, metformin induces suppression of ATP consumption by inhibiting energy needing processes, such as protein synthesis via mTOR [42, 137]. In addition, through downregulation of mTOR signalling and of insulin-like growth factor 1 (IGF-1), metformin influences cell growth, proliferation and autophagy [42].

NF- κ B pathway is another key mediator of ageing. As previously described, it is activated by genotoxic, oxidative and inflammatory stress and regulates the expression of cytokines, inflammation, growth factors and genes that regulate apoptosis [45]. Metformin has been demonstrated to inhibit NF- κ B resulting in suppressing the inflammatory response via AMPK-dependent and independent pathways [138]. Also, metformin seems to hold the ability to reduce the endogenous ROS production [93] by acting at a mitochondrial level through blockage of the reverse electron flow at the respiratory chain complex 1 [139].

Finally, a very recent pathway has been described by Chen et al. [140]. The authors showed through genetic manipulation that metformin extends the *Caenorhabditis elegans* lifespan and attenuates age-related fitness decline via a mechanism that requires v-ATPase-Ragulator-AXIN/LKB1 of the lysosomal pathway [140].

In toto, the possible molecular mechanisms by which metformin exerts anti-ageing effects are [13, 91]: (1) inhibition of mitochondrial complex 1 in the electron transport chain and decrease of ROS production [139, 141], (2) activation of AMPK [106, 124, 140, 142–144], (3) inhibition of mTOR [106, 134, 135, 140], (4) NF- κ B inhibition [101], and (5) reduced IGF-1 signalling [145].

6. Conclusions

Ageing encompasses a cluster of processes that induce a gradual decline of the human body functions, a condition that everyone faces in time. Also, ageing is a risk factor for a gamut of disturbances such as cancer, T2DM and cardiovascular and neurodegenerative diseases. Therefore, researchers worldwide strive to find the adequate tools in order to delay/avoid the onset of age-related diseases and hence promote healthspan. In keeping with this aim, metformin emerges as a drug that, beyond its main role to reduce hyperglycaemia, has antitumor effects and works as a protector against cardiovascular and neurodegenerative diseases making it a potential anti-ageing medicine. Importantly, metformin seems to possess positive effects even in nondiabetic subjects. However, the exact mechanisms of action and the molecular pathways involved in ageing that are modulated by metformin are not fully explained, and further studies are warranted for a better understanding of the beneficial effects of this drug.

Acknowledgements

Mr. Manfredi Rizzo serves as Director, Clinical Medical & Regulatory Affairs, Novo Nordisk Europe East and South. The publication was supported by funds from the National Research Development Projects to finance excellence (PFE)-37/2018–2020 granted by the Romanian Ministry of Research and Innovation.

IntechOpen

IntechOpen

Author details

Adriana Florinela Cătoi^{1*}, Andra Diana Andreicuț¹, Dan Cristian Vodnar², Katalin Szabo³, Andreea Corina^{4,5}, Andreea Arsene⁶, Simona Diana Stefan⁷, Roxana Adriana Stoica⁷ and Manfredi Rizzo^{8,9}

1 Department of Pathophysiology, Faculty of Medicine, 'Iuliu Hațieganu' University of Medicine and Pharmacy, Cluj-Napoca, Romania

2 Department of Food Science and Technology, University of Agricultural Sciences and Veterinary Medicine, Cluj-Napoca, Romania

3 Institute of Life Sciences, University of Agricultural Sciences and Veterinary Medicine, Cluj-Napoca, Romania

4 Lipids and Atherosclerosis Research Unit, Maimonides Biomedical Research Institute of Cordoba (IMIBIC), Reina Sofia University Hospital, University of Cordoba, Cordoba, Spain

5 CIBER Fisiopatología de la Obesidad y Nutrición (CIBEROBN), Instituto de Salud Carlos III, Cordoba, Spain

6 Department of General and Pharmaceutical Microbiology, Faculty of Pharmacy, "Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania

7 Diabetes, Nutrition and Metabolic Diseases Department, Faculty of Medicine, "Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania

8 PROMISE Department, University of Palermo, Italy

9 Clinical Medical and Regulatory Affairs, Novo Nordisk - Europe East and South

*Address all correspondence to: florinela12@yahoo.com

IntechOpen

© 2019 The Author(s). Licensee IntechOpen. This chapter is distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/3.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited. 

References

- [1] International Diabetes Federation Guideline Development Group. Global guideline for type 2 diabetes. Diabetes Research and Clinical Practice. 2014;**104**(1):1-52
- [2] Inzucchi SE, Bergenstal RM, Buse JB, et al. Management of hyperglycemia in type 2 diabetes: A patient-centered approach: Position statement of the American Diabetes Association and the European Association for the Study of Diabetes. Diabetologia. 2015;**58**(3):429-442
- [3] Garber AJ, Abrahamson MJ, Barzilay JI, et al. Consensus statement by the American Association of Clinical Endocrinologists and American College of Endocrinology on the comprehensive type 2 diabetes management algorithm—2017 executive summary. Endocrine Practice. 2017;**23**(2):207-238
- [4] Qaseem A, Barry MJ, Humphrey LL, Forciea MA, Clinical Guidelines Committee of the American College of Physicians. Oral pharmacologic treatment of type 2 diabetes mellitus: A clinical practice guideline update from the American College of Physicians. Annals of Internal Medicine. 2017;**166**(4):279-290
- [5] Bailey CJ. Metformin: Historical overview. Diabetologia. 2017;**60**(9):1566-1576
- [6] Wiernsperger NF. 50 years later: Is metformin a vascular drug with antidiabetic properties? The British Journal of Diabetes & Vascular Disease. 2007;**7**:204-210
- [7] Bailey CJ. Metformin: Effects on micro and macrovascular complications in type 2 diabetes. Cardiovascular Drugs and Therapy. 2008;**22**(3):215-224
- [8] UK Prospective Diabetes Study (UKPDS) Group. Effect of intensive blood-glucose control with metformin on complications in overweight patients with type 2 diabetes. Lancet. 1998;**352**(9131):854-865
- [9] Holman RR, Paul SK, Bethel MA, Matthews DR, Neil HA. 10-year follow-up of intensive glucose control in type 2 diabetes. The New England Journal of Medicine. 2008;**359**(15):1577-1589
- [10] Nathan DM, Buse JB, Davidson MB, et al. Medical management of hyperglycemia in type 2 diabetes: A consensus algorithm for the initiation and adjustment of therapy: A consensus statement of the American Diabetes Association and the European Association for the Study of Diabetes. Diabetes Care. 2009;**32**(1):193-203
- [11] Knowler WC, Barrett-Connor E, Fowler SE, et al. Reduction in the incidence of type 2 diabetes with lifestyle intervention or metformin. The New England Journal of Medicine. 2002;**346**(6):393-403
- [12] Diabetes Prevention Program Research Group. HbA 1c as a predictor of diabetes and as an outcome in the diabetes prevention program: A randomized clinical trial. Diabetes Care. 2015;**38**(1):51-58
- [13] Barzilai N, Crandall JP, Kritchevsky SB, Espeland MA. Metformin as a tool to target aging. Cell Metabolism. 2016;**23**(6):1060-1065
- [14] Hostalek U, Gwilt M, Hildemann S. Therapeutic use of metformin in prediabetes and diabetes prevention. Drugs. 2015;**75**(10):1071-1094
- [15] Diabetes Prevention Program Research Group. Long-term safety, tolerability, and weight loss associated with metformin in the diabetes

prevention program outcomes study. *Diabetes Care*. 2012;**35**(4):731-737

[16] Moin T, Schmitt diel JA, Flory JH, et al. Review of metformin use for type 2 diabetes prevention. *American Journal of Preventive Medicine*. 2018;**55**(4):565-574

[17] American Diabetes Association. Prevention or delay of type 2 diabetes: Standards of medical care in diabetes—2019. *Diabetes Care*. 2019;**42**(Suppl 1):S29-S33

[18] Van Ginneken V. Are there any biomarkers of aging? *Biomarkers of the brain. Biomedical Journal of Scientific & Technical Research*. 2017;**1**:193-206

[19] Lutz W, Sanderson W, Scherbov S. The coming acceleration of global population ageing. *Nature*. 2008;**451**(7179):716-719

[20] Weinert BT, Timiras PS. Invited review: Theories of aging. *Journal of Applied Physiology* (Bethesda, MD: 1985). 2003;**95**(4):1706-1716

[21] Garinis GA, van der Horst GT, Vijg J, Hoeijmakers JH. DNA damage and ageing: New-age ideas for an age-old problem. *Nature Cell Biology*. 2008;**10**(11):1241-1247

[22] López-Otín C, Blasco MA, Partridge L, Serrano M, Kroemer G. The hallmarks of aging. *Cell*. 2013;**153**(6):1194-1217

[23] Campbell JM, Bellman SM, Stephenson MD, Lisy K. Metformin reduces all-cause mortality and diseases of ageing independent of its effect on diabetes control: A systematic review and meta-analysis. *Ageing Research Reviews*. 2017;**40**:31-44

[24] Khan SS, Singer BD, Vaughan DE. Molecular and physiological manifestations and measurement of aging in humans. *Ageing Cell*. 2017;**16**(4):624-633

[25] Finlay BB, Pettersson S, Melby MK, Bosch TCG. The microbiome mediates environmental effects on aging. *BioEssays*. 2019;**2019**:e1800257

[26] Nagpal R, Mainali R, Ahmadi S, et al. Gut microbiome and aging: Physiological and mechanistic insights. *Nutrition and Healthy Aging*. 2018;**4**(4):267-285

[27] Valencia WM, Palacio A, Tamariz L, Florez H. Metformin and ageing: Improving ageing outcomes beyond glycaemic control. *Diabetologia*. 2017;**60**(9):1630-1638

[28] Yerevanian A, Soukas AA. Metformin: Mechanisms in human obesity and weight loss. *Current Obesity Reports*. 2019;**8**(2):156-164

[29] Seifarth C, Schehler B, Schneider HJ. Effectiveness of metformin on weight loss in non-diabetic individuals with obesity. *Experimental and Clinical Endocrinology & Diabetes*. 2013;**121**(1):27-31

[30] Zhou L, Liu H, Wen X, Peng Y, Tian Y, Zhao L. Effects of metformin on blood pressure in nondiabetic patients: A meta-analysis of randomized controlled trials. *Journal of Hypertension*. 2017;**35**(1):18-26

[31] Jenkins AJ, Welsh P, Petrie JR. Metformin, lipids and atherosclerosis prevention. *Current Opinion in Lipidology*. 2018;**29**(4):346-353

[32] Luo F, Das A, Chen J, Wu P, Li X, Fang Z. Metformin in patients with and without diabetes: A paradigm shift in cardiovascular disease management. *Cardiovascular Diabetology*. 2019;**18**(1):54

[33] Campbell JM, Stephenson MD, De Courten B, Chapman I, Bellman SM, Aromataris E. Metformin use associated with reduced risk of dementia in

- patients with diabetes: A systematic review and meta-analysis. *Journal of Alzheimer's Disease*. 2018;**65**(4):1225-1236
- [34] Kobiela J, Dobrzycka M, Jędrusik P, et al. Metformin and colorectal cancer—A systematic review. *Experimental and Clinical Endocrinology & Diabetes*. 2019;**127**(7):445-454
- [35] Bannister CA, Holden SE, Jenkins-Jones S, et al. Can people with type 2 diabetes live longer than those without? A comparison of mortality in people initiated with metformin or sulphonylurea monotherapy and matched, non-diabetic controls. *Diabetes, Obesity & Metabolism*. 2014;**16**(11):1165-1173
- [36] López-Otín C, Galluzzi L, Freije JMP, Madeo F, Kroemer G. Metabolic control of longevity. *Cell*. 2016;**166**(4):802-821
- [37] Piskovatska V, Strilbytska O, Koliada A, Vaiserman A, Lushchak O. Health benefits of anti-aging drugs. *Sub-Cellular Biochemistry*. 2019;**91**:339-392
- [38] Bérard E, Bongard V, Dallongeville J, et al. 14-year risk of all-cause mortality according to hypoglycaemic drug exposure in a general population. *PLoS One*. 2014;**9**(4):e95671
- [39] Bo S, Ciccone G, Rosato R, et al. Cancer mortality reduction and metformin: A retrospective cohort study in type 2 diabetic patients. *Diabetes, Obesity & Metabolism*. 2012;**14**(1):23-29
- [40] Claesen M, Gillard P, De Smet F, Callens M, De Moor B, Mathieu C. Mortality in individuals treated with glucose- lowering agents: A large, controlled cohort study. *The Journal of Clinical Endocrinology and Metabolism*. 2016;**101**(2):461-469
- [41] Mathers CD, Loncar D. Projections of global mortality and burden of disease from 2002 to 2030. *PLoS Medicine*. 2006;**3**(11):e442
- [42] Piskovatska V, Stefanyshyn N, Storey KB, Vaiserman AM, Lushchak O. Metformin as geroprotector: Experimental and clinical evidence. *Biogerontology*. 2019;**20**(1):33-48
- [43] Carmona JJ, Michan S. Biology of healthy aging and longevity. *Revista de Investigación Clínica*. 2016;**68**(1):7-16
- [44] Vijg J, Busuttil RA, Bahar R, Dollé ME. Aging and genome maintenance. *Annals of the New York Academy of Sciences*. 2005;**1055**:35-47
- [45] Kanigur Sultuybek G, Soydas T, Yenmis G. NF - κ B as the mediator of metformin's effect on ageing and ageing-related diseases. *Clinical and Experimental Pharmacology & Physiology*. 2019;**46**(5):413-422
- [46] Hoeijmakers JH. DNA damage, aging, and cancer. *The New England Journal of Medicine*. 2009;**361**:1475-1485
- [47] Niedernhofer LJ, Gurkar AU, Wang Y, Vijg J, Hoeijmakers JHJ, Robbins PD. Nuclear genomic instability and aging. *Annual Review of Biochemistry*. 2018;**87**:295-322
- [48] Lu T, Pan Y, Kao S, et al. Gene regulation and DNA damage in the ageing human brain. *Nature*. 2004;**429**(6994):883-891
- [49] Harman D. Aging: A theory based on free radical and radiation chemistry. *Journal of Gerontology*. 1956;**11**:298-300
- [50] Liochev SI. Reactive oxygen species and the free radical theory of aging. *Free Radical Biology & Medicine*. 2013;**60**:1-4

- [51] Birch-Machin MA, Bowman A. Oxidative stress and ageing. *The British Journal of Dermatology*. 2016;**175**:26-29
- [52] Janssens S, Tschopp J. Signals from within: The DNA-damage-induced NF- κ B response. *Cell Death and Differentiation*. 2006;**13**:773-784
- [53] Wu Z, Miyamoto S. Many faces of NF- κ B signaling induced by genotoxic stress. *Journal of Molecular Medicine*. 2007;**85**:1187-1202
- [54] Ishikawa N, Nakamura KI, Izumiyama-Shimomura N, et al. Changes of telomere status with aging: An update. *Geriatrics & Gerontology International*. 2016;**16**(Suppl. 1):30-42
- [55] Blackburn EH, Greider CW, Szostak JW. Telomeres and telomerase: The path from maize, Tetrahymena and yeast to human cancer and aging. *Nature Medicine*. 2006;**12**(10):1133-1138
- [56] Blasco MA. Telomere length, stem cells and aging. *Nature Chemical Biology*. 2007;**3**(10):640-649
- [57] Armanios M, Blackburn EH. The telomerase syndromes. *Nature Reviews. Genetics*. 2012;**13**(10):693-704
- [58] Armanios M, Alder JK, Parry EM, Karim B, Strong MA, Greider CW. Short telomeres are sufficient to cause the degenerative defects associated with aging. *American Journal of Human Genetics*. 2009;**85**(6):823-832
- [59] Fraga MF, Esteller M. Epigenetics and aging: The targets and the marks. *Trends in Genetics*. 2007;**23**(8):413-418
- [60] Freije JM, López-Otín C. Reprogramming aging and progeria. *Current Opinion in Cell Biology*. 2012;**24**(6):757-764
- [61] Greer EL, Maures TJ, Ucar D, et al. Transgenerational epigenetic inheritance of longevity in *Caenorhabditis elegans*. *Nature*. 2011;**479**(7373):365-371
- [62] Kaushik S, Cuervo AM. Proteostasis and aging. *Nature Medicine*. 2015;**21**(12):1406-1415
- [63] Hipp MS, Kasturi P, Hartl FU. The proteostasis network and its decline in ageing. *Nature Reviews. Molecular Cell Biology*. 2019;**20**(7):421-435
- [64] Klaips CL, Jayaraj GG, Hartl FU. Pathways of cellular proteostasis in aging and disease. *The Journal of Cell Biology*. 2018;**217**(1):51-63
- [65] Taylor RC, Dillin A. Aging as an event of proteostasis collapse. *Cold Spring Harbor Perspectives in Biology*. 2011;**3**(5):pii: a004440
- [66] Koga H, Kaushik S, Cuervo AM. Protein homeostasis and aging: The importance of exquisite quality control. *Ageing Research Reviews*. 2011;**10**(2):205-215
- [67] Vilchez D, Saez I, Dillin A. The role of protein clearance mechanisms in organismal ageing and age-related diseases. *Nature Communications*. 2014;**5**:5659
- [68] Efeyan A, Comb WC, Sabatini DM. Nutrient sensing mechanisms and pathways. *Nature*. 2015;**517**(7534):302-310
- [69] Fontana L, Partridge L, Longo VD. Extending healthy life span—From yeast to humans. *Science*. 2010;**328**(5976):321-326
- [70] Vermeij WP, Dollé ME, Reiling E, et al. Restricted diet delays accelerated ageing and genomic stress in DNA-repair-deficient mice. *Nature*. 2016;**537**(7620):427-431
- [71] Barzilai N, Huffman DM, Muzumdar RH, Bartke A. The critical

- role of metabolic pathways in aging. *Diabetes*. 2012;**61**:1315-1322
- [72] Laplante M, Sabatini DM. mTOR signaling in growth control and disease. *Cell*. 2012;**149**(2):274-293
- [73] Cantó C, Auwerx J. Calorie restriction: Is AMPK as a key sensor and effector? *Physiology (Bethesda, Md.)*. 2011;**26**(4):214-224
- [74] Anisimov VN, Berstein LM, Popovich IG, et al. If started early in life, metformin treatment increases life span and postpones tumors in female SHR mice. *Aging (Albany NY)*. 2011;**3**(2):148-157
- [75] Green DR, Galluzzi L, Kroemer G. Mitochondria and the autophagy-inflammation-cell death axis in organismal aging. *Science*. 2011;**333**(6046):1109-1112
- [76] Harman D. The free radical theory of aging: Effect of age on serum copper levels. *Journal of Gerontology*. 1965;**20**:151-153
- [77] Hekimi S, Lapointe J, Wen Y. Taking a “good” look at free radicals in the aging process. *Trends in Cell Biology*. 2011;**21**(10):569-576
- [78] Ristow M, Schmeisser S. Extending life span by increasing oxidative stress. *Free Radical Biology & Medicine*. 2011;**51**(2):327-336
- [79] Sena LA, Chandel NS. Physiological roles of mitochondrial reactive oxygen species. *Molecular Cell*. 2012;**48**(2):158-167
- [80] Pantea Stoian A, Mitrofan G, Colceag F, et al. Oxidative stress in diabetes: A model of complex thinking applied in medicine. *Revista de Chimie (Bucharest)*. 2018;**69**(9):2515-2519
- [81] Pantea-Stoian A, Ditu G, Diculescu M. Pancreatogenic type 3C diabetes. *Journal of Mind and Medical Sciences*. 2018;**5**(2):270-277
- [82] Safdar A, Bourgeois JM, Daniel I, et al. Endurance exercise rescues progeroid aging and induces systemic mitochondrial rejuvenation in mtDNA mutator mice. *Proceedings of the National Academy of Sciences of the United States of America*. 2011;**108**(10):4135-4140
- [83] Castello L, Maina M, Testa G, et al. Alternate-day fasting reverses the age-associated hypertrophy phenotype in rat heart by influencing the ERK and PI3K signaling pathways. *Mechanisms of Ageing and Development*. 2011;**132**:305-314
- [84] Haigis MC, Yankner BA. The aging stress response. *Molecular Cell*. 2010;**40**(2):333-344
- [85] Hawley SA, Ross FA, Chevtzoff C, et al. Use of cells expressing gamma subunit variants to identify diverse mechanisms of AMPK activation. *Cell Metabolism*. 2010;**11**(6):554-565
- [86] Muñoz-Espín D, Serrano M. Cellular senescence: From physiology to pathology. *Nature Reviews. Molecular Cell Biology*. 2014;**15**(7):482-496
- [87] McHugh D, Gil J. Senescence and aging: Causes, consequences, and therapeutic avenues. *The Journal of Cell Biology*. 2018;**217**(1):65-77
- [88] Kuilman T, Peeper DS. Senescence-messaging secretome: SMS-ing cellular stress. *Nature Reviews. Cancer*. 2009;**9**(2):81-94
- [89] Farr JN, Almeida M. The spectrum of fundamental basic science discoveries contributing to organismal aging. *Journal of Bone and Mineral Research*. 2018;**33**(9):1568-1584
- [90] Nelson G, Wordsworth J, Wang C, et al. A senescent cell bystander effect:

Senescence-induced senescence. *Aging Cell*. 2012;**11**(2):345-349

pii: glz058. DOI: 10.1093/gerona/glz058.
[Epub ahead of print]

[91] Prattichizzo F, Giuliani A, Mensà E, et al. Pleiotropic effects of metformin: Shaping the microbiome to manage type 2 diabetes and postpone ageing. *Ageing Research Reviews*. 2018;**48**:87-98

[98] Howell JJ, Hellberg K, Turner M, et al. Metformin inhibits hepatic mTORC1 signaling via dose-dependent mechanisms involving AMPK and the TSC complex. *Cell Metabolism*. 2017;**25**(2):463-471

[92] Halicka HD, Zhao H, Li J, et al. Genome protective effect of metformin as revealed by reduced level of constitutive DNA damage signaling. *Aging (Albany NY)*. 2011;**3**(10):1028-1038

[99] Halicka HD, Zhao H, Li J, et al. Potential anti-aging agents suppress the level of constitutive mTOR- and DNA damage-signaling. *Aging (Albany NY)*. 2012;**4**(12):952-965

[93] Algire C, Moiseeva O, Deschênes-Simard X, et al. Metformin reduces endogenous reactive oxygen species and associated DNA damage. *Cancer Prevention Research (Philadelphia, PA)*. 2012;**5**(4):536-543

[100] Andrzejewski S, Gravel S, Pollak M, St-Pierre J. Metformin directly acts on mitochondria to alter cellular bioenergetics. *Cancer & Metabolism*. 2014;**2**:12

[94] Garcia-Martin I, Penketh RJA, Janssen AB, et al. Metformin and insulin treatment prevent placental telomere attrition in boys exposed to maternal diabetes. *PLoS One*. 2018;**13**(12):e0208533

[101] Moiseeva O, Deschênes-Simard X, St-Germain E, et al. Metformin inhibits the senescence-associated secretory phenotype by interfering with IKK/NF- κ B activation. *Aging Cell*. 2013;**12**(3):489-498

[95] García-Calzón S, Perfilyev A, Männistö V, et al. Diabetes medication associates with DNA methylation of metformin transporter genes in the human liver. *Clinical Epigenetics*. 2017;**9**:102

[102] Hooten NN, Martin-Montalvo A, Dluzen DF, et al. Metformin-mediated increase in DICER1 regulates microRNA expression and cellular senescence. *Aging Cell*. 2016;**15**(3):572-581

[96] Hull RL, Shen Z, Watts MR, et al. Long-term treatment with rosiglitazone and metformin reduces the extent of, but does not prevent, islet amyloid deposition in mice expressing the gene for human islet amyloid polypeptide. *Diabetes*. 2005;**54**(7):2235-2244

[103] Xu G, Wu H, Zhang J, et al. Metformin ameliorates ionizing irradiation-induced long-term hematopoietic stem cell injury in mice. *Free Radical Biology & Medicine*. 2015;**87**:15-25

[97] Wolff CA, Reid JJ, Musci RV et al. Differential effects of rapamycin and metformin in combination with rapamycin on mechanisms of proteostasis in cultured skeletal myotubes. *The Journals of Gerontology. Series A, Biological Sciences and Medical Sciences*. 2019 March.

[104] Tizazu AM, Nyunt MSZ, Cexus O, et al. Metformin monotherapy downregulates diabetes-associated inflammatory status and impacts on mortality. *Frontiers in Physiology*. 2019;**10**:572

[105] Cabreiro F, Au C, Leung KY, et al. Metformin retards aging in *C. elegans* by altering microbial folate and methionine metabolism. *Cell*. 2013;**153**(1):228-239

- [106] Onken B, Driscoll M. Metformin induces a dietary restriction-like state and the oxidative stress response to extend *C. elegans* healthspan via AMPK, LKB1, and SKN-1. *PLoS One*. 2010;5(1):e8758
- [107] Martin-Montalvo A, Mercken EM, Mitchell SJ, et al. Metformin improves healthspan and lifespan in mice. *Nature Communications*. 2013;4:2192
- [108] Slack C, Foley A, Partridge L. Activation of AMPK by the putative dietary restriction mimetic metformin is insufficient to extend lifespan in *Drosophila*. *PLoS One*. 2012;7(10):e47699
- [109] Anisimov VN. Metformin: Do we finally have an anti-aging drug? *Cell Cycle*. 2013;12(22):3483-3489
- [110] Storelli G, Téfit M, Leulier F. Metformin, microbes, and aging. *Cell Metabolism*. 2013;17(6):809-811
- [111] Canfora EE, Jocken JW, Blaak EE. Short-chain fatty acids in control of body weight and insulin sensitivity. *Nature Reviews. Endocrinology*. 2015;11(10):577-591
- [112] Suceveanu AI, Pantea Stoian A, Parepa I, et al. Gut microbiota patterns in obese and type 2 diabetes (T2D) patients from Romanian Black Sea coast region. *Revista de Chimie (Bucharest)*. 2018;69(8):2260-2267
- [113] Song J, Jiang G, Zhang J, et al. Metformin prolongs lifespan through remodeling the energy distribution strategy in silkworm, *Bombyx mori*. *Aging (Albany NY)*. 2019;11(1):240-248
- [114] Anisimov VN, Berstein LM, Egormin PA, et al. Effect of metformin on life span and on the development of spontaneous mammary tumors in HER-2/neu transgenic mice. *Experimental Gerontology*. 2005;40(8-9):685-693
- [115] Anisimov VN, Egormin PA, Piskunova TS, et al. Metformin extends life span of HER-2/neu transgenic mice and in combination with melatonin inhibits growth of transplantable tumors in vivo. *Cell Cycle*. 2010;9(1):188-197
- [116] Anisimov VN, Berstein LM, Egormin PA, et al. Metformin slows down aging and extends life span of female SHR mice. *Cell Cycle*. 2008;7(17):2769-2773
- [117] Novelle MG, Ali A, Diéguez C, Bernier M, de Cabo R. Metformin: A hopeful promise in aging research. *Cold Spring Harbor Perspectives in Medicine*. 2016;6(3):a025932
- [118] An H, He L. Current understanding of metformin effect on the control of hyperglycemia in diabetes. *The Journal of Endocrinology*. 2016;228(3):R97-R106
- [119] Natali A, Ferrannini E. Effects of metformin and thiazolidinediones on suppression of hepatic glucose production and stimulation of glucose uptake in type 2 diabetes: A systematic review. *Diabetologia*. 2006;49(3):434-441
- [120] Buse JB, DeFronzo RA, Rosenstock J, et al. The primary glucose-lowering effect of metformin resides in the gut, not the circulation: Results from short-term pharmacokinetic and 12-week dose-ranging studies. *Diabetes Care*. 2016;39(2):198-205
- [121] El-Mir MY, Nogueira V, Fontaine E, Avéret N, Rigoulet M, Leverve X. Dimethylbiguanide inhibits cell respiration via an indirect effect targeted on the respiratory chain complex I. *The Journal of Biological Chemistry*. 2000;275(1):223-228

- [122] Owen MR, Doran E, Halestrap AP. Evidence that metformin exerts its anti-diabetic effects through inhibition of complex 1 of the mitochondrial respiratory chain. *The Biochemical Journal*. 2000;**348**(Pt 3):607-614
- [123] Hardie DG. The AMP-activated protein kinase pathway—New players upstream and downstream. *Journal of Cell Science*. 2004;**117**(Pt 23):5479-5487
- [124] Foretz M, Hébrard S, Leclerc J, et al. Metformin inhibits hepatic gluconeogenesis in mice independently of the LKB1/AMPK pathway via a decrease in hepatic energy state. *The Journal of Clinical Investigation*. 2010;**120**(7):2355-2369
- [125] Cao J, Meng S, Chang E, et al. Low concentrations of metformin suppress glucose production in hepatocytes through AMP-activated protein kinase (AMPK). *The Journal of Biological Chemistry*. 2014;**289**(30):20435-20446
- [126] He L, Wondisford FE. Metformin action: Concentrations matter. *Cell Metabolism*. 2015;**21**(2):159-162
- [127] Fullerton MD, Galic S, Marcinko K, et al. Single phosphorylation sites in Acc1 and Acc2 regulate lipid homeostasis and the insulin-sensitizing effects of metformin. *Nature Medicine*. 2013;**19**(12):1649-1654
- [128] Johanns M, Lai Y, Hsu M, et al. AMPK antagonizes hepatic glucagon-stimulated cyclic AMP signalling via phosphorylation-induced activation of cyclic nucleotide phosphodiesterase 4B. *Nature Communications*. 2016;**7**:10856
- [129] Rena G, Hardie DG, Pearson ER. The mechanisms of action of metformin. *Diabetologia*. 2017;**60**(9):1577-1585
- [130] Kim YD, Park K, Lee Y, et al. Metformin inhibits hepatic gluconeogenesis through AMP-activated protein kinase-dependent regulation of the orphan nuclear receptor SHP. *Diabetes*. 2008;**57**(2):306-314
- [131] Miller RA, Chu Q, Xie J, Foretz M, Viollet B, Birnbaum MJ. Biguanides suppress hepatic glucagon signaling by decreasing production of cyclic AMP. *Nature*. 2013;**494**(7436):256-260
- [132] Madiraju AK, Erion DM, Rahimi Y, et al. Metformin suppresses gluconeogenesis by inhibiting mitochondrial glycerophosphate dehydrogenase. *Nature*. 2014;**510**(7506):542-546
- [133] De Cabo R, Carmona-Gutierrez D, Bernier M, Hall MN, Madeo F. The search for antiaging interventions: From elixirs to fasting regimens. *Cell*. 2014;**157**(7):1515-1526
- [134] Nair V, Sreevalsan S, Basha R, et al. Mechanism of metformin-dependent inhibition of mammalian target of rapamycin (mTOR) and Ras activity in pancreatic cancer: Role of specificity protein (Sp) transcription factors. *The Journal of Biological Chemistry*. 2014;**289**(40):27692-27701
- [135] Pérez-Revuelta BI, Hettich MM, Ciociaro A, et al. Metformin lowers Ser-129 phosphorylated α -synuclein levels via mTOR-dependent protein phosphatase 2A activation. *Cell Death & Disease*. 2014;**5**:e1209
- [136] Kalender A, Selvaraj A, Kim SY, et al. Metformin, independent of AMPK, inhibits mTORC1 in a rag GTPase-dependent manner. *Cell Metabolism*. 2010;**11**(5):390-401
- [137] Towler MC, Hardie DG. AMP-activated protein kinase in metabolic control and insulin signaling. *Circulation Research*. 2007;**100**(3):328-341

[138] Saisho Y. Metformin and inflammation: Its potential beyond glucose-lowering effect. *Endocrine, Metabolic & Immune Disorders Drug Targets*. 2015;**15**(3):196-205

[139] Batandier C, Guigas B, Detaille D, et al. The ROS production induced by a reverse-electron flux at respiratory-chain complex 1 is hampered by metformin. *Journal of Bioenergetics and Biomembranes*. 2006;**38**(1):33-42

[140] Chen J, Ou Y, Li Y, Hu S, Shao L, Liu Y. Metformin extends *C. elegans* lifespan through lysosomal pathway. *eLife*. 2017;**6**:pii: e31268

[141] Bridges HR, Jones AJ, Pollak MN, Hirst J. Effects of metformin and other biguanides on oxidative phosphorylation in mitochondria. *The Biochemical Journal*. 2014;**462**(3):475-487

[142] Zheng Z, Chen H, Li J, et al. Sirtuin 1-mediated cellular metabolic memory of high glucose via the LKB1/AMPK/ROS pathway and therapeutic effects of metformin. *Diabetes*. 2012;**61**(1):217-228

[143] Lu J, Shi J, Li M, et al. Activation of AMPK by metformin inhibits TGF- β -induced collagen production in mouse renal fibroblasts. *Life Sciences*. 2015;**127**:59-65

[144] Cho K, Chung JY, Cho SK, et al. Antihyperglycemic mechanism of metformin occurs via the AMPK/LXR α /POMC pathway. *Scientific Reports*. 2015;**5**:8145

[145] Liu B, Fan Z, Edgerton SM, Yang X, Lind SE, Thor AD. Potent anti-proliferative effects of metformin on trastuzumab-resistant breast cancer cells via inhibition of erbB2/IGF-1 receptor interactions. *Cell Cycle*. 2011;**10**(17):2959-2966