Acta Dermatovenerol APA

Acta Dermatovenerologica
Alpina, Pannonica et Adriatica

2023;32:159-164
doi: 10.15570/actaapa.2023.29

New anti-aging strategies: a narrative review

Vana Stojić[1] ✉, Tea Štrbac[2], Andrija Stanimirović[3]

[1]Zagreb County Institute of Emergency Medicine, Velika Gorica, Croatia. [2]Department of Ophthalmology, Sveti Duh University Hospital, Zagreb, Croatia. [3]Department of Dermatology, School of Medicine, European University Cyprus, Nicosia, Cyprus.

Abstract

The objective of anti-aging medicine is to decelerate the aging process and mitigate its associated effects, such as susceptibility to cancer, diabetes, and cardiovascular and neurodegenerative diseases. This review provides an overview of the latest advancements in this field, considering both pharmaceutical and non-pharmaceutical approaches. Electronic literature search involved three databases: MEDLINE, Cochrane, and Google Scholar, supplemented by other available literature. Strategies for delaying aging and related diseases comprise pharmaceutical interventions and lifestyle choices. It is crucial for these strategies to be substantiated by research-based evidence. Lifestyle options include fasting, fasting-mimicking, and ketogenic diets. Anti-aging drugs and supplements operate through diverse mechanisms. Calorie restriction mimetics include the activator of AMP-activated protein kinase (metformin) and inhibitor of mTOR (rapamycin), alongside rilmenidine, exhibiting both effects. Rosmarinic acid, a natural product, functions through its anti-glycation properties. Age-related protein crosslinks are acknowledged as a causative factor in age-related diseases. Anti-aging medicine is an evolving field with a multitude of drugs and strategies, necessitating further clinical studies and long-term follow-up based on clinical experience and insights gained from delayed adverse events.

Keywords: aging, lifestyle, pharmaceuticals, ketones, anti-aging

Received: 12 October 2023 | **Returned for modification:** 31 October 2023 | **Accepted:** 19 November 2023

Introduction

Anti-aging medicine aims to eliminate, reverse, or reduce the effects of aging. There is growing interest in exploring various approaches to achieve this goal, leading to the rapid expansion of the anti-aging industry with the integration of new technologies and ingredients. Aging is a major risk factor for cancer, cardiovascular diseases, neurodegenerative disorders, and diabetes. The aging process is influenced by both intrinsic and extrinsic factors, with varying degrees of reducibility. In 2015, the World Health Organization officially recognized aging as a disease, sparking increased research on aging and aging-related diseases, along with the development of relevant therapeutic strategies. This review focuses on emerging interventions in anti-aging medicine. Aging is a complex, multifactorial process, and the identification of aging hallmarks aids in the conceptualization of aging research. Hallmarks of aging refer to biochemical changes occurring in all organisms that contribute to biological aging. For a process to qualify as a hallmark, it must exhibit changes with biological age and play a causal role in aging (Table 1) (1). Although aging may be a significant factor in many diseases, and is potentially classified as a disease itself, it is essential to acknowledge that it remains a natural part of the life cycle for all living organisms on Earth.

Ketone bodies

Ketone bodies (KB)—namely, β-hydroxybutyrate (β-HB), acetoacetate, and acetone—are produced from fatty acids by the liver in a process called ketogenesis. This metabolic shift, occurring during calorie restriction, forces the body to utilize fat instead of sugar for energy. Exogenous oral supplementation, including β-HB and its related forms, can also induce the presence of KB in patients (2).

Nutritional ketosis is a metabolically induced state with a plasma β-HB concentration ≥ 0.5 mM. This state can be achieved endogenously through a ketogenic diet or exogenously through oral supplementation, including β-HB and its related forms (2). Although ketones are typically present in minimal amounts in the blood, their levels increase when dietary carbohydrates are restricted, as seen in ketogenic diets. Some popular diets today are intermittent fasting and high-fat, moderate-protein, and low-carbohydrate ketogenic diets.

D-β-HB supplementation to *Caenorhabditis elegans*, a roundworm commonly used in aging research, has been reported to enhance health and extend lifespan. β-HB is thus regarded as an "anti-aging KB," demonstrating a mean lifespan extension of approximately 20% and providing protection against metabolic, thermal, and proteotoxic stress. This suggests potential therapeutic applications for β-HB in treating various human aging-associated disorders (3).

Research at the Buck Institute for Research on Aging showed that mice that were fed a ketogenic diet had a lower risk of dying as they aged from 1 to 2 years old, although their maximum lifespan was unchanged. Memory and cognitive skills in mice were also improved (4). In January 2023, the Buck Institute for Research

Table 1 | **Hallmarks of aging, adapted from Lopez-Otin et al. (1).**

Category	Hallmark
Primary	Genomic instability
	Telomere attrition
	Epigenetic alterations
	Loss of proteostasis
	Disabled macroautophagy
Antagonistic	Deregulated nutrient sensing
	Mitochondrial dysfunction
	Cellular senescence
Integrative	Stem cell exhaustion
	Altered intercellular communication
	Chronic inflammation
	Dysbiosis

✉ Corresponding author: vana.stojic@hotmail.com

on Aging launched its first human clinical trial. The BIKE (Buck Institute Ketone Ester) pilot study is the first trial in the world to investigate the anti-aging effect of ketone ester supplementation. Thirty healthy individuals over age 65 will take part in a 12-week double-blind, randomized, placebo-controlled study to see whether the benefits of KB in aging that have been observed in mice translate to human beings.

Ketone ester supplementation facilitates ketosis without the need for a restrictive diet. Throughout the trial, blood and other biospecimens will be collected, and participants will undergo standardized physical function tests (balance, walking speed, chair sits, leg press performance, walking speed, and grip strength) and standardized mental tests.

The biomarkers measured in the BIKE study align with those in the Targeting Aging with Metformin (TAME) trial. TAME investigates whether older adults given metformin versus placebo experience delayed development or progression of chronic conditions such as heart disease, cancer, and dementia (5).

Metformin

Metformin is an U.S. Food and Drug Administration (FDA)-approved drug that has been used to treat diabetes for more than 60 years, and it is the most widely used antidiabetic drug. Studies have already shown that it may delay aging in animals and may also influence fundamental aging factors that underlie multiple age-related conditions in humans. In addition to diabetes mellitus, metformin has been shown to be effective for aging-related diseases, such as degenerative skeletal diseases, cardiovascular diseases, neurodegenerative diseases, tumors, obesity, and other metabolic abnormalities (5, 6). The results from a randomized, double-blind, placebo-controlled, crossover trial revealed that metformin had both metabolic and non-metabolic effects associated with aging in the elderly, providing evidence for it's anti-aging effect (7).

The reported delay in aging is linked to its influence on key hallmark events of aging, addressing dysregulated nutrient sensing, loss of proteostasis, mitochondrial dysfunction, altered intercellular communication, telomere attrition, genomic instability, epigenetic alterations, stem cell exhaustion, and cellular senescence (6).

Metformin's ability to activate AMP-activated protein kinase (AMPK) and inhibit mTOR signaling pathways, which decline with age, contributes to its potential benefits on aging. It is known to reduce oxidative damage, induce positive stress, and promote cellular autophagy and apoptosis, processes crucial for eliminating damaged cellular components and improving cellular health (8). Autophagy, encouraged by AMPK activation, holds promise for promoting the health span through hormesis-like mechanisms.

Studies show a reduction in the incidence of multiple age-related diseases and all-cause mortality with metformin use, observed not only in diabetic patients but also in non-diabetic ones (9). Aging plays an important role in causing many cancers. Studies suggest that metformin can be used alone or in combination with other drugs in cancer treatment, including pancreatic, breast, colon, and other cancers, but further studies are needed to investigate these benefits (10). Its anti-cancer effect lies in impeding tumor cell proliferation, blocking progression of the tumor cell cycle, preventing genomic instability, inducing apoptosis, and impacting cellular energy metabolism (6).

Results from studies in cultured cells treated with a high concentration of metformin showed that it significantly inhibited angiogenesis, whereas lower doses had no effect (11). In even higher doses, it shows an antiangiogenic effect in endothelial and tumor cells. This means that high-dose administration of metformin may be beneficial as antiangiogenic therapy for diseases such as cancer (12).

Diabetes itself may represent a pro-aging state. Individuals with diabetes are more likely to develop age-related comorbidities, such as mild cognitive impairment, Alzheimer's disease, cardiovascular disease, osteoporosis, visual impairment, and renal dysfunction (13).

Metformin alone or in combination with other hypoglycemic drugs is effective in treating type 2 diabetes and its complications. Metformin can significantly reduce cardiovascular mortality, all-cause mortality, and the incidence of cardiovascular events in diabetic and non-diabetic patients with coronary heart disease (14). Preclinical studies show that the protective effect of metformin on cardiovascular diseases lies in its multiple effects on vascular endothelial cells, smooth muscle cells, and lipids as well as chronic systemic inflammation. It improves the function of vascular smooth muscle cells by inhibiting inflammation, contraction, proliferation, and calcification (15).

Recent research suggests that metformin may have anti-aging effects beyond its metabolic benefits and have an effect on skin aging (7). One way that metformin may affect skin aging is by reducing oxidative stress, which could help protect skin cells from oxidative stress and prevent premature aging (16). Another way that metformin may influence skin aging is by regulating inflammation, which is a common feature of aging. Metformin has been shown to modulate inflammatory pathways and reduce the production of pro-inflammatory cytokines, which could mitigate the effects of chronic inflammation on the skin (17). Metformin may also affect skin aging through its effects on cellular metabolism, which normally slows down as people age. It enhances cellular energy production and improves cellular functions (18). It also increases autophagy, a process by which cells recycle damaged or dysfunctional components. Autophagy also declines with age and has been linked to various age-related diseases, including skin aging (19).

More research is needed to confirm the efficacy and safety of these metformin properties because studies exploring its effects on skin aging are still limited. Metformin may be incorporated into skincare products or used as a preventative measure against skin aging in the future. It has the potential to mitigate skin aging through its effects on oxidative stress, inflammation, and cellular metabolism. Its anti-aging effects are not yet fully understood, but it is an exciting area of research with promising implications for the future of skin health. Metformin plays an important role in the treatment of musculoskeletal diseases by inhibiting the effect of inflammatory response, cartilage degeneration, mechanical hyperalgesia, cellular senescence, osteoclastic activity, oxidative stress, fibroblast ossification, and cellular proliferation and migration, as well as reducing body weight or improving osteogenic activity (6).

Metformin can also play a role in neuroprotection and neurorepair. However, the safety of metformin applied to neurodegenerative diseases needs further investigation (20).

Exactly how metformin affects aging is not yet clear, but it is suggested that many mechanisms are involved. The root of many age-related disorders is inflammation, and it is associated with glucose dysregulation and insulin resistance. Many age-related

neurodegenerative diseases are associated with the buildup of damaged proteins, and metformin appears to prevent protein damage in mouse models. It reduces pro-inflammatory molecules and increases anti-inflammatory molecules. In conclusion, before metformin finds its way into therapy for anti-aging, better understanding of the effects of the drug in humans is needed. Further research is needed to determine the optimal dose and duration of metformin treatment for anti-aging purposes.

The TAME study is a double-blind randomized placebo-controlled clinical trial investigating whether metformin can delay the onset of age-related diseases and improve the overall health span in older adults. It is the first-ever anti-aging study approved by the FDA (5). This 6-year trial to explore the role of metformin in longevity is still in its preparatory stage and will involve over 3,000 participants. A small randomized controlled trial provided preliminary evidence that metformin can lead to transcriptomic changes in pathways that affect ageing (7).

The most common side effect of metformin is gastrointestinal irritation causing diarrhea, nausea, vomiting, flatulence, and cramps. This can usually be avoided by starting with a low dose and gradually increasing it or using sustained-release formulations (6).

The most serious adverse effect of metformin is lactic acidosis, in which a buildup of lactic acid can be life-threatening. It is very rare and is usually linked to preexisting liver and kidney damage rather than metformin itself (21). It has been reported that high-dose and prolonged use of metformin may cause vitamin B12 deficiency, and so it would be helpful to screen patients for vitamin B12 deficiency (22).

Rapamycin

Sirolimus, also known as rapamycin, is a macrolide compound utilized to coat coronary stents as well as prevent organ transplant rejection due to its immunosuppressant effect. It is produced by the bacterium *Streptomyces hygroscopicus* (23).

Rapamycin binds to the enzyme mTOR and allows autophagy to proceed, and so in that way it mimics calorie restriction. Whereas metformin activates the AMPK pathway, rapamycin inhibits the mTOR pathway, leading to enhanced autophagy and improved cellular health. It is slowly becoming a new "anti-aging drug." Even though it is considered immunosuppressive, at anti-aging doses, rapamycin "eliminates hyperimmunity" or, more figuratively, it "rejuvenates immunity" (23). It has even been suggested that it may extend the lifespan by preventing cancer (24).

Calorie restriction and intermittent fasting extend the lifespan and health span in diverse species (25). However, calorie restriction is of little benefit when started in old age (26) because fasting inhibits the mTOR pathway in young mice but not old ones (27).

In contrast, rapamycin inhibits mTOR at any age, and so it extends the lifespan whether started late or early in life (28). It is important to caution that self-medication should be strongly discouraged. Blood levels of rapamycin should be measured because its concentration in blood varies among individuals taking the same dose, and so personalized dosing is very important (23). The optimal anti-aging dose is a personalized maximum dose that does not cause side effects. Life extension with rapamycin was shown to be dose-dependent in rodents, meaning the higher the dose, the higher the anti-aging benefits, including cancer prevention. On the other hand, side effects in humans are dose-dependent, and net benefits could potentially decrease at very high doses. Thus, the optimal dose in a particular human individual is determined by the emergence of side effects (Fig. 1) (29).

Although side effects are reversible, fear of them is the main reason mTOR inhibitors for life extension have been questioned. The second reason is skepticism among people about any "anti-aging drugs" and their effectiveness (3).

Rapamycin has been shown to have a positive effect on the cardiovascular system. It has been shown to delay the onset of age-related cardiac dysfunction, reduce inflammation in the heart, and improve cardiac function (30). These effects are due to inhibition of the mTOR signaling pathway, which mediates the aging process. It also preserves cardiac function and integrity by increasing autophagy, a process that removes damaged proteins and organelles from cells (31). It is important to mention that rapamycin persistently improved cardiac function in aged male and female mice, even following cessation of treatment (32).

Second, rapamycin also has a protective effect on the nervous system because it has been shown to delay the onset of neurodegenerative diseases such as Alzheimer's, Parkinson's, and Huntington's diseases. These disorders involve the accumulation of abnormal proteins, oxidative stress, and inflammation, and rapamycin administration reduces the accumulation of these abnormal proteins and oxidative stress. In this way it reduces the risk of neurodegeneration (33). Therefore, rapamycin acts as a potential therapeutic agent for prevention or treatment of neurodegenerative diseases.

Studies have shown that rapamycin has a beneficial effect on the musculoskeletal system. It can improve bone health by delaying bone loss and protecting against osteoporosis (34). This effect is due to the activation of autophagy in osteoblasts. This leads to increased production of new bone cells, as well as reduced bone resorption. It has also been shown to increase muscle mass and function, reducing muscle wasting during aging (35).

Rapamycin has a potential anti-aging effect in skin aging. Studies have shown that rapamycin may have beneficial effects on various skin aging-related conditions, including wrinkles, pigmentation, and loss of elasticity (36). One study found that application of topical rapamycin reduced wrinkles and improved skin elasticity in mice (37). Another study found that rapamycin treatment in aged rats increased collagen synthesis and inhibited matrix metalloproteinases (MMPs). MMPs are enzymes that break

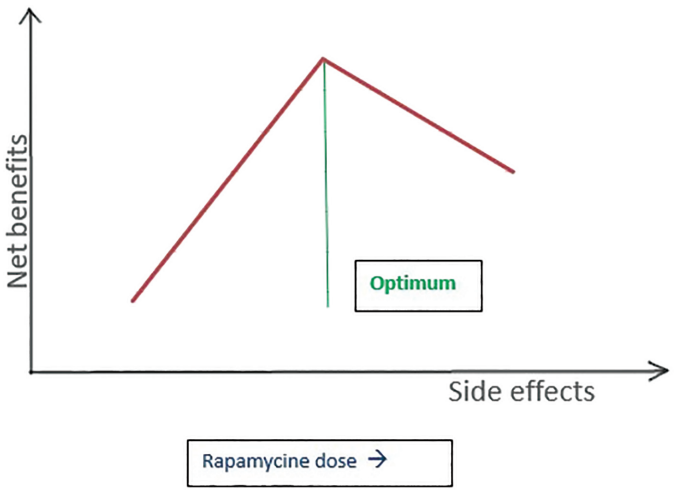

Figure 1 | In rodents, life extension by rapamycin exhibits a dose-dependent relationship. In humans, side effects also display a dose-dependent pattern, and the net benefits of treatment could potentially decline at very high doses. Therefore, the optimal dose for an individual is determined by the emergence of side effects. Adapted from Blagosklonny (29).

down collagen and elastin in the skin, which are implicated in skin aging (38).

Side effects of rapamycin include stomatitis, mucositis, noninfectious interstitial pneumonitis, and hyperglycemia, and it may increase the severity of some bacterial infections. These side effects require discontinuation of usage.

At higher doses, rapamycin slows cell proliferation, which can result in mild and reversible thrombocytopenia, anemia, and leukopenia. For anti-aging purposes, rapamycin may be used either intermittently (e.g., once a week) or at low daily doses (3).

To minimize side effects and optimize the anti-aging benefits of rapamycin, a recommended strategy involves extending the intervals between administrations while maintaining a consistent total dose (39). The key reason for the perceived exaggeration of rapamycin's side effects is that the frequency of these effects has frequently been assessed in studies that lacked a placebo group for comparison.

Combined treatment

The combination of rapamycin and metformin has shown promising results in enhancing each other's effectiveness while mitigating side effects, at least in studies involving mice. Rapamycin, when administered alone, has been observed to reduce weight gain, Homeostatic Model Assessment for Insulin Resistance (HOMA-IR), and inflammation. It also prevents hyperinsulinemia and pre-steatotic hepatic lipidosis. However, it exacerbates hyperglycemia, hypertriglyceridemia, and pancreatic islet degranulation. On the other hand, metformin, when used alone, reduces hyperinsulinemia and circulating C-reactive protein but exacerbates nephropathy (40).

The combined treatment of rapamycin and metformin retains the positive effects of each drug while preventing many of their deleterious effects (40). It is noteworthy that both rapamycin and metformin have been in use for many years and have established safety profiles. The synergy observed in combining metformin with rapamycin suggests a potent new approach to anti-aging therapy. However, it is imperative to conduct further research to determine the optimal therapeutic dose and duration for this combined therapy.

A study by Reifsnyder et al. demonstrated the benefits of combined treatment, highlighting a decrease in side effects and an increase in positive effects (40). These findings underscore the potential of synergistic drug combinations in anti-aging strategies, although continued investigation is crucial for a comprehensive understanding and successful translation to therapeutic applications.

Physiological hormesis

The induction of mild stress, leading to the activation of adaptive and protective pathways in cells, has been identified as having health-promoting, aging-modulatory, and lifespan-extending effects in organisms. Among the various stress-inducing agents known as hormetins, physical exercise stands out as the paradigm for physiological hormesis. It is not only a well-studied hormetin but is also considered the most prominent stress-inducing hormetic agent (41).

The nomenclature of different hormetins is established based on the primary stress response initiated by a potential hormetin, as illustrated in Figure 2 (41). This conceptual framework helps categorize and understand the diverse agents that induce mild stress and trigger beneficial adaptive responses in cells, contributing to the overall health and longevity of the organism.

Rilmenidine

Rilmenidine, a prescription medication primarily employed in the treatment of hypertension, has shown intriguing properties in geroprotection and potential caloric restriction mimetic effects. Research conducted on the nematode *Caenorhabditis elegans* indicates that administering rilmenidine at both young and older ages can lead to an increased lifespan. It also has a potential caloric restriction mimetic effect, which was found in liver and kidney tissue. Rilmenidine is both an activator of AMPK and an inhibitor of mTOR (42).

Consistent with this, rilmenidine did not extend the lifespan of animals under calorie restriction (42). It also improves plasma lipid and blood glucose in patients with hypertension and metabolic syndrome (43). Further examinations of the potential use of this drug are needed.

Rosmarinic acid and glycation

Rosmarinus officinalis L., commonly known as rosemary, is a natural product with well-established safety. Extensive studies on its antioxidant and anti-aging potential have been conducted through both in vitro and in vivo assays, particularly using the nematode *C. elegans*. In the context of these studies, *R. officinalis* flowers have demonstrated the ability to extend the lifespan of *C. elegans* by up to 18% (44).

Glycation, a significant contributor to the aging process, involves the generation of toxic compounds that contribute to cellular aging. This process is implicated in various conditions, including metabolic disorders, skin aging, and cognitive decline.

HORMETIN	*Primary stress response*	*Examples*
Hormetin - A	Autophagy	Fasting, CR mimetics
Hormetin - D	DNA damage/repair response	Radiation, genotoxic agents
Hormetin - E	Energy deficiency response	Sirtuin activators, flavonoids
Hormetin - H	Heat shock stress response	Physical exercise, celasterol, temperature shock
Hormetin - I	Inflammatory stress response	Microbes, prebiotics, probiotics
Hormetin - O	Oxidative stress response	Pro-oxidants, spices

Figure 2 | Proposed nomenclature of hormetins, adapted from Rattan (41). CR = calorie restriction.

Advanced glycation end-products (AGEs) are the final outcomes of complex chemical reactions, resulting in the formation of intra- and inter-molecular cross-links between proteins. Protein crosslinking is a recognized pathophysiological mechanism contributing to age-related diseases.

Rosmarinic acid, a component of rosemary, has been identified for its anti-glycation properties. Research indicates that rosmarinic acid has the potential to reverse AGE-related crosslinks, presenting a promising avenue for intervention. This property positions rosmarinic acid as a potential treatment for conditions associated with glycation, including diabetes, skin aging, and age-related vascular diseases such as nephropathy, neuropathy, and retinopathy (45).

Calorie restriction and fasting

Calorie restriction, when implemented without malnutrition, has been demonstrated to have anti-aging effects and increase the lifespan in model organisms and rodents. In addition, calorie restriction has been associated with a reduction in the incidence of age-related diseases such as obesity, hypertension, cardiovascular disease, diabetes, and cancer in humans, ultimately contributing to an extended lifespan (46). Despite these positive outcomes, the molecular mechanisms underlying the anti-aging effects of calorie restriction remain unclear, necessitating further research. One challenge associated with traditional calorie restriction diets is the potential for adverse effects, which can limit their widespread application.

Unlike classic calorie restriction diets, a fasting-mimicking diet is a specific meal plan formulated to simulate the fasting state while providing nutrients and calories. Fasting-mimicking diets (FMDs) are emerging as an effective dietary measure that has the potential to improve the health span and decrease the incidence of cancer and other age-related diseases. Unlike chronic dietary restrictions or water-only fasting, FMDs are safer and less challenging options for cancer patients. FMD diets have been shown to increase protection in healthy cells while sensitizing cancer cells to various therapies (47).

Human growth hormone

Low-dose growth hormone (GH) treatment is used for adults with GH deficiency only. Clinical effects include an increase in muscle mass, a decrease in fat mass, an increase in bone density and muscle strength, and improvement of cardiovascular parameters (e.g., a decrease in LDL cholesterol), indicative of a potential halt in premature aging. It is not approved for use in healthy aging patients (48).

Estrogen

Long-term use of estrogen decreases the risk of bone fractures or postmenopausal osteoporosis caused by aging, but it increases the risk of cardiovascular diseases, endometrial cancer, and breast cancer (49).

Administration of estrogen is controversial, and research into its long-term effects is continuing. Physicians that prescribe hormones prescribe only low doses of the drugs, and they are generally only recommended for postmenopausal women that are at a high risk of osteoporosis when non-hormonal treatments are not suitable (50).

Conclusions

Studies of anti-aging drugs and methods are rapidly expanding, attracting significant interest among researchers. Although promising results have been demonstrated in numerous animal studies, human trials are limited due to the potential risks and side effects associated with these interventions. The effectiveness of anti-aging strategies in humans is still not fully understood.

Given the aging global population, there is a growing imperative to discover ways to alleviate the burden of age-related diseases. Researchers play a crucial role in this endeavor, exploring new approaches to enhance the quality of life for aging individuals. However, it is equally important for researchers to maintain a careful balance, considering both the potential benefits and risks associated with these interventions.

As the pursuit of anti-aging interventions continues, it remains essential to prioritize safety and thoroughly evaluate the impact of these approaches on human health. The ongoing exploration of innovative strategies holds promise for addressing age-related challenges, with the ultimate goal of improving the wellbeing and longevity of individuals as they age.

References

1. López-Otín C, Blasco MA, Partridge L, Serrano M, Kroemer G. Hallmarks of aging: an expanding universe. Cell. 2023;186:243–78.
2. Poff AM, Koutnik AP, Egan B. Nutritional ketosis with ketogenic diets or exogenous ketones: features, convergence, and divergence. Curr Sports Med Rep. 2020;19:251–9.
3. Edwards C, Canfield J, Copes N, Rehan M, Lipps D, Bradshaw PC. D-beta-hydroxybutyrate extends lifespan in C. elegans. Aging. 2014;6:621–44.
4. Newman JC, Covarrubias AJ, Zhao M, Yu X, Gut P, Ng CP, et al. Ketogenic diet reduces midlife mortality and improves memory in aging mice. Cell Metab. 2017; 26:547–57.
5. Barzilai N, Crandall JP, Kritchevsky SB, Espeland MA. Metformin as a tool to target aging. Cell Metab. 2016;23:1060–5.
6. Chen S, Gan D, Lin S, Zhong Y, Chen M, Zou X, et al. Metformin in aging and aging-related diseases: clinical applications and relevant mechanisms. Theranostics. 2022;12:2722–40.
7. Kulkarni AS, Brutsaert EF, Anghel V, Zhang K, Bloomgarden N, Pollak M, et al. Metformin regulates metabolic and nonmetabolic pathways in skeletal muscle and subcutaneous adipose tissues of older adults. Aging Cell. 2018;17:e12723.
8. Kulkarni AS, Gubbi S, Barzilai N. Benefits of metformin in attenuating the hallmarks of aging. Cell Metab. 2020;32:15–30.
9. Campbell JM, Bellman SM, Stephenson MD, Lisy K. Metformin reduces all-cause mortality and diseases of ageing independent of its effect on diabetes control: a systematic review and meta-analysis. Ageing Res Rev. 2017;40:31–44.
10. Vancura A, Bu P, Bhagwat M, Zeng J, Vancurova I. Metformin as an anticancer agent. Trends Pharmacol Sci. 2018;39:867–78.
11. Virtanen A, Huttala O, Tihtonen K, Toimela T, Heinonen T, Laivuori H, et al. Therapeutic doses of metformin do not have impact on angiogenesis in presence of sera from pre-eclamptic, IUGR and healthy pregnancies. Pregnancy Hypertens. 2020;22:7–13.
12. Dallaglio K, Bruno A, Cantelmo AR, Esposito AI, Ruggiero L, Orecchioni S, et al. Paradoxic effects of metformin on endothelial cells and angiogenesis. Carcinogenesis. 2014;35:1055–66.
13. Palmer AK, Gustafson B, Kirkland JL, Smith U. Cellular senescence: at the nexus between ageing and diabetes. Diabetologia. 2019;62:1835–41.

14. Han Y, Xie H, Liu Y, Gao P, Yang X, Shen Z. Effect of metformin on all-cause and cardiovascular mortality in patients with coronary artery diseases: a systematic review and an updated meta-analysis. Cardiovasc Diabetol. 2019;18:96.
15. Deng M, Su D, Xu S, Little PJ, Feng X, Tang L, et al. Metformin and vascular diseases: a focused review on smooth muscle cell function. Front Pharmacol. 2020; 11:635.
16. El-Mir MY, Nogueira V, Fontaine E, Avéret N, Rigoulet M, Leverve X. Dimethylbiguanide inhibits cell respiration via an indirect effect targeted on the respiratory chain complex I. J Biol Chem. 2000;275:223–8.
17. Madiraju AK, Erion DM, Rahimi Y, Zhang XM, Braddock DT, Albright RA, et al. Metformin suppresses gluconeogenesis by inhibiting mitochondrial glycerophosphate dehydrogenase. Nature. 2014;510:542–6.
18. Harman D. Aging: a theory based on free radical and radiation chemistry. J Gerontol. 1956;11:298–300.
19. Rubinsztein DC, Mariño G, Kroemer G. Autophagy and aging. Cell. 2011;146:682–95.
20. Du MR, Gao QY, Liu CL, Bai LY, Li T, Wei FL. Exploring the pharmacological potential of metformin for neurodegenerative diseases. Front Aging Neurosci. 2022; 14:838173.
21. Eppenga WL, Lalmohamed A, Geerts AF, Derijks HJ, Wensing M, Egberts A, et al. Risk of lactic acidosis or elevated lactate concentrations in metformin users with renal impairment: a population-based cohort study. Diabetes Care. 2014;37: 2218–24.
22. Wu H, Esteve E, Tremaroli V, Khan MT, Caesar R, Mannerås-Holm L, et al. Metformin alters the gut microbiome of individuals with treatment-naive type 2 diabetes, contributing to the therapeutic effects of the drug. Nat Med. 2017;23:850–8.
23. Blagosklonny MV. Aging and immortality: quasi-programmed senescence and its pharmacologic inhibition. Cell Cycle. 2006;5:2087–102.
24. Ehninger D, Neff F, Xie K. Longevity, aging and rapamycin. Cell Mol Life Sci. 2014; 71:4325–46.
25. Martin B, Mattson MP, Maudsley S. Caloric restriction and intermittent fasting: two potential diets for successful brain aging. Ageing Res Rev. 2006;5:332–53.
26. Carter CS, Khamiss D, Matheny M, Toklu HZ, Kirichenko N, Strehler KYE, et al. Rapamycin versus intermittent feeding: dissociable effects on physiological and behavioral outcomes when initiated early and late in life. J Gerontol A Biol Sci Med Sci. 2016;71:866–75.
27. Sengupta S, Peterson TR, Laplante M, Oh S, Sabatini DM. mTORC1 controls fasting-induced ketogenesis and its modulation by ageing. Nature. 2010;468:1100–4.
28. Chen C, Liu Y, Liu Y, Zheng P. mTOR regulation and therapeutic rejuvenation of aging hematopoietic stem cells. Sci Signal. 2009;2:ra75.
29. Blagosklonny MV. Rapamycin for longevity: opinion article. Aging. 2019;11: 8048–67.
30. Flynn JM, Melov S. SOD2 in mitochondrial dysfunction and neurodegeneration. Free Radic Biol Med. 2013;62:4–12.
31. Gouda NA, Elkamhawy A, Cho J. Emerging therapeutic strategies for Parkinson's disease and future prospects: a 2021 update. Biomedicines. 2022;10:371.
32. Quarles E, Basisty N, Chiao YA, Merrihew G, Gu H, Sweetwyne MT, et al. Rapamycin persistently improves cardiac function in aged, male and female mice, even following cessation of treatment. Aging Cell. 2020;19:e13086.
33. Fu YF, Liu X, Gao M, Zhang YN, Liu J. Endoplasmic reticulum stress induces autophagy and apoptosis while inhibiting proliferation and drug resistance in multiple myeloma through the PI3K/Akt/mTOR signaling pathway. Oncotarget. 2017;8:61093–106.
34. Wu J, Wang A, Wang X, Li G, Jia P, Shen G, et al. Rapamycin improves bone mass in high-turnover osteoporosis with iron accumulation through positive effects on osteogenesis and angiogenesis. Bone. 2019;121:16–28.
35. Tang H, Shrager JB, Goldman D. Rapamycin protects aging muscle. Aging. 2019; 11:5868–70.
36. Lamming DW, Ye L, Sabatini DM, Baur JA. Rapalogs and mTOR inhibitors as anti-aging therapeutics. J Clin Invest. 2013;123:980–9.
37. Chung CL, Lawrence I, Hoffman M, Elgindi D, Nadhan K, Potnis M, et al. Topical rapamycin reduces markers of senescence and aging in human skin: an exploratory, prospective, randomized trial. Geroscience. 2019;41:861–9.
38. Tamaki Z, Asano Y, Kubo M, Ihn H, Tada Y, Sugaya M, et al. Effects of the immunosuppressant rapamycin on the expression of human α2(I) collagen and matrix metalloproteinase 1 genes in scleroderma dermal fibroblasts. J Dermatol Sci. 2014;74:251–9.
39. Johnson SC, Kaeberlein M. Rapamycin in aging and disease: maximizing efficacy while minimizing side effects. Oncotarget. 2016;7:44876–8.
40. Reifsnyder PC, Flurkey K, Doty R, Calcutt NA, Koza RA, Harrison DE. Rapamycin/metformin co-treatment normalizes insulin sensitivity and reduces complications of metabolic syndrome in type 2 diabetic mice. Aging Cell. 2022;21:e13666.
41. Rattan SIS. Physiological hormesis and hormetins in biogerontology. Curr Opin Toxicol. 2022;29:19–24.
42. Bennett DF, Goyala A, Statzer C, Beckett CW, Tyshkovskiy A, Gladyshev VN, et al. Rilmenidine extends lifespan and healthspan in Caenorhabditis elegans via a nischarin I1-imidazoline receptor. Aging Cell. 2023;22:e13774.
43. De Luca N, Izzo R, Fontana D, Iovino G, Argenziano L, Vecchione C, et al. Haemodynamic and metabolic effects of rilmenidine in hypertensive patients with metabolic syndrome X. A double-blind parallel study versus amlodipine. J Hypertens. 2000;18:1515–22.
44. Moliner C, López V, Barros L, Dias MI, Ferreira ICFR, Langa E, et al. Rosemary flowers as edible plant foods: phenolic composition and antioxidant properties in Caenorhabditis elegans. Antioxidants. 2020;9:811.
45. Daniel J, Maryse P, Claude D. Evaluation in vitro of AGE-crosslinks breaking ability of rosmarinic acid. Glycative Stress Res. 2015;2:204–7.
46. Al-Regaiey KA. The effects of calorie restriction on aging: a brief review. Eur Rev Med Pharmacol Sci. 2016;20:2468–73.
47. Blaževitš O, Di Tano M, Longo VD. Fasting and fasting mimicking diets in cancer prevention and therapy. Trends Cancer. 2023;9:212–22.
48. Klatz R, Kahn C. Grow young with HGH. New York: HarperCollins; 1998.
49. Jick H, Walker AM, Rothman KJ. The epidemic of endometrial cancer: a commentary. Am J Public Health. 1980;70:264–7.
50. Marjoribanks J, Farquhar C, Roberts H, Lethaby A, Lee J. Long-term hormone therapy for perimenopausal and postmenopausal women. Cochrane Database Syst Rev. 2017;1:CD004143.