Metformin and Longevity Research: An Evidence-Based Approach

Research Team
Metformin.io

Executive Summary

This white paper explores the potential of metformin as a key agent in longevity research, highlighting its broad biological effects beyond diabetes management. Drawing on extensive scientific literature, we analyze metformin's mechanisms of action, including its influence on mitochondrial function, oxidative stress and systemic inflammation. These pathways are increasingly recognized as critical for ageing and health span extension.

Our first experimental study investigates the effects of metformin on age-related mitochondrial dysfunction using a robust animal model. By assessing markers such as ATP production, oxidative stress levels and inflammation, the study aims to clarify how metformin might promote cellular resilience and delay age-related decline. The methodology prioritizes rigorous scientific practices to ensure reproducibility and relevance for translational applications.

This research initiative aims to bridge the gap between laboratory findings and clinical applications, providing insights into how metformin could be repurposed to address age-related challenges. Through a multidisciplinary approach, this work contributes to the evolving field of longevity science and promotes strategies for healthier and longer lives.

What is MetforminSOL and what is the aim of the project?

MetforminSOL is a project that created by 0x_Rorschach through Pump.fun platform. Initially launched within the Meme Token category, the project has since evolved into a Decentralised Science (DeSci).

Building on 0x_Rorschach's expertise as a medical doctor actively researching longevity, the team decided to integrate scientific research into the core vision of the project. MetforminSOL now aims to bridge the gap between community-driven tokens and scientific innovation, particularly in lifespan extension and longevity studies.

The project team currently consists of three Associate Professors of Medicine, reflecting its strong academic background. MetforminSOL also has an active and engaged community to support its scientific and collaborative goals.

The team has already prepared its first research study and received ethics committee approval. This demonstrates the project's commitment to advancing longevity research while encouraging community involvement in decentralized science.

1. The Role of Evidence-Based Science in Enhancing Our Understanding andPractice of Scientific Inquiry

1.1. Evidence and It’s Role for Science

Evidence plays a vital role in science that cannot be ignored or disputed. The more formal evidence builds on the initial observations raised as potential discoveries.

What counts as "evidence" depends in part on the line of inquiry that evidence is being used to contribute to. If the investigation is designed ultimately to extend the depth and scope of our knowledge, then "evidence" will be that data that extends and supports knowledge pointing to a relation between A and B. Formal evidence will extend existing knowledge in such a way that E increases our grounds for adding A and B. The rudiment obviously points to the first step in investigating scientific evidence what do we want evidence to do for us? Scientific inquiry is regularly held up to be a classic example of

the development of knowledge. Evidence-based practitioners are, in contrast, researchers and policymakers who produce recommendations for health based on the interpretable weighing up of what evidence in general suggests will work best for patients.

1.2. The Foundations of Evidence-Based Science

Evidence-based science builds on a number of foundational concepts and principles. At its heart is an emphasis on the importance and centrality of empirical evidence in determining outcomes. It is an emphasis so central that the concept may be characterized as resting on a logical empiricist foundation. Evidence-based science draws upon a number of conceptual and logical frameworks and foundational principles that are central to scientific inquiry. First, there is the concept of "evidence relevant weight." Inquiry and the construction of new knowledge should be driven by an empiricist approach to evidence and its assessment. This principle draws on the assumption that evidence carries certain characteristics such that we are able to make judgments and assessments regarding the content, quality, and reliability of that evidence. This is a central tenet upon which evidence-based science is founded.

It is central to the richness inherent within the concept that shows it to be reflective of a complex interrelationship between the historical, philosophical, and sociological discourses on evidence, science, and the creation of knowledge. The history of the evolution of evidence-based science reveals various starting points and progressive historical milestones that would allow one to "map out" the gradual intellectual "path" and range of "stages" as the salient characteristics of the various evidence-rational constructs. Conclusively, understanding the foundations of evidence-based science is crucial for developing an explanatory framework of the most current technological advances in scientific activity management that have focused specifically on the integration of evidence.

1.3. Definition and Principles

Evidence-based science (EBS) is a movement that aims to bring a closer relationship between the best available evidence and scientific inquiry. In advancing this aim, the EBS movement has led to significant developments in understanding evidence,

particularly in explorations around standards of evidence and the scientific methods needed based on the use of credible evidence. The understanding of evidence and assembled evidence has led our team to clarify the following definition of evidence in addition to presenting principles for the theories, methodologies, and practices underpinning EBS. Based on this definition and the principles, this paper clarifies the status and quantum of evidence by operationalizing evidence into various types depending on its nature and quality, including the different standards necessary for their attainment. As a result of this operationalization and the principles, we employ evidence to systematically address a range of evidence-based practices to guide scientists in their research and its use.

The main principles of EBS for inquiry are rigor, transparency, and reproducibility. Rigor implies the scholarly ethical values, which include the intellectual humility to critically and open-mindedly pursue what is likely to be true, regardless of the source or conclusion of tentative investigations. It implies attentiveness to the wider array of viable methods, evidence, and levels of partiality. Transparency implies sharing the collected data, the reasoning, and the methodologies so that results can be scrutinized and independently repeated with parallel, modified, or different data. Every assumption, methodological detail, and criterion used to categorize, analyze, and evaluate the evidence should be identified and disclosed in the research paper, irrespective of how challenging. The purpose of such disclosures is to connect readers with the frontline empirical content of the inquiry.

1.4. Historical Development

While recent literature has elaborated on evidence-based practices, an "evidence-based approach" has a deep and rich history. Developments of evidence-based science can be traced back to specific locations and individuals that have significantly contributed to historical milestones and have established the methodological norms in embracing an evidence-based approach in the later development of science. Although traditional, "pre-

allopathic" medicine either relied on a single case study or on authorities known from translated texts, some physicians rejected authority and evidence in favor of their individual direct or vicarious experience. Approximately 2500 years ago, a system of care was launched that is not based on a corporeal nature but designed to reinforce the constitution of patients so that the disease defied the substances that exceed the body. Treatment was mostly observational. It was intended to embellish nature and make it as practical as possible. A detailed individual clinical trial was believed to be necessary for understanding the natural story of the outcome of patient care. Indigenous tribal doctors have appreciated the importance of evidence-based methods to evaluate the effectiveness of therapy in individual cases.

Two distinct periods of history also manifested evidence-based approaches. The stagnation of the Roman medical period hindered the advancement of clinical practices, the significance of which evidence produced a decreased demand. It wasn't until the 19th century that evidence-based clinical practices made a resurgence, which aligned with the modern definition of evidence-based research. Involved descriptions of best practices were based on the exposure of large patient populations, efforts to quantify the effectiveness of novel therapies in a clinical setting, and the use of randomized controlled trials in case descriptions. Different descriptors with similar definitions have momentarily existed on the ethereal evidence of a previous era, which manages to shape much of the randomized control and evidence-based research methodology that exists today.

These examples have provided prototypical evidence-based methodologies that were later further developed by collaborators in the late 1950s. Today, there are many experimental articles on anthropological evidence. Since the beginning of the 19th century until the early part of this century, four main factors have led to the rise of the evidence-needing period in biomedicine. One, there were no effective drugs and only remedies were needed. Two relatively few doctors were the results of barbers, surgeons, and diets, not authorized medical schools. Three, the causes of most diseases were still unknown, leading to an elusive understanding of natural processes. Four, no diagnostic tests were available, so all diseases were not subject to physical evidence. An important question now is to what extent the concept of evidence-based practice should be used. The

historian of science suggested, "Remembering that the next time doctors recognize the importance of the evidence, the chance is that the present fashion will change again."

1.5. Applications of Evidence-Based Science in Scientific Inquiry

One of the most important ways that evidence-based science is integrated into our understanding and practice of scientific inquiry is through the collection and application of data within an experiment. In this section, we present ways in which evidence-based practices are useful when dealing with investigators and questions in the laboratory. We introduce specific strategies that enable the incorporation of evidence-based science into research design, methodology, and statistical power, highlighting evidence-based insights that can aid in data collection and statistical analyses. This will allow researchers to change more than just their questioning: the evidence-based science used to pursue the answers to our questions will be altered as well.

First and foremost, the design and methodology of an experiment should aid in answering the questions being posed. Evidence-based guidelines for treatment comparisons can be used to aid in the design, development, and conduct of laboratory-based experiments and support the knowledge base upon which we can base interpretations and conclusions. These decision frameworks help facilitate greater understanding of the questions being asked, as well as the results that can be anticipated. This, in turn, enhances the ability of investigators to deduce whether interventions or treatment groups differ or whether these differences are by chance. Crafting an experiment that results in reliable conclusions with the greatest certainty based upon valid, well-established evidence is favorable, and adhering to evidence-based principles for the conduct of an experiment is crucial.

1.6. Research Design and Methodology

Application of evidence-based principles can substantially improve the quality of scientific inquiry. Evidence-based clinical practice guideline developers acknowledge that powerful evidence comes from one or more reliable studies. Hence, they utilize systematic review as a central determinant in making evidence-based recommendations.

Systematic approaches to the planning and execution of research are emphasized in different research frameworks, such as Thyer's outcome research model, Coutons' contribution-based community research framework, and Guba and Lincoln's naturalistic inquiry. Different research texts have summarized criteria that specify what should be demonstrated to be valid to judge that a study's outcomes are credible and meaningful. These criteria address how the studies are designed in a way that will produce practically significant and scientifically valid results. Criteria for the quality of studies can be seen as a systematic way of classifying methodologies that have been developed to incorporate evidence-based principles and improve research reliability and validity. These criteria for evidence-based designs are built, in essence, to ensure that the studies would provide reliable and valid data.

Despite some differences, well-established sampling techniques can increase the generalizability of research findings. Researchers design research by sampling either individuals from a general population or a specific population for certain symptoms or disorders. Sampling procedures must be meticulously documented in order to demonstrate that they are appropriate to the research question, the research's goals, and what is required for subsequent inductive inference. Control Group: Examination of the independent variable provides the least biased association between the independent and dependent variables. To draw causal inferences, individuals exposed to an independent variable must differ from individuals not exposed, except for that exposure. A good research design systematically indicates the use of a control group, if possible, or at least rules out other possible causes of between-group differences. Other Methodological Elements to Be Accounted For: While they may not always be obvious, a study can be made more or less methodologically sound or valid based on these design and methodology features. Often, what is the "best design" is a matter of judgment and depends on the specific research question being asked. Random Assignment to Treatment Conditions: Impact of uniform reduction in speed limit on urban street accidents of different severity. Historical evidence suggested that higher speed limit environments were associated with a greater proportion of crashes that resulted in serious injury or death. Therefore, evidence from post-implementation accident data needs to be

interpreted cautiously, as lower-severity accidents could be artificially increased by road design that aimed to slow traffic.

Data analysis typically involves asking what the evidence is saying about the association between the independent variable (IV) and the dependent variable (DV). A significant question is whether the IV actually has an effect on the DV. When evaluating analysis results, it is important to maintain objectivity, avoiding prematurely jumping to conclusions about the findings. Use statistical tools and models that conform with evidence-based principles. 'Best practice' reporting of results adopts an evidence-based framework in which information about methods, results, and the data itself is obtained and interpreted through an understanding of the basic concepts of statistical inference.

Research articles that report original results typically provide information on the number of subjects, the mean of the scores, and a measure of statistical variability, usually the standard deviation of the scores. There are many techniques by which people have used evidence to guide how they interpret a set of findings. Making sure to carefully attend to the evidence contained in the data is critical for success in scientific inquiry. Just as bad is not knowing how to extract evidence from the data.

2. Biological Aging: Mechanisms and Implications

2.1. Introduction to Biological Aging

Aging as a biological phenomenon occurs in all living organisms and is universal. This concept came into existence when humans showed interest in longevity. As a result, considerable information regarding the mechanisms that underpin aging is available due to research in the fields of biology, wherein a large number of resources have been spent to understand different processes encompassing aging. Understanding aging is important for health-related purposes. It has been defined as a multifactorial process that results in a progressive, cumulative, intrinsic, deleterious, dynamical, and heterogeneous deteriorating phenomenon of most living organisms accompanied by metabolic disturbances and increased susceptibilities to divergent types of diseases that ultimately lead to morbidity and mortality. The term biological aging or senescence is generally used interchangeably to convey the aging process or phenomenon.

Aging is quite interesting and challenging due to its complex nature. Because of its major consequences on human health and due to the relatively large number of the older population worldwide, aging research has become an emerging science. In the last five decades, aging has been considered an interdisciplinary field, which includes research in all branches of biology. The number of research articles and research laboratories clearly indicates this. Despite the availability of this vast knowledge and research outputs in aging, human aging research has not taken off as expected. The expansion and significant increase of the older population have become a great concern for both developed and developing countries. For universal aging research purposes, Critical Age Theory might be more useful than working on human aging alone. The biological sciences are mechanistic at their core, and biology is the science of dynamical systems. As a result, in the midst of these myriad ways of looking at aging, the focus of aging research has slowly shifted away from aging as a disease to aging as a process. In the following sections, age-related changes at the cellular and molecular levels are explored.

2.2. Cellular and Molecular Changes in Aging

Aging is the progressive decline in the functional capacity of an organism. Several cellular and molecular alterations in a wide variety of species have been documented, with major implications for the possible causes of the aging process. Evidence points towards the critical roles of cellular senescence and apoptosis in aging. At the molecular level, environmental factors and genetic factors converge upon related signaling pathways that are significantly involved in aging. Furthermore, aging organisms tend to accumulate damaged or mutated biomolecules and have diminished repair mechanisms. The accumulation of mutated DNA, or of damaged proteins, lipids, or carbohydrates is toxic and can lead to the progression of many adult diseases such as cancer, cataracts, arthritis, and cardiovascular and cerebrovascular diseases, as well as to aging. By better understanding the mechanisms implicated in the aging process, we might be able to develop therapies to prevent or ameliorate degenerative diseases.

The loss of stem cells is especially damaging in tissues with reduced capacity for regeneration from stem cells and large energy demands, such as the heart, some parts of the brain, kidney, or liver, leading to many age-related diseases when their function is

lost. Aging is also associated with chronic low-grade inflammation expressed by increased levels of proinflammatory cytokines. Increased sensitivity to stress and other adaptive responses, such as the elevation of antioxidants or heat shock proteins, which are protective against further challenges, characterize the aging strategy in the elderly of many species. Neurodegenerative diseases and the susceptibility of the brain are perhaps the most noticeable functional consequences of the aging process. The second section examines the primary biological theories of aging.

2.3. Theories of Biological Aging

The aging process is due to the interplay of several distinct mechanisms. Aging is widely recognized as a multifactorial, complex, and dynamic process. This complex and elusive nature of aging is mirrored by the multitude of theories that have been proposed to explain why organisms age. These theories can essentially be divided into two contrasting and mutually exclusive classes: those that claim that aging occurs as a consequence of biological processes that keep the individual alive, and those that claim that aging is the result of these very processes. A third class of theories, which can be considered an offshoot of the latter class, was developed to harmonize the two extremes.

Theories within the first class generally maintain that the aging process is encoded in our genes. In contrast, theories in the second class tend to focus on the degenerative processes that take place within the bodies of aging organisms, resulting from the second law of thermodynamics, which states that the disorder of an isolated system cannot decrease. These theories postulate that aging results from the accumulation of one or more abnormalities, such as damage to or in somatic cells caused by stresses that challenge their metabolic efficiency and/or genetic fidelity throughout the life history of an organism. Finally, epigenetic theories suggest that aging is an anticipatory and adaptive genomic response to changes that naturally occur during ontogeny. The programmed aging theory posits that the aging process is actively regulated by biological clocks that consist of genes which control aging. One well-known version of this theory is the antagonistic pleiotropy theory, wherein natural selection favors early life benefits even if the genes responsible for this decrease the chances of survival later in life, so long as the benefits of the former are greater than the detriments of the latter. While a plethora of

factual observations support programmed theories, they also face numerous counterfactual observations, such as individuals that live for a prolonged period of time in environments poor in resources, largely independent of their genotype. Damage accumulation theories, on the other hand, do not need to invoke active genic control of aging, instead suggesting that damage accumulates with time and that such degenerative changes in a variety of somatic cells determine age.

There is a consensus in the gerontology field that both of these theories hold kernels of truth. However, a number of observations seem to contradict the possible universal application of the damage accumulation theory. The view of aging as occurring for manifested causes of adaptive degeneration rather than random assault is persuasive, given the energy requirements for DNA, cellular, and body macromolecular repair, and evidence that lowered dietary and some other stressors increase life and health span in a variety of model organisms by reducing the total damage load. It is also advocated that aging is adaptive and occurs in both germ and soma, that aging is localized, and that age-related stimuli from damaged tissue may be partly responsible for general aging. Any one and possibly any imaginable type of molecular damage, from cells and tissues to disease, takes on a minor effect at first age with time due to energy limitations of repair that are related to the sequence of damage. Although the above view represents merely one of the possible suppositions of how the theories of aging may interlace, a comprehensive theory is yet to be developed.

2.4. Impact of Aging on Health and Longevity

Complete ablation of the aging process would have profound physiological, psychosocial, and ethical consequences and abolish all the genetic and environmental factors that modulate the rate at which we age. Altering aging would also have a beneficial downward impact on a variety of disorders, particularly multifactorial age-associated diseases involving lost or degenerating cells or intracellular components, by increasing the average proportion of the life in which one is both disease- and disability-free. The inability of human populations to maintain their health—both physiological and psychological—combined with the decline in the reparatory capacity of the body over time increases the susceptibility to and the impact of environmental insults which, in turn,

hasten the aging process and deepen the downward spiral of physical and mental impairments associated with growing older. In the disadvantaged and frail elderly, the close interplay with chronic conditions produces a vicious cycle which leads to premature death, while in those at relatively low risk of multimorbidity the association of accumulating age-related alterations with chronic conditions contributes to the increased risk of mortality and the progressive decline in physical and mental function. People can expect to live for a long time; in 1955, life expectancy was 69 years, by 2015 it was 81, and the same trend can be seen in most countries where complete individual vital statistics are available. While not everyone reaches old age due to early mortality, at all ages, the estimated gains in life expectancy are typically better for women than for men and, overall, for populations at higher levels of income. However, advances in aging and lifespan are not universal. Variation in maximum attainable longevity between genetically identical organisms demonstrates that the environment cannot account for all differences in longevity of real organisms. Furthermore, the wide range in the age at death between different mammalian and bird species cannot be explained only by differences in living conditions. Voluntary lifestyle choices have a significant impact on aging; for example, heavy tobacco smoking and alcohol consumption, poor diet, physical inactivity, and obesity are all known to accelerate morbidity and mortality, especially in the frail and elderly.

2.5. Strategies for Anti-Aging and Longevity

Many of the strategies for anti-aging and longevity are based on improved health throughout life. Regular exercise and a diet rich in natural antioxidants can help preserve the body's systems, reducing the risk of developing chronic diseases and increasing both healthspan and lifespan. In recent years, a state of peace of mind has also been linked to an increase in telomeres and the preservation of the immune system, which in turn has improved human health parameters. Focused and evidence-based practices such as food philosophy, exercise philosophy, stress management, art therapy, and relationships to enhance and integrate both mental and physical components have become the latest trends to promote the extension of lifespan and anti-aging. Young individuals also look for foods, medications, and supplements to promote longevity and anti-aging. Increasing

evidence shows that both calorie restriction and dietary interventions based on human needs have the potential to extend maximum lifespan and independence from chronic diseases. To improve human health during aging, several drugs and supplements that act on biochemical pathways are currently being investigated in animals and are candidates for clinical trials.

Reprogramming aged cells or regenerating damaged or lost cells is also a method for extending lifespan. In recent years, the approval of the first gene therapy for immunodeficiency has opened avenues for future genetically modifiable disease vaccines that will further extend lifespan. The development of brain-machine interfaces and neuromodulation also holds potential for improving cognitive function and extending human life. Through the development of artificial intelligence and virtual reality, bio-psycho-subject models are created to explore the potential of anti-aging and cognitive impairment in the brain and the equilibrium of key life channels. In the near future, emerging exosome technology may also play a role in understanding inter-cell communication in the brain and in exchanging information between cells in the body, potentially preventing cognitive decline.

Currently, potential areas of basic neuromodulation are being researched to extend lifespan, mental health, and memory health. It is possible that by the next decade or so, low-power electromagnetic neuromodulation could be incorporated as a cognitive enhancement and life-extending neuromodulation within the lifespan strategy, along with nutrition, senolytic drugs, hormone replacement, fecal transplant, vaginal therapy, exercise, and relaxation practices. Ethical, philosophical, scientific, business, and medical hygiene issues are intertwined in neuromodulation that goes beyond social responsibility and human rights. Anti-aging and lifespan strategies should focus on not only physical and mental health but also cognitive power, memory health, and emotional health.

Many preceding research associated aging with our living environment, including food. Some recent work observes aging from the perspective of genes, and scientists have found that chronic oxidative stress can mediate age-related harmful reactions. In fact, aging is a natural phenomenon that also occurs in rats. Moreover, the body may not be able to defend itself in older age. Therefore, old age is considered to be a state of

inability. Lifespan and health span are both extended by the balance of external and internal factors of the body. In the human body, there are three metabolic pathways: the immune system, inflammation, and oxidative stress. Infections are partly the result of increased inflammatory and oxidative stress pathways. Caring for your home and your body can help us to lead a longer life. According to some reports, the habit of thinking too much about a trouble triggers the production of cortisol, which contributes to inflammation and harms the body. In sum, it is clear that a balanced mix of exercise, nutrition, relaxation, and other techniques will provide anti-aging and longevity.

3. The Potential of Metformin: Beyond Diabetes Management

3.1. Introduction to Metformin

Metformin, a biguanide first isolated from the French lilac plant, has come a long way since its use, over 1200 years ago, as a traditional remedy for frequent urination. Its approval as a pharmaceutical over 50 years ago marked it as the first line of treatment for insulin-sensitive diabetes, which remains the fifth leading cause of death worldwide. Poor management of this so-called developed-world malady leads, within two decades of diagnosis, to a twofold higher risk of depression, anxiety, and dementia, five times the prevalence of hypertension and cardiovascular disease, and a whopping eightfold spike in the prevalence of neuropathy and chronic kidney disease. Despite its wide clinical appeal, topical interest in Metformin has surged in the past decade, spurring calls for an exposé on the breadth of its biological effects. Thus, the time is ripe for a comprehensive review of Metformin's manifold actions, in order to avoid fruitless off-target drug development and 'chase a drug in search of a disease,' as well as to understand, at the smallest logical level, the points of intervention in the network of pathways affected by Metformin.

At an industrial level, the hype over off-target actions has not gone unnoticed, with companies targeting Metformin lookalike drugs having attracted funding of up to 1 billion. Thus, it feels particularly poignant to go back-to-back with a pop review which reiterates the importance of understanding the breadth of action of drugs as a means of predicting adverse drug reactions and drug repurposing. By providing a comprehensive

and unbiased overview of Metformin, we hope that this primer makes further investigation of Metformin's molecular mechanism of action as a whole, and those responsible for its hypoglycemic and anticancer effects in particular, a priority for research in diabetes and cancer fields.

3.2. Historical Development and Traditional Use

The first descriptions of the medicinal use of the plant Galega officinalis are found in the work of ancient Greek physicians. It was included in many herbals of the time, used as a diuretic and to stimulate lactation. There are records from the fourteenth and fifteenth centuries of its use in Europe for these conditions, both in humans and animals. It was also recognized as a source of toxicity for grazing animals, particularly when it made up a significant part of their diet. This recognition of active constituents in the plant was one of the early steps in the development of understanding of poisons, and the methods and concepts developed played a significant role in the 20th century in the establishment of the modern pharmaceutical industry. It also had an important place in the development of ideas about what causes diabetes.

The active compounds of the plant were isolated at the end of the 19th century, and called guanidine and galegine. In the sticks of the plant taken during the Second World War in Canada, insulin was found to be the active component. This collection led to studies in which two major extensions in understanding occurred. The first was the observation that it reduced the blood sugar of diabetics, especially when other medications were given at the same time. The second was that it did not cause hypoglycemia, and hence was not a fully insulinotropic drug. This indicated that it worked in part by increasing insulin sensitivity, and so led to the discovery of the mechanism of action of the biguanides. The discovery led to the launch of metformin in 1957 as a treatment option for diabetes in Europe. Metformin was used widely in some European countries and was picked up by Australian endocrinologists. It took longer to be accepted in British diabetes practice, partly because it was seen as too closely related to another compound, which did cause hypoglycemia and was withdrawn for this reason. Although some physicians used it because of its apparent lack of hypoglycemic effect and its weight-reducing ability, it did not achieve wide acceptance as a treatment option until new facts about the pathological

significance of insulin resistance changed clinical practice in the 1990s. The possibility that metformin also inhibits cancer in both experimental animals and humans has recently been suggested. The historical importance of this drug in enabling an understanding of the basic abnormality underlying Type 2 diabetes is rarely noted, yet it was this conceptual advance that marked the point at which clinical and laboratory research complemented one another, resulting in the eventual widespread acceptance of the concept of insulin resistance. It suggests the importance of listening to traditional knowledge as an essential partner to emerging potential benefits identified through contemporary research.

3.3. Mechanism of Action

To understand Metformin's broad potential, one must first look closer at the very complex processes at the helm of our metabolism. The pathophysiology of metabolic disorders lies within the way the body clears blood glucose levels. Following dietary intake, insulin is secreted by the pancreas and inhibits hepatic glucose production, while promoting glucose clearance by peripheral tissues like muscle and fat. Patients with diabetes develop resistance to insulin and have inadequate insulin production. Metformin addresses the resistance issue and enhances insulin sensitivity. The main sites of Metformin action in muscles and fat are not yet fully understood, as therapeutic concentrations are required to be oxidized. What is understood quite well is how Metformin alters hepatic glucose production through a mitochondrial mechanism.

Metformin has been shown to inhibit hepatic glucose production on a molecular level, likely due to its inhibitory effect on Complex I of the mitochondrial respiratory chain. This leads to alterations in the AMP: ATP ratio within the hepatocyte and allows for the activation of adenosine monophosphate-activated protein kinase. This hormonal effect subsequently activates the downstream factors involved in glucose and lipid metabolism, accompanied by a package of biophysical effects and activation of alternative signaling pathways involved, outside the scope of those routinely derived from AMPK. Although these biophysical actions of Metformin serve its place in therapy, they seem to be especially relevant in non-diabetic or so-called normal metabolic circumstances.

3.4. Improving Insulin Sensitivity

When tissues resist insulin, the maintenance of normal blood glucose levels is challenged. Therefore, insulin resistance increases the requirement for insulin production to facilitate glucose uptake and utilization. The improvement of insulin sensitivity has been a primary goal in diabetes management. When sensitivity to the action of insulin increases, glucose uptake increases as well, resulting in lower blood glucose levels. Various studies have reported that Metformin significantly enhanced insulin sensitivity and lowered fasting insulin levels in subjects with obesity. This effect can be noteworthy in patients with severe hyperglycemia, decreasing insulin concentrations up to 28 weeks.

Insulin responses to oral glucose loads are also improved by Metformin treatment in patients with obesity. Insulin sensitivity in human tissues is regulated by the function of transporters that facilitate glucose transfer into the cells. Glucose transporter type 4 (GLUT4) occurs in muscle and fat cells and plays a fundamental role in transporting glucose into these cells via activation through binding to insulin receptors. The most important example of improved insulin sensitivity by Metformin treatment is its effect on the increased expression of GLUT4 and activation of insulin receptors. It is widely accepted that strategies decreasing insulin resistance might have preventive effects not only for diabetes but also for other more frequent disorders such as metabolic syndrome. Metformin is an antihyperglycemic drug of effective choice, with beneficial systemic effects. Thus far, Metformin has several multifaceted effects apart from the enhancement of glycemic control.

3.5. Reducing Glucose Production in the Liver

Hepatic Effects When metformin is administered, it preferentially inhibits glucose production by the liver. It does so by reducing the synthesis of liver enzymes through the hepatic protein kinase. This protein that gets phosphorylated is involved in the regulation of genes that encode for enzymes involved with glucose production and fatty acid formation. This multifactorial approach also involves inhibiting energy formation, leading to ATP depletion, which will finally lead to AMPK activation. Metformin is seen

to decrease most of the gluconeogenic enzymes, including the first step conversion of pyruvate to oxaloacetate in the mitochondria. The mitochondria are the energy factory of the cell, and we know that part of the patient's problems is the lack of energy. The diabetic condition is like trying to fill a leaking tank. Everything that comes in would be immediately used up, and there would be nothing left to store. By decreasing the production of glucose by the liver, metformin could make the liver more sensitive to insulin.

3.6. Biological Significance

As mentioned earlier, the liver plays a very important role in the body in regulating the concentration of glucose in the blood. This is achieved by producing glucose and also storing it. The stored glucose is called glycogen and is stored in the liver, but it is the main source of glucose in the blood when you are in between meals. By inhibiting the conversion of pyruvate to oxaloacetate, metformin only interferes with the excess production of glucose by the liver, which has no direct impact for a non-diabetic fasting person whose sugar level is within normal range. This could help to reduce the high blood sugar level in a diabetic person who has a hyperglycemia problem. Both liver production and retention of glucose could cause the high blood serum level, and as a result, the reabsorption of glucose. It has also become obvious that metformin tends to improve liver function of the patient. A clinical study was conducted to examine the effect of metformin in diabetic patients with nonalcoholic fatty liver disease. At the end of the 12-month period of metformin treatment, a remarkable reduction in body mass index, serum transaminase, and FPG levels were observed in the treated group. Furthermore, histopathologic benefits, including hepatic steatosis, inflammation, and fibrosis, were also observed in the treated group when compared with the placebo group. This showed the role of metformin in reducing the body burden of asymptomatic diabetic patients with fatty liver disease, among other advantages.

3.7. Metformin’s Role in Aging

Despite being over a century old, metformin—a drug originally derived from the botanical plant—has been experiencing something of a renaissance. Current global and

national efforts to mitigate the burden of age-related disease, decrease medical comorbidities, and improve human health span have encouraged researchers to re-evaluate standard treatments and delve deeper into interventions that could be repurposed to avoid pathologies associated with age-related conditions. The intersection between health span (i.e., the period for which an individual is optimally healthy and free from disease) and longevity (i.e., the maximum length of time that a person or animal can live) is a paradigm shift for those eschewing a disease-based model for an aging-based one. As such, this model requires new targets to change, for instance, the development of novel interventions, new approaches to nutritional practices, and/or combination therapies, including existing pharmaceuticals repurposed to prevent aging and its concomitant decline.

Epidemiologic studies have shown that individuals with maintenance of metabolic health into later years have a significant impact on the prediction of remaining lifespan, further highlighting the interconnections between metabolic health and aging processes. Metformin targets many of these age-related biological alterations and dysregulated pathways implicated in the aging process. A wealth of preclinical evidence stemming largely from hypothesis-driven model organisms supports the anti-aging properties of metformin. Metformin's effect on longevity has been widely studied in preclinical models, including invertebrates like worms, flies, and mice. More recent and faith-altering evidence from human-based studies is steering the reframing of aging, including findings that diabetics prescribed metformin lived longer than those without diabetes and that small adjuvant metformin doses improved cellular oxygen consumption rates in isolated mitochondria, whereas increasing inflammation and reducing antioxidant protection, in addition to decreasing glutathione levels in adipose tissue and plasma, the main storage and circulation site for this peptide. Metformin may exalt the storage-disposal-immune pathway that characterizes health and fitness in both women and men. Indeed, current research support and investigations of the drug's role in extending lifespan are significantly advancing

3.8. Longevity and Anti-Aging Potential

A broad selection of studies proposes that Metformin users may be less prone to age-associated diseases compared to non-users. It is suggested that Metformin seemingly attenuates both the hallmarks of aging and the biology of age-related disease. As a direct result, older people have an improved lifespan as well as a reduced risk of the pathology that will eventually kill them. Among women, a meta-analysis of age-related survival concluded that diabetes increased the risk of all-cause mortality equally by 80% and all-cause mortality by 40%. However, diabetes treatment with Metformin reduced mortality rates to those of women without diabetes. Metformin has been shown to decrease the prevalence of death due to aging in a number of similar research studies. Because they continue to polymedicate from a young age, new research reports that older adults with diabetes on Metformin had a lower risk of subsequent cardiovascular-related hospitalization or death than similarly aged people without diabetes. Metformin may be described as prolonging the onset of age-related decline and serving a protective function as we see a decrease in the steady-state mortality rate due to various age-related diseases.

Metformin is a biguanide anti-diabetic agent that is used to improve insulin sensitivity and decrease hepatic glucose output by increasing lactate and Metformin conversion. Metformin can apparently act on the cellular and molecular pathways via a method analogous to its starting mechanism. Metformin is thought to execute an OXPHOS process, as indicated by decreased ATP production and mitochondrial respiration. In cells, the shift away from glycolysis to OXPHOS is mediated by the lack of ATP, which in turn inhibits cyclic AMP and protein kinase activity. The findings of the study are the first lines of evidence linking Metformin's previously reported molecular and cellular bioenergetic use in both cellular and mitochondrial pathways of aging. This effect was most profound in the liver and muscle tissue, underscoring the therapeutic potential of applying Metformin at the tissue-specific level for age-related protective benefit. The genes under the influence appear to be linked to the hallmarks of aging previously identified. Studies using model organisms have shown that Metformin increases the lifespan of healthy non-diabetic subjects. Among its pleiotropic effects are a decrease in inflammation and oxidative stress. These patients enrolled in the Diabetes Prevention Program indicated that overweight pre-diabetic patients reduced their incidence of later diabetes for twenty years. Use of Metformin in the continuation of the diabetes

prevention program produced a long-term decrease in diabetes Medicare requirements. Among its pleiotropic effects are a reduction in inflammation and oxidative stress. It is proposed that Metformin's most likely mechanism of reducing mortality risks is by direct genetic and biochemical shifts that influence the rate of metabolic processes of aging at the cellular and molecular stage.

3.9. Pathways for Longevity Enhancement

Cellular damage connected with age is the result of accumulating alterations, not all of which are degenerative. In response to these damage signals, the body can initiate mechanisms that, when chronically activated, help protect from future stressors and can extend the lifespan of a number of organisms. The link between stress response pathways and longevity is seen in many model organisms. Some key pathways include mTOR and sirtuins, which are involved in energy metabolism. Their activation usually has a positive effect on health, as part of discrete stimuli to help prepare for future stress. Activation through periods of fasting has a role in diabetes and even in neuroprotection.

The adenosine monophosphate-activated protein kinase (AMPK) acts as a fuel sensor: it switches on catabolic pathways in energy stress and off anabolic/gluconeogenic pathways, inhibiting glucose output from the liver, lipogenesis, and stimulating lipolysis. The effect of AMPK connections to mTOR and sirtuins may directly link bioenergetic changes that extend lifespan, as seen with calorie restriction through ATP/AMP ratios, dependent on AMPK. The beneficial effect of exercise (but with a high-dose stimulus, detrimental moderate chronic exercise) is generally seen with diseases, especially in aging. Increased AMPK glycogen, increased NAD+, and sirtuin deacetylation then SIRT1 PGC1 can increase mitochondrial capacity. Sirtuins are multifaceted, acting to increase warmth and longevity. With SIRT1 activation, DNA repair gene expression is upregulated, and NF-kB is downregulated, helping to increase health span: SIRT1 is contained in the cytoplasm and in the endoplasmic reticulum as much as in the nucleus.

3.10. Current Research and Clinical Trials

Several clinical trials and observational research studies are progressing to investigate the therapeutic potential of metformin beyond diabetes. However, apart from these, a few

recent studies are using this well-established, safe, and effective medicine to find its efficacy in various health-related conditions. Metformin is widely tested for weight loss, various cancers, management of various types of cardiovascular diseases either as primary prevention or secondary prevention, and polycystic ovary syndrome. The majority of metformin's trials and research are following a conventional methodology such as interesting RCTs, and observational studies are ongoing. Although each trial has different study designs and objectives, all are focused on the development aspects of metformin, providing a new door to focus on metformin to manage other conditions. These trials will validate the metabolic robustness of metformin based on the collected data on different population genetics and geographical locations, which are a few challenges in these trials because of long study duration and patient compliance in the ongoing studies.

It has records of 57 completed trials on various conditions and a discussion about the potential development of metformin that may have brought new hope into the medical future, attaining almost 10,000 citations. This discussion allows us to go back in time and study the path that has been traveled. More clinical research and observational studies are ongoing to investigate the therapeutic aspect of metformin, used for various conditions instead of diabetes management, which may outline its potential role, as metformin is the first drug of choice and widely practiced medication for the treatment of diabetes mellitus type 2 for decades.

3.11. Ongoing Studies and Trials

A suite of ongoing studies and clinical trials is looking at the use of metformin for preventing age-related chronic diseases and other dosing regimens in different target populations. While most of these trials address one of metformin's common comorbidities, there is also growing interest in its effect on a range of geriatric syndromes, which we know increase with age and are often comorbid with such conditions as physical frailty, cognitive dysfunction, incontinence, and polypharmacy. The chronic dosing trial has a plethora of clinical outcomes to address many aspects of health and aging, including sarcopenia, frailty, quality of life, morbidity, ADLs, safety, mental health, body composition, metabolism, and biomarkers of aging.

The importance of a pharmaceutical approach to weight management has been highlighted recently, particularly in populations vulnerable to obesity and already experiencing weight-related comorbidities, where lifestyle interventions may offer suboptimal improvement in cardiometabolic health. With an efficacy-oriented approach to managing a condition as pernicious as obesity, it is vital to think long-term and consider both overall morbidity and healthy life extension. The safety and reversibility of metformin mean it is a promising candidate to begin to address age-related metabolic dysfunction, and we have therefore designed a clinical trial to look at metformin in the same context as a targeted intervention trialed for quality-of-life improvements in a pre-frail non-diabetic population. Metformin is also currently being researched as an adjuvant therapy in a growing number of cancer clinical trials across the globe, in relation to cancer prognosis, and as part of cancer prevention strategies. The number of registered studies provides a snapshot of current global research trends.

4. Challenges in Longevity Research

The field of longevity research faces several significant challenges:

4.1. Longevity and Anti-Aging Research's

Longevity research, or the understanding of why and how long people live, has been actively pursued for almost a century. The first large-scale survey in longevity research was conducted in 1926 in California and focused on the longest-living people. This was followed by research demonstrating genetic influences on longevity in the mid- and late-20th century—specifically in the relationships between close relatives' lifespans. However, with a longer-term approach, a consensus has been established and has recently been extended to include social, cultural, and environmental factors. Systematic studies of livestock started in the late 1950s and have converged with human studies in the early 21st century. The understanding of longevity is an ongoing field of research and a rapidly progressing one, partly due to the multidisciplinary nature of the field. Several research groups are involved in the field of genetics—as well as those of genetic and non-genetic components of aging and age-related diseases.

The field of longevity research asks: where should we start in understanding the biology of aging and the differences of aging in model systems, and what should be measured? From there, several scientific questions follow: are the longest-living organisms similar to normal aging organisms, and should we study the intersubject variability in the aging process? Should we also focus on 'time to and cause of death,' or should we focus on delayed aging and, ultimately, on the improvement in health? Do aging mechanisms have a limited lifespan and, therefore, might the effects on aging become exhausted, as suggested by longitudinal duration studies? How and at what age do we measure aging biomarkers, and what biomarkers should we study? Based on the answers, several intervention studies have been developed, and some randomized intervention studies integrated with observational studies were initiated. The main rationales to elucidate the physiology of aging are based on changes in lifestyle and increased health span. In vitro studies of aging have included characterization of the aging process in several cell types of different species, the search for immortal cell substrates among cancer cells, and comparative studies of senescence in human and non-human species.

4.2. Long Study Durations and Observing Effects

There is a huge challenge in longevity research when it comes to study durations. To see "life extension" effects in longevity studies, one might argue we need a study duration to observe statistical significance in lifespan from treatments that are relevant to the maximum lifespan of the species we are attempting to extend. There are many factors that complicate this argument, but it does represent the conceptual challenge of drug development for indications that span generations, as in humans. Of course, there are also other complexities in using maximum lifespan as an endpoint in humans, including generating a dataset of centenarians we could use as matched controls. For these and other reasons, researchers studying longevity tend to focus on "healthspan," or simply time-to-illness/incidence of adverse health outcomes. Given all these factors, an incredibly long duration is needed to see potential anti-aging effects, so the challenge is great. Even if we utilize animal models with accelerated rates of aging, a long duration of several years is necessary to follow up treated subjects until death. Finally, since we know disappointingly long durations are required to observe the relevant outcome,

participants in the study will have to be engaged for many years. Maintaining the interest and compliance of participants over so many years is another major challenge.

There are also biases introduced by studying a population that wants to live longer and healthier lives. Observing the effects of a drug early on and then inferring changes in future lifespan would require mechanisms that we have not characterized or validated and would fall outside of observing causality. Techniques to track health outcomes over such long periods do exist and are currently used in the pharmaceutical industry in the form of pharmacovigilance or observational studies of patients who have taken a drug to market. The observational data obtained in these timeframes can be informative but cannot be used to conclude drug effects or lifespan. Adaptive trial designs could allow researchers to improve how to track "longer-term" health span quickly. However, a long duration of time would still be required to demonstrate objective changes in aging and observe a lifespan effect.

4.3. Complex Interactions Between Aging Pathways

Aging is a result of a complex process involving the interactions of multiple pathways, including genetic, environmental, and lifestyle factors, and ultimately leads to an increase in susceptibility to aging-associated diseases. A proper understanding of the relationships and interactions between these pathways is important if we are to cure or intervene in aging-associated diseases. The detailed interactions between these pathways and molecular mechanisms that regulate aging can be easily elucidated using systems biology approaches. Models such as aging gene networks and aging pathway diagrams or age-associated biological networks can provide a holistic view of the regulatory mechanisms involved in the cell or organ tissue type, and molecular interactions that occur with aging. The frameworks of aging pathways are built on the molecular and cellular interactions that associate with aging.

Systems biology also catalogs proteins that interact either physically or functionally. Protein-protein interaction networks that name the combination of the entire group of protein-protein interactions within an organism may offer another source of aging-associated proteins that could be targeted for lifespan interventions. Thus, various

overrepresented or age-dependent protein-protein or protein-metabolic regulations in the aging process could be used as potential therapeutic targets for promoting lifespan. Despite this, one of the current challenges is a two-fold one.

Mapping the cellular dysfunction and longevity-insurance functions for humans from such topologically oriented networks can sometimes be a difficult task, and the choice of what to target is often based on the unverified hypothesis that a network with biologically significant nodes and edges is somehow mechanistically associated, even though this jump cannot be made. Whether we can translate what goes on at the molecular level into what happens to phenotype is one big problem. Simultaneously, we want to see longevity assessment studies as an example of target isolation and identification and hit validation in model organisms; however, only a fraction of this research will scale up into studies in mammalian models and people. Although animal models act as valuable guides, we expect better insight into human longevity research before moving forward to high-quality samples of individuals, whether subjects or not of disease.

4.4. Translation of Findings from Animal Models to Humans

The most critical challenge in longevity research lies in translating findings from short-lived invertebrates and rodent models to significantly longer-lived human populations. The most common organisms used in aging studies include the mouse and the nematode, each with its own species-specific intervention and feature. However, its typical lifespan makes mice difficult to study long-term, and consequently, most mouse-based research focuses on pathological aging. Similarly, the lifespan of C. elegans may correspond to only a very short life phase in humans. Despite this, several interventions that substantially increase the maximum lifespan of animals have been shown to lessen the long-term effects of aging in humans.

While in some cases a similar biology of aging can be inferred from a strong conservation of aging mechanisms, this is not sufficient to establish a direct translation of animal research to humans. Obstacles to understanding human aging include the significant differences in basic biology, as well as the major differences in the aging process between short-lived model organisms and humans. Regarding genetic variability

and variations in environmental and lifestyle factors, natural populations of model organisms can provide important insights into the impact of genetics and the environment/lifestyle.

However, such heterogeneity plays a complicated role in detailing the underlying factors influencing aging and aging-related diseases. Biomarkers are important tools for this investigation when dealing with causative relationships. In principle, human trajectories that better represent model organisms using this approach can be used to narrow the biological difference. In order to reach a sounder translation of animal research to applications in humans, the concept of a humanized animal model is emerging. Humanized animal models are important, but translational aspects of animal studies should be investigated with caution and take human ethics into account with careful consideration, if it involves the creation of genetically modified animals. Humanized animals translate to the degree to which aging mechanisms are similar between animal and human models but are not human individuals and should not be treated in practice.

4.5. Regulatory and Ethical Considerations

This has led to the emergence of a series of emerging biotechnologies—from gene editing in the human germline to developing anti-aging interventions on a population-wide level—with their own unique set of regulatory considerations and oversight needs. This shift has the potential to affect how research is conducted, from participant consent to study design and implementation. There are potential ethical concerns that might arise as a result of using any of these biotechnologies, and review by a Research Ethics Committee will be required where investigators are applying them in a study involving human subjects. Finally, an appropriate public discourse about biotechnologies and possible societal concerns related to their applications is necessary. Ethical concerns arise in relation to how such technologies should be constrained and when this amounts to unjustifiable repression of technological innovation. A research agenda into these areas should be developed in collaboration with researchers, research ethics boards, and relevant authorities, as the exercise of biotechnological innovation is not value-neutral. This section provides an overview of the regulatory framework surrounding longevity research in humans before outlining some examples of ethical issues that might need to

be considered, such as changes in our understanding of health and disease and the social and economic effects of increasing average lifespan. Moreover, we propose the inclusion of a section detailing who might be involved in discussing the issues related to international best practices in research governance and regulation for longevity research in humans. In summary, the aim of this review is to establish a respectful, open-minded, and inclusive collaboration between scientists, bioethicists, and regulatory authorities to ensure that innovation is not hampered by unfounded concerns.

5. Project Vision and Objectives

Our team consists of three Associate Professors with specialized expertise in longevity research and clinical medicine. With years of combined experience in academic and clinical settings, we bring a multidisciplinary approach to studying the complexities of aging and healthspan extension. Collectively, we have authored and co-authored over 100 peer-reviewed publications, contributing to advancements across various medical disciplines, including endocrinology, gerontology, and translational medicine.

Our mission is to drive innovation in longevity science through rigorous, evidence-based research. By focusing on metformin's potential as a therapeutic agent, we aim to unravel its mechanisms of action in aging processes, including its effects on mitochondrial function, oxidative stress, and systemic inflammation. Through our work, we strive to bridge the gap between laboratory findings and real-world clinical applications, ultimately contributing to the development of effective strategies for promoting healthier, longer lives.

6. Experimental Studies: Design and Methodology

6.1. Effects of Metformin on Aging-Induced Mitochondrial Dysfunction and Longevity: An Animal Model Study

Introduction

Ageing is a complex biological process characterized by a gradual decline in cellular and physiological functions (57-59). This process is associated with increased susceptibility to disease and increased risk of mortality. Among the mechanisms underlying ageing, mitochondrial dysfunction, which plays a critical role in energy production and redox balance, is a major

contributor. Mitochondrial dysfunction is recognized as one of the major determinants of ageing and results in impaired cellular energy metabolism, increased reactive oxygen species (ROS) and an inability to clear damaged organelles. These mechanisms play an important role in the pathogenesis of age-related diseases (60). Metformin, in addition to being a widely used anti-diabetic drug, is attracting attention for its anti-aging effects due to its properties such as increasing mitochondrial efficiency via AMP-activated protein kinase (AMPK) and reducing oxidative stress (61).

There is increasing evidence that metformin extends health and lifespan in animal models through effects that mimic caloric restriction (62,63). However, how metformin regulates mitochondrial function, how this regulation affects age-related biological processes such as oxidative stress and inflammation, and its direct effects on longevity are not fully understood. In this study, we test the hypothesis that metformin improves energy metabolism by reducing age-related mitochondrial dysfunction, reduces reactive oxygen species (ROS) and lipid peroxidation, and thus promotes longevity by both protecting cellular functions and preventing age-related damage. To evaluate this hypothesis, an experimental study was designed using a mouse model to analyses in detail the effects of metformin on mitochondrial biogenesis, energy production and oxidative stress.

Materials and Methods

Study design and ethical approval

This experimental study was designed according to ARRIVE guidelines and approved by Istanbul Medipol University Animal Experiments Ethics Committee. All experimental procedures were performed in accordance with national and international guidelines for the care and use of laboratory animals.

Animal Model

- **Species and Breed**: C57BL/6 mice are a well-characterized model for the study of ageing and longevity.
- **Age Groups**:
    - **Young group**: 3-month-old mice at the beginning of the study.
    - **Old group**: 18-month-old mice at the beginning of the study.
- **Sample Size**: A total of 60 mice, equally divided for each experimental group (n=10).
- **Housing Conditions**: 12 h light/12 h dark cycle, 22 ± 2°C temperature, standard diet and ad libitum access to water.

Experimental Groups

1. **Control (Young)**: Standard diet, no intervention.
2. **Control (Elderly)**: Standard diet, no intervention.
3. **Metformin (Elderly)**: Standard diet plus 0.1% (w/w) metformin supplementation for 6 months.

4. **High Dose Metformin (Elderly)**: Standard diet with 1% (w/w) metformin supplementation for 6 months.

Intervention

- **Metformin Administration**: Based on the literature (7), metformin was administered by mixing with standard diet to reach the desired concentration.
- **Duration**: Continuous dietary supplementation was administered for a total of 6 months.

Outcome Measures

1. **Primary Outcome**:

Survival: To be assessed by Kaplan-Meier survival analysis.

1. **Secondary Outcomes**:

Mitochondrial Function

ATP Production: ATP levels will be quantitatively measured using luciferase-based ATP measurement kits to assess cellular energy metabolism.

Mitochondrial Membrane Potential: JC-1 fluorescent dye will be used to assess mitochondrial membrane integrity and energy production capacity.

Oxidative Stress

Reactive Oxygen Species (ROS): Cellular oxidative stress levels will be measured using fluorescence microscopy with dihydroethidium (DHE) dye.

Lipid Peroxidation: Lipid peroxidation levels will be evaluated by measuring malondialdehyde (MDA) levels. High performance liquid chromatography (HPLC) method will be used to provide high sensitivity and specificity in this measurement.

Gene Expression

PGC-1α and Nrf2: will be analyses by qPCR for mitochondrial biogenesis and antioxidant response. Expression levels of mitochondrial biogenesis (PGC-1α) and antioxidant response (Nrf2) markers will be analyses by quantitative polymerase chain reaction (qPCR).

Inflammation

Serum IL-6 and TNF-α levels: IL-6 and TNF-α levels, which are markers of systemic inflammation, will be measured using enzyme-linked immunosorbent assay (ELISA) method.

Physical Health Indicators

Body Weight: In order to evaluate the effect of metformin on body weight, the weights of all mice will be measured monthly with a precision balance.

Exercise Capacity: A rotarod test will be performed to assess muscle strength and coordination capacity. The residence time of the mice on the rotarod will be measured and the effects of metformin on physical performance will be analyses based on these data.

Sample Collection and Analysis

At the end of the study, liver, skeletal muscle and brain tissues will be removed from mice for biochemical and histological analyses. Immediately after surgical removal, the tissues will be washed with cold physiological saline solution and frozen with liquid nitrogen and stored at -80°C for biochemical analyses. For the evaluation of mitochondrial ultrastructure, transmission electron microscopy (TEM) will be used as part of histological analysis. Mitochondrial membrane integrity, cristae structure and general mitochondrial morphology will be evaluated in TEM analyses. For TEM analyses, tissues will be fixed with 2.5% glutaraldehyde and 1% osmium tetroxide, followed by dehydration with ethanol and propylene oxide, respectively. After dehydration, tissues will be embedded with epoxy resin, ultrathin sections will be taken and stained with uranyl acetate and lead citrate.

Statistical Analysis

Statistical analysis of the study was performed using different methods to assess the primary and secondary outcome measures. In survival analysis, the Kaplan-Meier method was applied to assess differences in survival between groups and statistical significance was tested by the log-rank test. One-way analysis of variance (ANOVA) was used to compare parameters such as mitochondrial function, oxidative stress markers (ATP production, reactive oxygen species, lipid peroxidation) and inflammatory cytokine levels (IL-6, TNF-α) between groups. In cases where significant differences were found, Bonferroni correction was applied to determine which groups were different. Pearson correlation analysis was performed to examine the relationship between mitochondrial markers (e.g., PGC-1α and Nrf2 expression) and survival.

All statistical analyses were performed in accordance with appropriate assumptions depending on whether the data were normally distributed. In cases where the data were not normally distributed, nonparametric tests (e.g. Kruskal-Wallis test) were applied to confirm the results. In statistical analyses, $p<0.05$ was accepted as significance criterion and all analyses were performed using SPSS (IBM SPSS Statistics for Windows, Version 25.0) software.

Expected Impact

This study aims to elucidate the mechanisms by which metformin extends lifespan by reducing age-related mitochondrial dysfunction and oxidative stress. The results of this study will not only contribute to the development of strategies for the treatment of age-related diseases but will also provide a new perspective for translational research into health span extension. This study may be an important step in understanding the fundamental mechanisms of ageing biology through the regulation of mitochondrial function and may provide a scientific basis for the development of anti-aging therapeutic approaches.

6.2. Effects of Metformin on Lung Cancer Cell Proliferation and Apoptosis: An Animal Study in the Lewis Lung Cancer Model

Introduction

Lung cancer is one of the most common and lethal types of malignancy in terms of both incidence and mortality rates worldwide (64). Non-small cell lung cancer (NSCLC) accounts for approximately 85% of all lung cancer cases and is particularly notable for its potential for treatment resistance and metastasis. Current treatment options generally have limited efficacy and the need for new therapeutic approaches continues (65-67). Energy metabolism and apoptotic responses of cancer cells play critical roles in the progression of this disease and these processes are considered as therapeutic targets (68).

Although metformin is a biguanide derivative commonly used in the treatment of type 2 diabetes, it has recently attracted attention for its potential effects in cancer therapy. Metformin regulates cellular energy metabolism by activating the AMP-activated protein kinase (AMPK) pathway and reduces cellular proliferation by suppressing mTOR signaling. In addition, metformin has been shown to reduce oxidative stress, support apoptotic processes and contribute to immunological regulation in the tumor microenvironment (69,70).

In this study, the effects of metformin on lung cancer cell proliferation and its potential to activate apoptotic mechanisms were investigated using a Lewis lung cancer animal model. The study aims to evaluate both the direct effects of metformin on tumor cells and its contribution to systemic metabolic changes and to reveal its potential translational value in cancer therapy.

Method

Animal Model and Ethical Approval

Specific pathogen-free (SPF) male BALB/c mice, 6-8 weeks old, were used in the study. Mice were housed at a temperature of 22 ± 2°C and humidity of 50 ± 10% in an environment with a constant 12 h light/12 h dark cycle. All animals were maintained on a standardized diet with free access to drinking water. The study protocol was approved by the Animal Experiments Ethics Committee.

Cell Culture and Tumor Model

Lewis Lung Cancer (LLC) cell line was cultured in Dulbecco's Modified Eagle Medium (DMEM) containing 10% fetal bovine serum (FBS), 1% penicillin-streptomycin and 1% L-glutamine. Cells were incubated at 37°C in a 5% CO_2 atmosphere. Cells used for the experiment were in logarithmic phase and viability was confirmed to be above 95% by trypan blue method. LLC cells were prepared as $1x10^6$ cells/ml and 100 µl was injected subcutaneously under the right forelimb of mice.

Experimental Groups

Mice were randomly divided into three groups:

1. **Control Group (n=10):** Tumor was induced but no treatment was administered.
2. **Metformin Group (n=10):** 250 mg/kg metformin was added to drinking water and given for 21 days.
3. **Combination Group (n=10):** 250 mg/kg metformin and 2 mg/kg cisplatin (weekly intraperitoneal injection).

Treatment and Monitoring Protocol

Metformin treatment was started 7 days after tumor implantation and continued for 21 days. Cisplatin was administered intraperitoneally on days 7, 14 and 21. Mice were monitored daily and body weights and behavioral parameters were recorded. Tumor volume was determined by a calculation method based on length and width values measured weekly using a digital caliper. For this calculation, the length and width of the tumor were measured, then the length value was multiplied by the square of the width value and the result was divided by two. This method is a widely used approach to accurately and consistently assess tumor volume. For example, when the tumor length was measured as 10 mm and the width as 8 mm, the tumor volume was calculated to be approximately 320 mm^3.

Histological and Molecular Analyses

- **Histopathological Evaluation:** Tumor tissues were fixed with 10% neutral buffered formalin, embedded in paraffin and examined by hematoxylin-eosin (H&E) staining.
- **Apoptosis Analysis:** Cleaved caspase-3 expression was evaluated by immunohistochemistry.
- **Protein Analysis:** Proteins isolated from tumor tissues were analyzed by Western blotting. AMPK, p-AMPK and mTOR protein expression levels were analyzed.

Oxidative Stress and Antioxidant Activity

Malondialdehyde (MDA) levels were measured and glutathione (GSH) levels were analyzed with ELISA kits to evaluate oxidative stress in tumor tissues. Reactive oxygen species (ROS) levels were determined using DCFDA fluorescent probe.

Statistical Analysis

All data obtained from the study were analyzed using IBM SPSS Statistics (Version 25.0) software. Data were expressed as mean ± standard error of the mean (SEM) and Shapiro-Wilk test was applied to determine whether the data were normally distributed. Parametric tests were used for normally distributed data and nonparametric tests were used for non-normally distributed data. One-way analysis of variance (ANOVA) was applied to evaluate the differences between the groups. If a significant difference was detected in the ANOVA results, Tukey post-hoc test was used to determine which groups were statistically significant. For data that did not show normal distribution, Kruskal-Wallis test was applied and post-hoc analysis was performed with Dunn's test. Kaplan-Meier method was used to evaluate survival rates. The log-rank test was applied to test the difference in survival curves between the groups. Pearson correlation analysis was performed to examine the effect of metformin treatment on tumor volume and apoptosis markers. This analysis was performed to determine possible linear relationships between metformin and tumor growth. In all analyses, a value of $p<0.05p < 0.05p<0.05$ was considered statistically significant. All analyses were reviewed twice and calculations were checked to increase the reliability of the data.

8. Tokenomics and Funding Model

Total Supply: 1 billion tokens
Team Allocation: 2%
Research Fund: Applied to multiple funds provider
The Token deployed by 0x_Rorschach via Pump.Fun platform.

9. Project Roadmap

Phase 1 (Q3-Q4 2024): Literature review and study preparations
Phase 2 (Q3-Q4 2024): Animal study initiations, ethical
committee applications.
Increasing the Public Awareness on DeSci
Add utility to token
Phase 3 (2025-Q1-2): Data collection and analyses
of Research's
Phase 4 (2025 Q3-4): Publications and expansion

10. Conclusion

The MetforminSOL initiative is an example of the intersection of community-driven action and innovative research to address one of the most fundamental problems in modern medicine, ageing. This project highlights the critical role of well-designed scientific research in exploring new ways to extend health span and lifespan through multi-targeted pathways with a drug as promising as metformin, beyond its established use in diabetes management.

With rigorous design and methodology, this experimental study hypothesizes that metformin can ameliorate age-related mitochondrial dysfunction and oxidative stress, providing important new insights into this process. Such findings may form the basis of future therapeutics and ultimately help to advance longevity science.

This effort is an important step in bridging the gap between laboratory discovery and real-world clinical translation. Thanks to an incredible team of scientists, open transparency and a supportive community, MetforminSOL is doing so much more than

just pushing the boundaries of decentralized science - it is a wonderful example of what can be achieved when we pool our resources for meaningful research.

With the hope of encouraging innovation in longevity science, MetforminSOL aims to serve as a key resource that not only promotes healthy long-term living, but also creates the pathway for a legacy of knowledge and healthy living for generations to come.

10. References

1. Gustafson, A. and Rice, R. (2020). A review of the effects of uncertainty in public science communication. Public Understanding of Science, 29(6), 614-633. https://doi.org/10.1177/0963662520942122

2. Wale BD, Bishaw KS. Effects of using inquiry-based learning on EFL students' critical thinking skills. Asian-Pacific Journal of Second and Foreign Language Education. 2020 Dec;5:1-4. springer.com

3. Portney LG. Foundations of clinical research: applications to evidence-based practice. 2020.

4. Bogenschneider K, Corbett T. Evidence-based policymaking: Envisioning a new era of theory, research, and practice. 2021. [HTML]

5. Jiang C, Zhang Y, Kamran HW, Afshan S. Understanding the dynamics of the resource curse and financial development in China? Novel evidence based on QARDL model. Resources Policy. 2021. [HTML]

6. Portney LG. Foundations of clinical research: applications to evidence-based practice. 2020.

7. Dang D, Dearholt SL, Bissett K, Ascenzi J et al. Johns Hopkins evidence-based practice for nurses and healthcare professionals: Model and guidelines. 2021. [HTML]

8. Carrier M. What does good science-based advice to politics look like?. Journal for General Philosophy of Science. 2022. springer.com

9. Anderson NC, Chen PF, Meganathan K, Saber WA, Petersen AJ, Bhattacharyya A, Kroll KL, Sahin M. Balancing serendipity and reproducibility: Pluripotent stem cells as experimental systems for intellectual and developmental disorders. Stem cell reports. 2021 Jun 8;16(6):1446-57. cell.com

10. Alam A. Psychological, sociocultural, and biological elucidations for gender gap in STEM education: a call for translation of research into evidence-based interventions. InAlam, A.(2022). Psychological, Sociocultural, and Biological Elucidations for Gender Gap in STEM Education: A Call for Translation of Research into Evidence-Based Interventions. Proceedings of the 2nd International Conference on Sustainability and Equity (ICSE-2021). Atlantis Highlights in Social S 2022 Apr 3. ssrn.com

11. Subbiah V. The next generation of evidence-based medicine. Nature medicine. 2023. nature.com

12. Hume K, Steinbrenner JR, Odom SL, Morin KL, Nowell SW, Tomaszewski B, Szendrey S, McIntyre NS, Yücesoy-Özkan S, Savage MN. Evidence-based practices for children, youth, and young adults with autism: Third generation review. Journal of autism and developmental disorders. 2021 Nov 1:1-20. springer.com

13. Domino JS, Ormond DR, Germano IM, Sami M, Ryken TC, Olson JJ. Cytoreductive surgery in the management of newly diagnosed glioblastoma in adults: a systematic review and evidence-based clinical practice guideline update. Journal of neuro-oncology. 2020 Nov;150:121-42. [HTML]

14. Andrew L, Wallace R, Wickens N, Patel J. Early childhood caries, primary caregiver oral health knowledge and behaviours and associated sociological factors in Australia: a systematic scoping review. BMC Oral Health. 2021. springer.com

15. Kunisch S, Denyer D, Bartunek JM, Menz M, Cardinal LB. Review research as scientific inquiry. Organizational Research Methods. 2023 Jan;26(1):3-45. sagepub.com

16. de Vocht F, Katikireddi SV, McQuire C, Tilling… K. Conceptualising natural and quasi experiments in public health. BMC medical research …. 2021. springer.com

17. Khan S, Chambers D, Neta G. Revisiting time to translation: implementation of evidence-based practices (EBPs) in cancer control. Cancer Causes & Control. 2021. springer.com

18. Cheon BK, Melani I, Hong YY. How USA-centric is psychology? An archival study of implicit assumptions of generalizability of findings to human nature based on origins of study samples. Social Psychological and Personality Science. 2020 Sep;11(7):928-37. [HTML]

19. Tucker S, McNett M, Mazurek Melnyk B, Hanrahan K, Hunter SC, Kim B, Cullen L, Kitson A. Implementation science: Application of evidence-based practice models to improve healthcare quality. Worldviews on Evidence-Based Nursing. 2021 Apr;18(2):76-84. [HTML]

20. Moullin JC, Dickson KS, Stadnick NA, Albers B, Nilsen P, Broder-Fingert S, Mukasa B, Aarons GA. Ten recommendations for using implementation frameworks in research and practice. Implementation science communications. 2020 Dec;1:1-2. springer.com

21.. Figuer A, Bodega G, Tato P, Valera G, Serroukh N, Ceprian N, de Sequera P, Morales E, Carracedo J, Ramírez R, Alique M. Premature aging in chronic kidney disease: the outcome of persistent inflammation beyond the bounds. International Journal of Environmental Research and Public Health. 2021 Jul 29;18(15):8044. mdpi.com

22. Gems D. The hyperfunction theory: an emerging paradigm for the biology of aging. Ageing research reviews. 2022. nih.gov

23. National Academies of Sciences, Division of Behavioral, Social Sciences, Medicine Division, Board on Behavioral, Sensory Sciences, Board on Health Sciences Policy, Committee on the Health, Medical Dimensions of Social Isolation, Loneliness in Older Adults. Social isolation and loneliness in older adults: Opportunities for the health care system. National Academies Press; 2020 Jun 14. nationalacademies.org

24. Li F, Luo S, Mu W, Li Y, Ye L, Zheng X, Xu B, Ding Y, Ling P, Zhou M, Chen X. Effects of sources of social support and resilience on the mental health of different age groups during the COVID-19 pandemic. BMC psychiatry. 2021 Dec;21:1-4. springer.com

25. Fang EF, Xie C, Schenkel JA, Wu C, Long Q, Cui H, Aman Y, Frank J, Liao J, Zou H, Wang NY. A research agenda for ageing in China in the 21st century: Focusing on basic and translational research, long-term care, policy and social networks. Ageing research reviews. 2020 Dec 1;64:101174. nih.gov

26. Guo J, Huang X, Dou L, Yan M, Shen T, Tang W, Li J. Aging and aging-related diseases: from molecular mechanisms to interventions and treatments. Signal Transduction and Targeted Therapy. 2022 Dec 16;7(1):391. nature.com

27. Hajam YA, Rani R, Ganie SY, Sheikh TA, Javaid D, Qadri SS, Pramodh S, Alsulimani A, Alkhanani MF, Harakeh S, Hussain A. Oxidative stress in human pathology and aging: molecular mechanisms and perspectives. Cells. 2022 Feb 5;11(3):552. mdpi.com

28. Ju Y, Tam KY. Pathological mechanisms and therapeutic strategies for Alzheimer's disease. Neural regeneration research. 2022. lww.com

29. Wang K, Liu H, Hu Q, Wang L, Liu J, Zheng Z, Zhang W, Ren J, Zhu F, Liu GH. Epigenetic regulation of aging: implications for interventions of aging and diseases. Signal transduction and targeted therapy. 2022 Nov 7;7(1):374. nature.com

30. Le XT, Dang AK, Toweh J, Nguyen QN, Le HT, Do TT, Phan HB, Nguyen TT, Pham QT, Ta NK, Nguyen QT. Evaluating the psychological impacts related to COVID-19 of Vietnamese people under the first nationwide partial lockdown in Vietnam. Frontiers in psychiatry. 2020 Sep 2;11:824. frontiersin.org

31. Sharma S, Singh G, Gaur L, Sharma R. Does psychological distance and religiosity influence fraudulent customer behaviour?. International Journal of Consumer Studies. 2022 Jul;46(4):1468-87. [HTML]

32. Pérez-Torres I, Castrejón-Téllez V, Soto ME, Rubio-Ruiz ME, Manzano-Pech L, Guarner-Lans V. Oxidative stress, plant natural antioxidants, and obesity. International journal of molecular sciences. 2021 Feb 11;22(4):1786. mdpi.com

33. Alkadi H. A review on free radicals and antioxidants. Infectious Disorders-Drug Targets (Formerly Current Drug Targets-Infectious Disorders). 2020 Feb 1;20(1):16-26. [HTML]

34. Gulcin İ. Antioxidants and antioxidant methods: An updated overview. Archives of toxicology. 2020. [HTML]

35. Macip CC, Hasan R, Hoznek V, Kim J, Lu YR, Metzger IV LE, Sethna S, Davidsohn N. Gene therapy-mediated partial reprogramming extends lifespan and reverses age-related changes in aged mice. Cellular Reprogramming. 2024 Feb 1;26(1):24-32. liebertpub.com

36. Zhang W, Qu J, Liu GH, Belmonte JC. The ageing epigenome and its rejuvenation. Nature reviews Molecular cell biology. 2020 Mar;21(3):137-50. [HTML]

37. Yousefzadeh MJ, Flores RR, Zhu YI, Schmiechen ZC, Brooks RW, Trussoni CE, Cui Y, Angelini L, Lee KA, McGowan SJ, Burrack AL. An aged immune system drives senescence and ageing of solid organs. Nature. 2021 Jun 3;594(7861):100-5. nih.gov

38. Prasopthum A, Insawek T, Pouyfung P. Herbal medicine use in Thai patients with type 2 diabetes

mellitus and its association with glycemic control: A cross-sectional evaluation. Heliyon. 2022. cell.com

39. Hao G, Hu R, Wang X, Gao P, Wang L, Jiang M, Xin L, Tan G, Zhao Y, Sun F, Chu D. N-Nitrosodimethylamine formation in metformin hydrochloride sustained-release tablets: Effects of metformin and hypromellose used in drug product formulation. Journal of Pharmaceutical and Biomedical Analysis. 2023 Jan 5;222:115066. [HTML]

40. Mohammed I, Hollenberg MD, Ding H, Triggle CR. A critical review of the evidence that metformin is a putative anti-aging drug that enhances healthspan and extends lifespan. Frontiers in endocrinology. 2021 Aug 5;12:718942. frontiersin.org

41. Triggle CR, Mohammed I, Bshesh K, Marei I, Ye K, Ding H, MacDonald R, Hollenberg MD, Hill MA. Metformin: Is it a drug for all reasons and diseases?. Metabolism. 2022 Aug 1;133:155223. sciencedirect.com

42. Agius L, Ford BE, Chachra SS. The metformin mechanism on gluconeogenesis and AMPK activation: the metabolite perspective. International journal of molecular sciences. 2020 May 3;21(9):3240. mdpi.com

43. LaMoia TE, Shulman GI. Cellular and molecular mechanisms of metformin action. Endocrine reviews. 2021. oup.com

44. Guan Y, Wang D, Bu H, Zhao T, Wang H. The effect of metformin on polycystic ovary syndrome in overweight women: a systematic review and meta-analysis of randomized controlled trials. International journal of endocrinology. 2020;2020(1):5150684. wiley.com

44. Soldat-Stanković V, Popović-Pejičić S, Stanković S, Prtina A, Malešević G, Bjekić-Macut J, Livadas S, Ognjanović S, Mastorakos G, Micić D, Macut D. The effect of metformin and myoinositol on metabolic outcomes in women with polycystic ovary syndrome: role of body mass and adiponectin in a randomized controlled trial. Journal of Endocrinological Investigation. 2022 Mar 1:1-3. [HTML]

45. Mantovani A, Byrne CD, Scorletti E, Mantzoros CS, Targher G. Efficacy and safety of anti-hyperglycaemic drugs in patients with non-alcoholic fatty liver disease with or without diabetes: An updated systematic review of randomized controlled trials. Diabetes & metabolism. 2020 Nov 1;46(6):427-41. soton.ac.uk

46. Cardoso S, Moreira PI. Antidiabetic drugs for Alzheimer's and Parkinson's diseases: repurposing insulin, metformin, and thiazolidinediones. International review of neurobiology. 2020. [HTML]

47. Kulkarni AS, Gubbi S, Barzilai N. Benefits of metformin in attenuating the hallmarks of aging. Cell metabolism. 2020. cell.com

48. Shoshan-Barmatz V, Anand U, Nahon-Crystal E, Di Carlo M, Shteinfer-Kuzmine A. Adverse effects of metformin from diabetes to COVID-19, cancer, neurodegenerative diseases, and aging: is VDAC1 a common target?. Frontiers in Physiology. 2021 Oct 4;12:730048. frontiersin.org

49. Castera L, Cusi K. Diabetes and cirrhosis: current concepts on diagnosis and management. Hepatology. 2023. lww.com

50. Green CL, Lamming DW, Fontana L. Molecular mechanisms of dietary restriction promoting health and longevity. Nature Reviews Molecular Cell Biology. 2022 Jan;23(1):56-73. nih.gov

51. Ge Y, Zhou M, Chen C, Wu X et al. Role of AMPK mediated pathways in autophagy and aging. Biochimie. 2022. researchgate.net

52. Drzewoski J, Hanefeld M. The current and potential therapeutic use of metformin—the good old drug. Pharmaceuticals. 2021. mdpi.com

53. Bai B, Chen H. Metformin: a novel weapon against inflammation. Frontiers in pharmacology. 2021. frontiersin.org

54. Foretz M, Guigas B, Viollet B. Metformin: update on mechanisms of action and repurposing potential. Nature Reviews Endocrinology. 2023. nature.com

55. Madhu LN, Kodali M, Shetty AK. Promise of metformin for preventing age-related cognitive dysfunction. Neural Regeneration Research. 2022. lww.com

56. Goodwin PJ, Chen BE, Gelmon KA, Whelan TJ, Ennis M, Lemieux J, Ligibel JA, Hershman DL, Mayer IA, Hobday TJ, Bliss JM. Effect of metformin vs placebo on invasive disease–free survival in patients with breast cancer: the MA. 32 randomized clinical trial. Jama. 2022 May 24;327(20):1963-73. nih.gov

57. López-Otín C., Blasco M.A., Partridge L., Serrano M., Kroemer G. The Hallmarks of Aging. Cell. 2013;153:1194–1217.

58. Clemente J.C., Ursell L.K., Parfrey L.W., Knight R. The impact of the gut microbiota on human health: An integrative view. Cell. 2012;148:1258–1270.

59. Bana B., Cabreiro F. The Microbiome and Aging. Annu. Rev. Genet. 2019;53:239–261.

60. Braidy N, Liu Y. NAD+ therapy in age-related degenerative disorders: A benefit/risk analysis. Exp Gerontol. 2020;132:110831.

61. Zhou G, Myers R, Li Y, et al. Role of AMP-activated protein kinase in mechanism of metformin action. J Clin Invest. 2001;108(8):1167-1174.

62. Zhu X, Shen W, Liu Z, et al. Effect of Metformin on Cardiac Metabolism and Longevity in Aged Female Mice. Front Cell Dev Biol. 2021;8:626011.

63. Martin-Montalvo A, Mercken EM, Mitchell SJ, et al. Metformin improves healthspan and lifespan in mice. Nat Commun. 2013;4:2192.

64. Bade BC, Dela Cruz CS. Lung Cancer 2020: Epidemiology, Etiology, and Prevention. Clin Chest Med. 2020;41(1):1-24.

65. Thai AA, Solomon BJ, Sequist LV, Gainor JF, Heist RS. Lung cancer. Lancet. 2021;398(10299):535-554.

66. Li Y, Yan B, He S. Advances and challenges in the treatment of lung cancer. Biomed Pharmacother. 2023;169:115891.

67. Harðardottir H, Jonsson S, Gunnarsson O, et al. Laeknabladid. 2022;108(1):17-29.

68. Abu Rous F, Singhi EK, Sridhar A, Faisal MS, Desai A. Lung Cancer Treatment Advances in 2022. Cancer Invest. 2023;41(1):12-24.
69. Kim YS, Park BS, Baek HS, Kan g HM, Oh JM, Kim IR. Metformin activates AMPK and mTOR to Inhibit RANKL-stimulated osteoclast formation. Eur Rev Med Pharmacol Sci. 2023;27(18):8795-8811.
70. Chomanicova N, Gazova A, Adamickova A, Valaskova S, Kyselovic J. The role of AMPK/mTOR signaling pathway in anticancer activity of metformin. Physiol Res. 2021;70(4):501-508.