Aging Hallmarks and Anti-aging Strategies: A Landscape of Research Advancement

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Abstract

Aging is a dynamic, time-dependent process characterized by a gradual accumulation of cell damage. Continual functional decline in the intrinsic ability of living organisms to accurately regulate homeostasis leads to increased susceptibility and vulnerability to diseases. Anti-aging research has a long history throughout civilization, with many efforts put forth to understand and prevent the effects of aging. Thus, the major cellular and molecular hallmarks of aging have been identified and multiple strategies aiming to promoting healthy aging and extending the lifespan including lifestyle adjustments, medical treatments, and social programs, have been developed. Here, we use data from the CAS Content Collection to analyze the publication landscape of recent research. We review the advances in knowledge and delineate trends in research advancement on aging factors and attributes, as well as the anti-aging strategies across time, geography, and development pipelines. We also review the current concepts related to the major aging hallmarks on the molecular, cellular, and organismic level, the age-associated diseases, with attention to brain aging and brain health support, as well as the major biochemical processes associated with aging. We further assess the state-of-the-art anti-aging strategies and explore their correlations with age-related diseases. Well-recognized and novel, currently evaluated anti-aging agents have been summarized. Finally, we review clinical applications of anti-aging products with their development pipelines. We hope this review will be helpful for apprehending the current knowledge in the field of aging progression and prevention, in effort to further solve the remaining challenges and fulfil its potential.

Keywords: aging; longevity; antiaging strategy; epigenetic; senescence; inflammaging; telomere; stem cell; brain aging

1. Introduction

The growing social and economic concern of an aging world population has catapulted aging-related research into the spotlight. Indeed, over the last decades, progress in medicine has powered a significant increase in life expectancy worldwide. Thus, over 2 billion individuals are expected to be older than the age of 60 by 2050. [1] This demographic milepost will come with a major increase in age-related diseases, such as Alzheimer's disease, cardiovascular disorders, and cancer, which effectively double in incidence every 5 years passing the age of 60. [2] The relationship between aging and these diseases has triggered fundamental research into the aging mechanisms and approaches to attenuate its effect.

The attributes of aging include a variety of interconnected molecular and cellular mechanisms that act jointly to control the aging process. [3] Thus, aging has been characterized as a progressive degenerative status accompanied by processes like stem cell exhaustion, tissue inflammation, extracellular matrix modifications, cellular senescence, and metabolic dysfunction. [4] These cellular and tissue modifications reflect inherent molecular deviations in mitochondria, epigenetics, DNA maintenance, proteostasis, intercellular interactions, and nutrient sensing, which give rise to genomic instability and impairment, including telomere dysfunction. [4]

Aging is broadly defined as a gradual functional decline in the living organism's intrinsic ability to defend, maintain and repair itself in order to keep working efficiently, and has attracted attention throughout the history of civilization. [5, 6] The health and survival of an organism presents a dynamic equilibrium between the processes of damage and repair, alteration and maintenance, a conventional concept of which is homeostasis. This concept, recently replaced by homeodynamics, involves the constantly changing interrelations of body constituents while an overall equilibrium is maintained. [7] Thus, aging is characterized as a multidimensional process involving a gradual contraction of the homeodynamic space. It affects many different aspects of life, including physical health, cognitive functioning, emotional well-being, and social relationships. Therefore, it is important to consider the multidimensional nature of aging when designing interventions and policies aimed at promoting healthy aging. Furthermore, aging is influenced by multiple factors, including genetics, lifestyle aspects such as diet, exercise, and stress, environmental factors such as pollution and climate change, and social factors including social support and socioeconomic status. Understanding the complex interactions between these factors is essential for promoting healthy aging. [8]

There is a consensus that aging is associated with two key aspects: (i) the progressive decline of numerous physiological processes, such as the body's ability to accurately regulate homeostasis, and (ii) the enhanced risk of developing severe diseases like cancer or cardiovascular disease. However, while aging is a major risk factor for many chronic diseases, it is important to recognize that aging and disease are not synonymous. Many older adults maintain good physical and mental health well into old age, and there is growing interest in promoting "successful aging" by focusing on factors that contribute to overall health and well-being. [9, 10]

Researchers have distinguished between two categories of age: the chronological age based on the birthdate, and the biological age, which measures the true age at which the cells, tissues and organs appear to be, based on biochemistry. [11] While it is impossible to alter the chronological age, there are ways to manage the biological age. There is growing evidence that interventions such as lifestyle adjustments, medical treatments, and social programs can help promote healthy aging and extend the

lifespan. As our understanding of the aging process continues to advance, it is likely that new interventions will emerge that can further improve health and well-being in later life.

Along with the whole organism, the functional capabilities of the **brain** gradually degrade upon aging, manifesting as declines in learning and memory, attention, decision-making capacity, sensory perception, and motor management. The aging brain exhibits significant indicative signs of impaired bioenergetics, weakened adaptive neuroplasticity and resilience, anomalous neuronal network activity, dysfunctional neuronal calcium homeostasis, accumulation of oxidatively modified molecules and organelles, and inflammation. [12-18] Reduced number and maturity of dendritic spines in aging organisms, along with alterations in synaptic transmission, may indicate abnormal neuronal plasticity directly related to impaired brain functions. [16] At worst, a neurodegenerative and cerebrovascular disease, which strongly damages the basic functions of the brain, may develop. Thus, age-associated alterations render the aging brain vulnerable to degenerative disorders including Alzheimer's and Parkinson's diseases, stroke, and various kinds of dementia. [19, 20] While currently there is no cure for these age-related brain disorders, early detection by recognizing their symptoms can help slow the progression of the disease.

In fact, most vital organs and tissues of the body undergo certain age-related decline in function. Thus, muscle strength decays with age, bones weaken losing mass and/or density, skin exhibit visible changes, and also may show signs of impaired wound healing. Blood level of certain hormones (e.g., growth hormone, androsterone, testosterone) decline with age, while others (e.g., gonadotropins, insulin) increase with age. Overall immune function deteriorates, becoming slower to respond, leading to an increased susceptibility to various infectious diseases. Sleep worsens, certain sleep disorders develop. Vision and hearing decline. Kidney tissue decreases and kidney function diminishes, along with multiple other age-related changes. [10, 21-23]

The efforts to understand and prevent the effects of aging date back centuries, so the anti-aging research has a long history (Figure 1). In ancient times many cultures developed traditional remedies and practices aimed at promoting longevity and slowing the aging process. For example, ancient Chinese medicine includes herbal remedies and acupuncture techniques designed to promote health and longevity. In 16th century Italian physician and philosopher Girolamo Cardano wrote a treatise on aging and longevity, in which he discussed the physical and mental changes that occur with age and proposed strategies for promoting health and extending lifespan. [24] In 19th century, French physiologist Claude Bernard proposed that aging is caused by changes in the internal ambiance of the body, including changes in metabolism and the accumulation of toxins. [25] In the early 20th century, scientists began to study the effects of diet and lifestyle on aging, with researchers like Elie Metchnikoff proposing that the gut microbiome plays a role in aging. [26] The free radical theory of aging was proposed by Denham Harman, who suggested that aging is caused by damage to cells and tissues by unstable molecules called free radicals. [27]

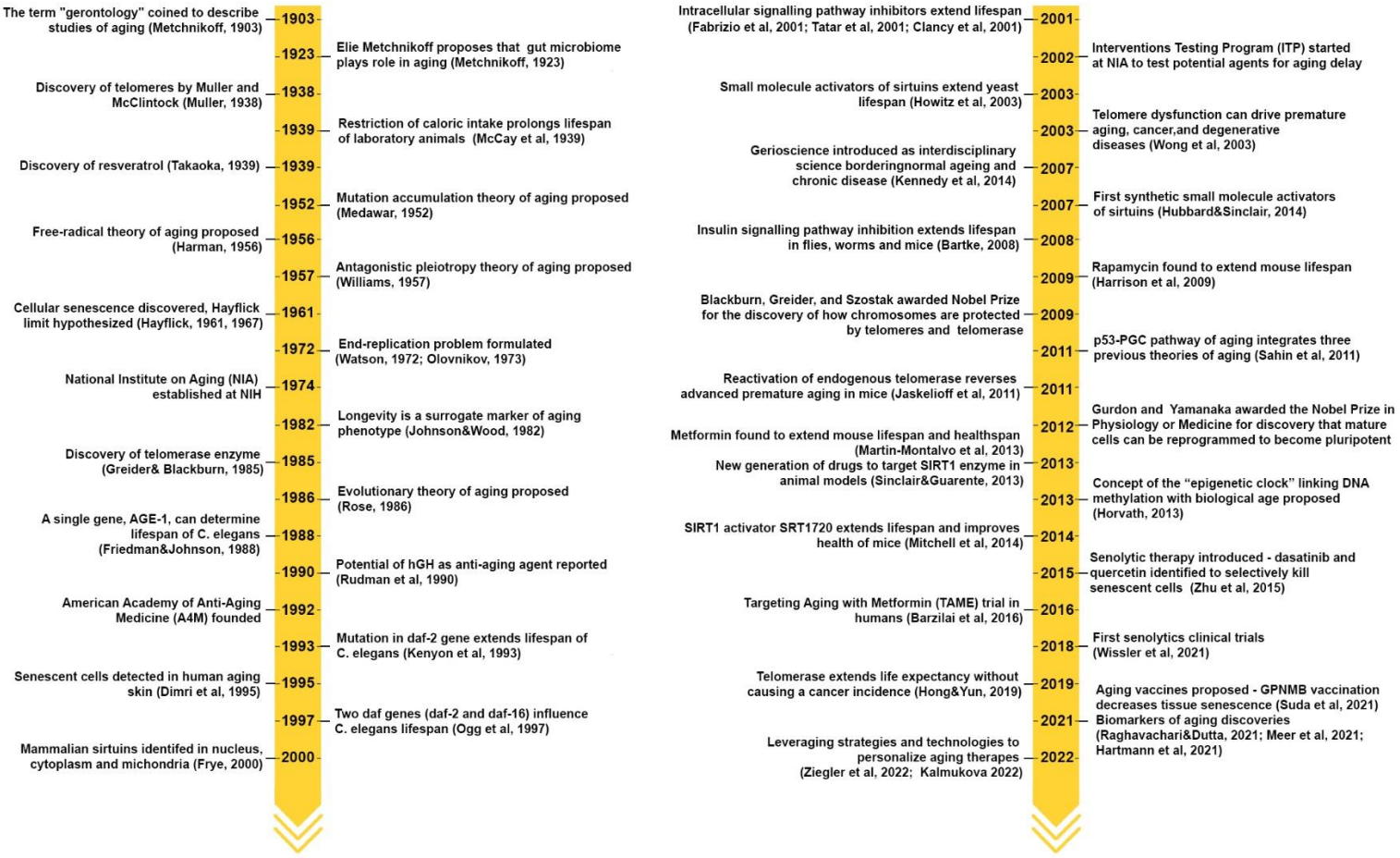


Figure 1. Timeline of key events and discoveries in anti-aging research. [28-78]

An early step in the field of aging exploration has been the observation that restriction of caloric intake increases lifespan – first demonstrated in mice and rats [30], and later in other species as well, including primates. [79, 80] Moreover, it was detected that dietary restrictions enhanced not only the lifetime but also the healthspan, suppressing the development of age-related diseases. [81] Thus, a concept emerged that correlation exists between lifespan and healthspan, described as the portion of lifespan free from disease. [5] Mutation accumulation theory that aging is a result of decline in the power of natural selection after reproduction was proposed in 1952. [32] Over 30 years later, landmark research in the nematode *Caenorhabditis elegans* demonstrated that the mutant nematode strain exhibits a 40-60% extended lifespan, showing that a single gene, the AGE-1 gene, can control the lifespan of an organism. [43]

The close link between telomeres and aging originates in the discovery of a unique structure at the last portions of the *Zea mays* and *Drosophila melanogaster* chromosomes, hypothesized to play a significant role in preventing chromosome end fusion. [29, 82] In the early 1960s, Hayflick noted that human cells in tissue culture cease dividing after a certain number of cell divisions by a process termed replicative senescence. [34, 35] It was shown later that human fetal cells exhibited finite replication

potential of 50-60 doublings, labelled as replicative senescence or the ‘‘Hayflick limit”. [34, 35] The link between the ending of cell division and replication of telomeres was revealed in the early 1970s. [36, 37] It was established that telomere attrition takes place in parallel with the replication lifespan of human primary cells, thus demonstrating that shortened telomeres cause the Hayflick limit. [83] It took nearly two decades for the suggested causative relationship between replication senescence and telomere shortening to be established. [84, 85] Thus, the aging of cells could be associated with alterations in telomere length on genomic DNA. [86, 87] Further on, via animal models, the function of telomeres in aging was authenticated and identified as an essential signaling pathway guiding the aging process [3] Telomere dysfunction was shown to accelerate signs and symptoms of aging. [88, 89] Later, transcriptomic studies revealed the p53 aging pathway, promoting apoptosis, thus integrating three previously distinct theories of aging: genotoxicity (telomere dysfunction), oxidative damage, and mitochondrial decay. [62, 90, 91] Reactivation of endogenous telomerase, an enzyme that could extend telomere sequence, was demonstrated to reverse advanced premature aging in mice. [63] Noteworthy, adenoviral delivery of telomerase in aged mice was demonstrated to enhance cardiac function after acute myocardial infarction, improve muscle coordination and kidney and liver performance, reduce insulin resistance and subcutaneous fat reduction, increase bone mineral density, and extend life expectancy without triggering a growth in cancer frequency. [3, 72]

In the early 1990s, the potential of human growth hormone (hGH) as an anti-aging agent acting to increase lean body mass, decrease adipose tissue mass, and increase bone mineral density was reported [44], and hGH supplements became available, sparking controversy questioning their safety and effectiveness. Later on, aging research was focused on the genetic pathways of aging, revealing a complex system of intracellular signaling pathways and higher-order processes. [5, 92]

Throughout the 1990s, researchers identified genes that are linked to aging, including the SIRT1 gene. A substantial attention to sirtuins was attracted after it was reported that overexpression of SIR2 gene can prolong yeast lifespan by as much as 70%. [93] In the 2000s, advances in biotechnology and genetics lead to the development of new anti-aging therapies, including stem cell therapy, gene therapy, and telomerase activation. [53, 54, 56, 58, 59] In the 2010s and 2020s, the focus of anti-aging research shifted towards advanced understanding of the mechanisms of aging and developing interventions that can slow or reverse the aging process, with ongoing studies exploring a range of potential interventions, including senolytics (drugs that clear out senescent cells), metformin (a diabetes drug that may have anti-aging properties), and various forms of gene therapy. [62, 63, 67-78] Advances in gene editing technology, such as CRISPR/Cas9, allow for more precise manipulation of genes involved in the aging process. [94] It is important to note that while anti-aging research has made significant strides over the years, most of these researches are still in the non-human systems and there is still much that is not yet understood about the aging process and how to effectively prevent or reverse its effects.

The aging research area is still moderate in size, by an order of magnitude less for the number of related publications relative to more advanced areas, including those focused specifically on major age-associated diseases such as cancer, cardiovascular, and Alzheimer’s disease. However, understanding that advanced age is the major risk factor for all of these disorders has brought the rapidly growing field of aging research to the forefront.

The research focused on aging mechanisms and attributes, and the anti-aging strategies and medical interventions, has undergone a steady growth, especially intense in the last decade (Figure 2).

Indeed, the desire for enjoying a lifespan in a healthy, youthful condition is a universal human appeal. A characteristic feature of anti-aging medicine is that it is proactive rather than reactive, trying to avoid disease via health preservation rather than cure disease after it occurs. Its basic approach is replacement therapy. [95] Since appropriate knowledge and expertise allowing to slow or halt the homeostatic decline in basic informational, regulatory, and protective molecules upon aging has not yet been established, replacement of those products such as hormones, cofactors, antioxidants, and others, is routinely employed. Such a proactive approach meant to slow down or escape the development of age-associated disease is more rational than a reactive, symptomatic approach. [95]

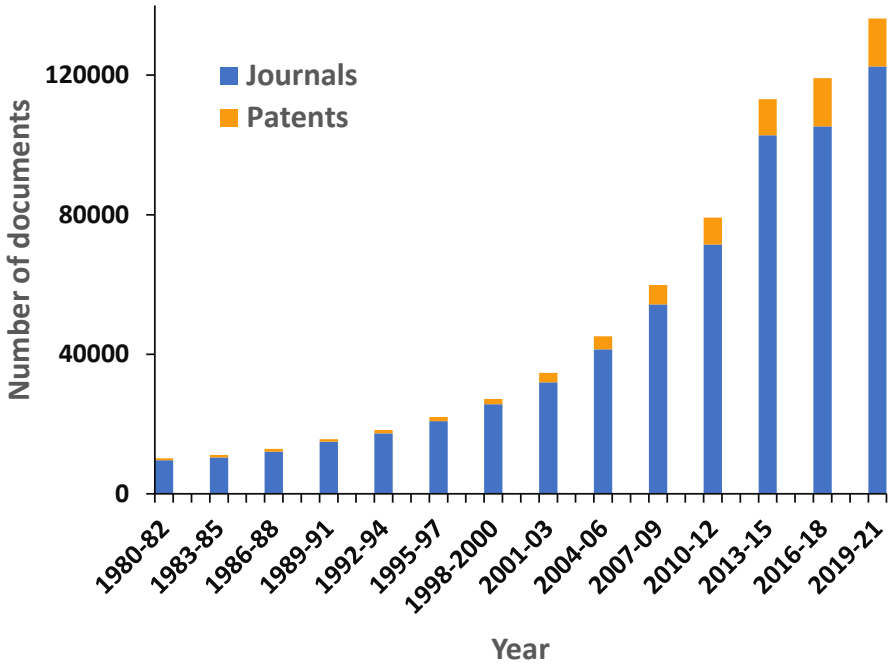


Figure 2. Yearly growth of the number of documents (patents and non-patents) in the CAS Content Collection™ related to the aging mechanisms and anti-aging strategies.

Various studies have revealed how aging takes place and how it is controlled by cellular and molecular mechanisms. Factors influencing the aging process and longevity have been identified including telomere shortening, mitochondrial dysfunction, oxidative stress, deregulated nutrient-sensing, DNA repair deterioration, DNA damage, protein homeostasis alterations resulting in accumulation of misfolded proteins, and changes in epigenetic control. [96-99]

In this paper, we review the advances in the research on aging factors and anti-aging strategies. We examine data from the CAS Content Collection [100], the largest human-compiled collection of published scientific information, and analyze the publication landscape of recent research in this area in effort to provide insights into the research advances and developments. We review the current concepts related to the major aging hallmarks on the molecular, cellular, and organismic level, age-associated diseases, as well as the major biochemical processes associated with aging. We further assess the state-of-the-art anti-aging strategies and explore their correlations with age-related diseases, based on the data from the CAS Content Collection. Well-known and currently examined anti-aging

agents have been specified. Finally, we review clinical applications of anti-aging products with their development pipelines. We hope this review will be helpful for apprehending the current knowledge in the research field related to aging mechanisms and anti-aging strategies, for evaluating challenges and growth opportunities, in effort to further solve the remaining challenges and fulfilling its potential.

2. Mechanisms and physiology of aging

Aging is typified by a gradual loss of physiological fitness, leading to deteriorated functions and enhanced vulnerability. Such deterioration is the key risk factor for critical pathologies such as cancer, diabetes, cardiovascular and neurodegenerative disorders, and many other maladies. Various studies have examined how aging takes place and how it is controlled by sophisticated cellular and molecular mechanisms at different periods of life. [97, 98] Multiple factors involved in the aging process and longevity have been described. The finding that the rate of aging is regulated, at least to a certain extent, by genetic routes and biochemical processes conserved in evolution has triggered remarkable advances in aging research. Aging is basically damage that accumulates over time and is manifested in certain physiological forms, considered as the hallmarks of aging. [4, 96, 101-104]

A hallmark of aging is a distinct attribute that occurs upon normal aging and correlates with the decline in biological function and increased risk of age-associated diseases. Moreover, in order to qualify as a hallmark, the attribute needs to play a causative role in the process of aging. [4, 103] The definition of nine molecular and cellular hallmarks of aging in 2013 provided a background framework to channel future aging research. [4] These hallmarks included:

- Genomic instability: It refers to the accumulation of DNA damage and mutations over time, which can lead to a variety of age-related diseases such as cancer and neurological disorders.
- Telomere attrition: Telomeres are the protecting caps at the chromosome ends, which shorten with each cell division. This process is associated with cellular senescence, a state of permanent growth arrest that can contribute to aging.
- Epigenetic alterations: Changes in gene expression patterns that are not caused by changes in the DNA sequence itself. These changes can contribute to the development of age-associated diseases.
- Loss of proteostasis: Refers to the gradual breakdown of protein homeostasis or the ability of cells to maintain their proper protein folding and degradation.
- Dysregulated nutrient sensing: The body's ability to sense and respond to changes in nutrient availability becomes impaired with age, which can lead to metabolic dysfunction and an increased risk of age-associated diseases such as diabetes.
- Mitochondrial dysfunction: Mitochondria are the powerhouses of the cell and play a critical role in energy production. With age, mitochondrial function can decline, leading to decreased energy production efficiency and more oxidative stress, therefore an increased risk of age-related diseases.
- Cellular senescence: As mentioned earlier, cellular senescence is a status of permanent growth arrest that can contribute to the aging process.

- Stem cell exhaustion: Stem cells have the capability to differentiate into various different cell types and play a critical role in tissue repair and regeneration. With age, the number and function of stem cells can decline, leading to decreased tissue repair and an increased risk of age-related diseases.
- Altered intercellular communication: The process of aging implicates alterations at the level of intercellular interaction, including endocrine, neuroendocrine, or neuronal communication. Thus, neurohormonal signaling is likely to be deregulated in aging thereby modifying the mechanical and functional properties of all tissues.

The first four hallmarks – genomic instability, telomere attrition, epigenetic alterations, and loss of proteostasis, have been classified as primary hallmarks because they are the primary causes of cellular damage (Figure 3). [4, 104] They are all, unequivocally, negative. The next three hallmarks – dysregulated nutrient sensing, mitochondrial dysfunction, and cellular senescence – are classified as antagonistic because they are related to the responses to the primary hallmarks. Contrary to the primary hallmarks, antagonistic ones have multidimensional effects – initially these responses mitigate the damage caused by the primary hallmarks, but eventually become harmful themselves. For example, cellular senescence, or cell cycle arrest, can protect the organism from cancer, but also promote aging. The last two hallmarks – stem cell exhaustion and altered intercellular communication – have been characterized as integrative since they take place when the accumulated damage, resulting from the primary and antagonistic hallmarks cannot be compensated by the cellular homeostatic mechanisms and reparative processes. [4, 104] Integrative hallmarks further promote deterioration of cells that are responsible in due course for aging. Both of these hallmarks influence tissue homeostasis and function.

As a result of the scientific research progress, additional aging attributes have been identified with time. A decade after the initial nine hallmarks were suggested, an additional five aging characteristic features surfaced [103, 105], including:

- Compromised autophagy
- Microbiome disturbances (dysbiosis)
- Splicing dysregulation
- Chronic low-level inflammation (inflammaging)
- Mechanical properties alterations

Recently, additional mechanisms of aging related to lipid metabolism have been implied:

- Accumulation of sphingolipids. Ceramides, a common class of sphingolipids, accumulate in aging muscle and reduce its function, impacting the functional capacity of older adults. [106-108]
- Dysregulation of cholesterol metabolism. Senescent cells pile up cholesterol in the lysosomes to support the senescence-associated secretory phenotype (SASP). [109]

Furthermore, multiple other common physiological features of aging have been identified and examined in research publications:

- Decline in immune function: Upon aging, the immune system becomes less effective at fighting off infections and diseases, which can lead to an increased risk of infections and certain types of cancer. [110, 111]

- Hormonal changes: The levels of many hormones in the body change upon aging, e.g., the levels of growth hormone, testosterone, and estrogen tend to decrease, while levels of cortisol (the stress hormone) tend to increase. [112-114]
- Changes in body composition: With age, there is a tendency to lose muscle mass and gain fat. This can increase the risk of metabolic disorders such as type 2 diabetes and cardiovascular disease. [115-117]
- Decreased cognitive function: Cognitive decline has been widely observed upon aging, particularly in areas such as memory and processing speed. [118-120]
- Increased risk of falls and fractures: With aging, bones become weaker, and balance may decline, increasing the risk of falls and fractures. [121-123]

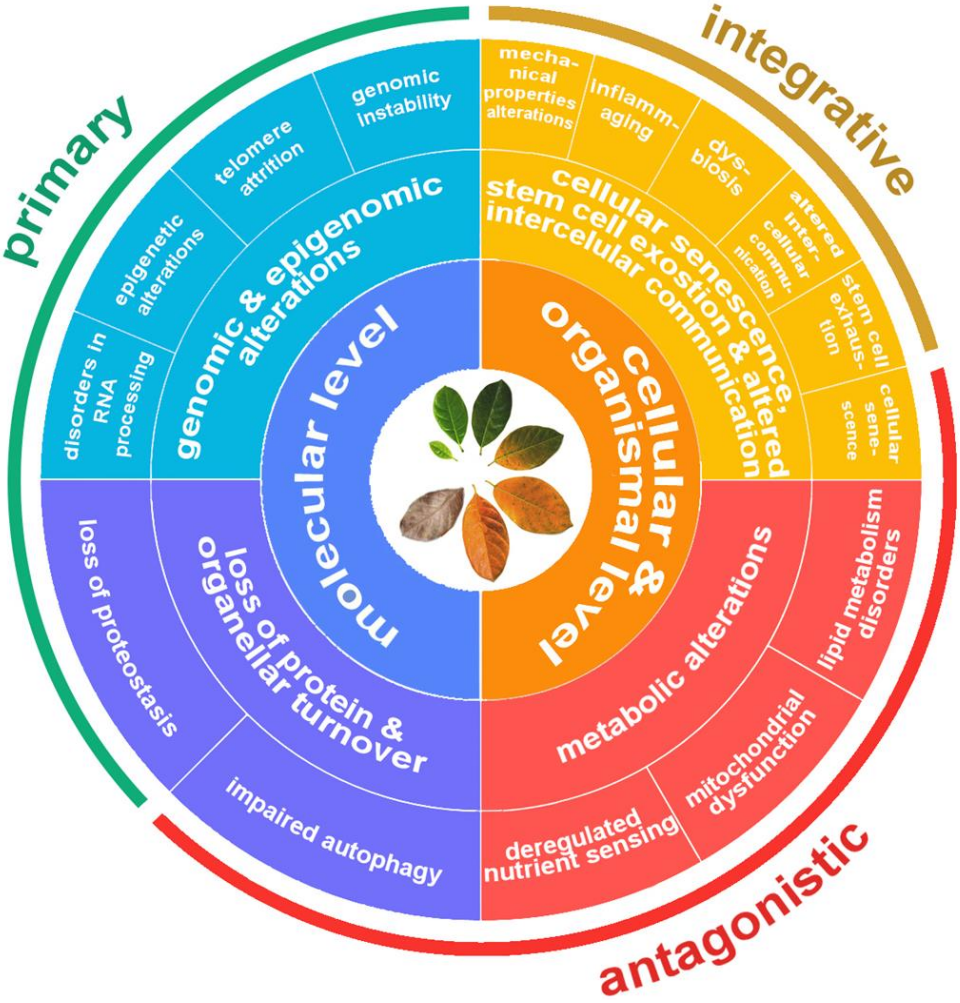


Figure 3. Scheme of the currently considered hallmarks of aging along with their classification.

2.1. Genomic and Epigenomic Alterations

- Genomic instability

Accumulation of genetic impairment over a lifetime is a common feature of aging. Indeed, the human genome is under continual challenges by DNA-destructive attacks, which endanger cellular homeostasis. These include various exogenous physical, chemical, and biological threats such as viruses, UV damage, and chemicals, as well as endogenous hazards such as DNA replication inaccuracies, spontaneous hydrolytic reactions, and reactive oxygen species (ROS). [124] Thus, **somatic mutations of the nuclear DNA** accumulate within cells of aged organisms. [125] **Mutations and deletions in mitochondrial DNA** may also contribute to aging. [126] Accumulated defects in the **nuclear lamina** are another possible source of genomic instability, except for the genomic damage affecting nuclear or mitochondrial DNA. [127] Further, cell cycle stress, alterations in gene expression and gene regulation take place as a direct consequence of genomic instability. Eventually, it results in age-related cellular degeneration and functional decay. Aging and degenerative disease happen as the ultimate outcome of genomic instability. [128] Thus, there is extensive evidence that **genomic damage** accompanies and is causatively related to aging. The produced genetic damages are diverse, including impairments such as point mutations, translocations, telomere shortening, and others. [4] To counteract these DNA damages, organisms have developed repair mechanisms such as specific processes for maintaining the appropriate length and functionality of telomeres and for ensuring the integrity of mitochondrial DNA. [129-131]

- Telomere attrition

Although DNA damage accumulation with age involve the genome generally, certain regions of the chromosomes, such as **telomeres**, are especially vulnerable to **age-associated deterioration** (Figure 4A). [130, 132] Telomeres are chromatin structures at the distal ends of chromosomes, including conserved microsatellite repeats TTAGGG, which cap and protect the end of a chromosome from recombination and degradation. [133] They allow the chromosome to replicate properly during cell division. The telomere length in humans at birth is ~10,000 – 19,000 base pairs. [134] They are known to shorten during cell division, as a result of imperfect replication, losing ~50 – 200 base pairs per cell division. [83, 135, 136] Such gradual telomere shortening restricts the number of times a cell can divide. It is considered to act as a 'molecular clock' correlated to organism aging. [137, 138] Telomere length is one of the biomarkers of aging and biological age. Specifically, telomere shortening below a critical length causes telomere protection deficiency, chromosomal instability, and weakened cell viability. This excludes germ cells and certain cancerous cells which are known to express high levels of telomerase, thus avoiding significant telomere shortening and supporting cell viability. [132, 138]

Telomerase is a specialized DNA polymerase with the ability to replicate the distal ends of DNA molecules. Telomerase is highly expressed in embryonic stem cells, however, it is not expressed in most mammalian somatic cells, which results in cumulative loss of telomere-shielding sequences at the chromosome ends. [139] Such telomere exhaustion explicates the limited proliferation ability of some cells cultured *in vitro*, the so-called Hayflick limit specified as the number of times a normal somatic cell population will keep dividing until cell division halts. [35] Studies on genetically modified animals have reported causative relationships between telomere loss, cellular senescence, and aging, indicating that aging can be reverted by telomerase activation. [4] Thus, normal aging is associated with **telomere attrition**, while pathological telomere dysfunction accelerates aging. [140]

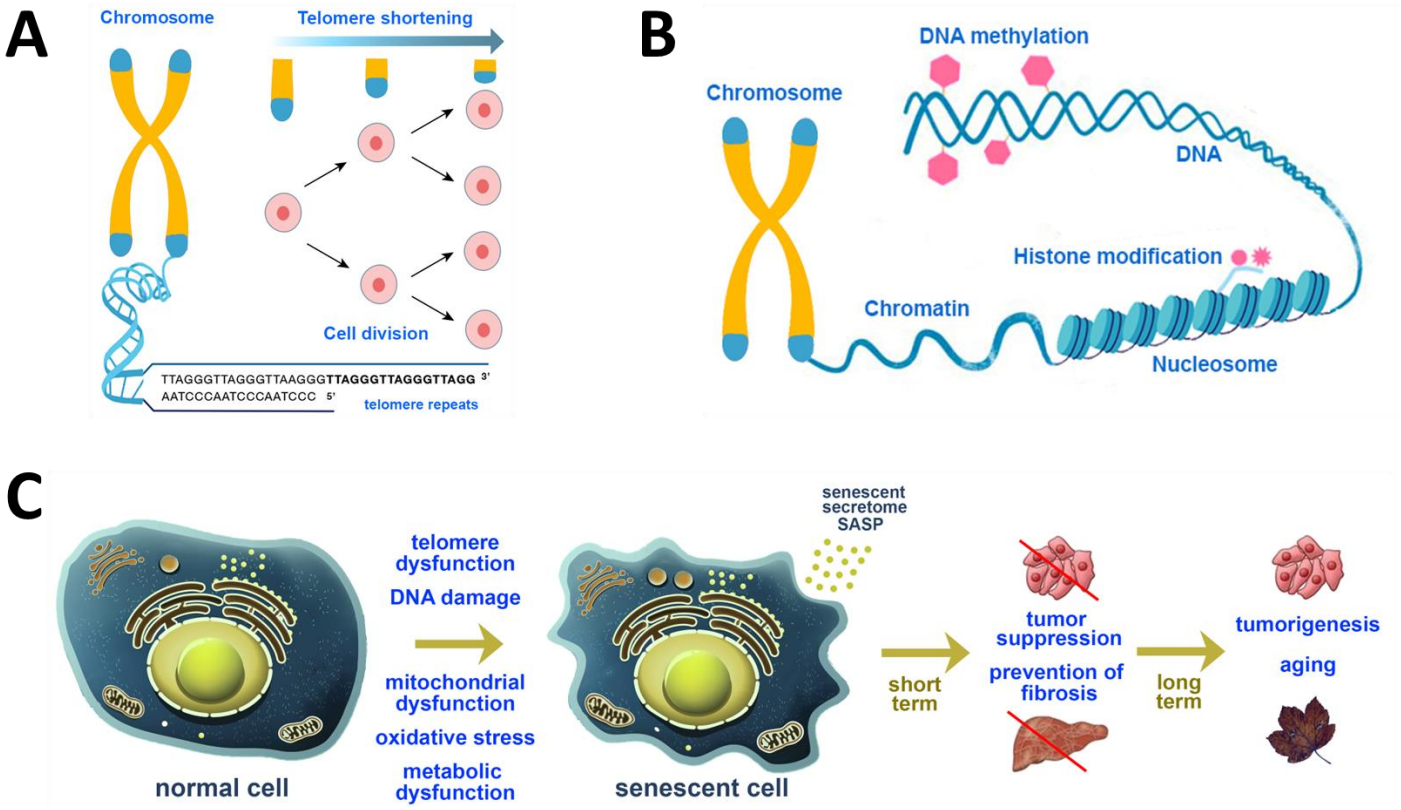


Figure 4. (A) Scheme of the structures of chromosomes and telomeres. At their ends chromosomes exhibit repeated base segments called telomeres, which truncate with each replication cycle. Telomeres are known to shorten upon cell division, as a result of incomplete replication. (B) Scheme of the common epigenetic alterations including DNA methylation and histone modifications. (C) Schematic presentation of normal cell and senescent cell, secreting senescence-associated secretory phenotype (SASP). In short term, senescence growth arrest prevents tumorigenesis and fibrosis. The loss of proliferative capacity, which accompanies senescence, impairs tissue regeneration and stimulates aging. SASP can promote tumor growth and progression by stimulating angiogenesis and extracellular matrix remodeling.

Telomeres are particularly predisposed to age-associated deterioration because of the existence of complexes termed **shelterins**. [141, 142] The key function of shelterins is forming T-loops at the ends of chromosomes, which safeguard telomeres by avoiding them being identified as DNA damage by the DNA polymerase. This has the undesirable effect though of making it difficult for DNA polymerase to repair telomere damage. [4] Thus, both extending telomeres and developing a mechanism to repair DNA damage in telomeres is required in order to deal with this aging pathway.

- Epigenetic alterations

Although a great amount of research has been devoted to the genetic factors that directly affect aging, nongenetic control of aging has gained considerable interest lately as an important aspect in

getting awareness of the process of aging. Nongenomic modifications that affect gene expression and modify the chromatin structure are referred to as epigenetic alterations, and are generally defined as alterations in genomic regulation not directly encoded by DNA, i.e., alterations that do not change the DNA sequence, but instead control gene operations. [143] Such changes take place when methyl groups are added to or removed from DNA (DNA methylation/demethylation), when post-translational modifications are made to the histones, and upon chromatin remodeling (Figure 4B). These changes may occur upon aging and/or exposure to environmental factors, they can be also inherited. They can also be called epimutations. [144] The enzymatic systems ensuring the generation and maintenance of epigenetic alterations include the enzymes DNA methyltransferases, histone acetylases, deacetylases, methylases and demethylases. They also include proteins involved in chromatin remodeling. [4, 145] Epigenetic alterations are profoundly involved in the process of aging, resulting in disturbances in the wide-ranging genome architecture, thus understanding the epigenome holds promise for amending age-related pathologies and prolonging healthy lifespan. [146, 147] In addition to age-related epigenomic changes, many other systems become dysfunctional with age. [4, 103, 105] Noteworthy, chromatin and transcription regulation have been identified to play major roles in the age-associated symptoms of these aging hallmarks. [148] Given the reversible nature of epigenetic pathways, their understanding provides a promising approach for therapeutics against age-related decline and disease. [149]

As mentioned above, epigenetic alterations take place when methyl groups are added to or removed to a cytosine base (C) of DNA or when modifications are made to proteins called histones that bind to the DNA in chromosomes. DNA methylation produces DNA condensation, the form in which genes are not being transcribed. DNA methylation in mammals primarily occurs on the C5 of the cytosine base (5 methylcytosine, 5-mC) of CpG dinucleotides. [150] Nearly 70-80% of CpG dinucleotides are methylated in somatic cells. Methylation levels change throughout life, but generally tend to decrease upon aging. Global 5-methylcytosine (5mC) variations have been first described during aging in rats. [151] DNA methylation affects a wide range of developmental and pathological processes. Further on, vast literature has documented genome-wide DNA methylation changes that occur in response to aging across multiple species. [152]

Although aging is largely correlated with changes in DNA methylation, the relationship between DNA methylation and aging is complicated. The general trends involve largescale hypomethylation (non-CpG islands) and regions of hypermethylation (primarily CpG islands) upon aging. [153, 154] It is currently believed that DNA methylation biomarkers can verify biological age throughout the entire human lifespan. This phenomenon, known as epigenetic clock, is based on CpG sites (cytosine and guanine bases separated by only one phosphate group in the DNA sequence) associated with age and the methylation profile of which can be used as an accurate indicator of biological age. [67, 155, 156] Moreover, DNA methylation-based clocks are suggested as biomarkers of early disease risk and as forecasters of life expectancy and mortality. [152, 157] Thus, the Horvath Clock defines a pattern of DNA methylation changes and considers the global decline in genomic CpG methylation as a well-documented predominant event in aging. [67, 158, 159] A strong causative link between DNA and H3K9 methylation and aging is considered likely [160-162], however, the mechanisms underlying age-related DNA methylation alterations and aging mechanism are yet to be fully understood.

Histones are a family of basic proteins that provide structural support for the chromosomes. DNA winds around them to form nucleosomes, which are then wrapped into the chromatin fibers. In addition to their role in compacting genomic DNA into nuclei, histones also perform a structural function

in regulating gene expression. [143] An important feature of histone biology is their ability to acquire a large set of post-translational modifications that modulate their interaction with DNA or chromatin-associated proteins. Especially, the H3 and H4 histones, which exhibit long tails protruding from the nucleosome, which can be covalently modified at several places (Table 1). These modifications have been reported to be important in gene expression profiles. [163] Histone modifications also affect transcriptional accuracy, it is therefore conceivable that the observed loss in transcriptional precision with aging is causally related to histone modifications. [164] The major modifications that impact them are methylation and acetylation (addition of an acetyl chemical group -$COCH_3$). Methylation abnormalities result in enhanced cancer relapse with low survival rate. Alterations in methylation upon aging are accompanied by a loss in the acetylation level of certain histones (hypoacetylation). Animal model studies have reported that prevention of age-related hypoacetylation inhibits cognitive impairment and moderates illnesses such as Parkinson's disease, osteoporosis, and stroke. [96, 165]

Alterations in **histone methylation** has been considered as another characteristic feature of aging. In cases in which histone methylation has been found to impact aging, it does so by regulating transcription, categorizing it as a major mechanism of its action. Moreover, histone methylation regulates or is regulated by additional cellular pathways that contribute to or prevent aging. [166] Cells from aged organisms show a large-scale loss of histones, specifically a gradual loss of histone H3 trimethylation at lysines 9 and 27 (H3K9me3 and H3K27me3), which are considered as repressive marks that promote chromatin compaction. Another trend is the increase in "activating" histone marks (H3K4me2/3, H3K36me3). [4, 96, 166] Some of these trends are exemplified in Table 1. Furthermore, it is worth noting that the role of histone methylation in aging is only starting to be appreciated.

Table 1. Age-associated changes in histone methylation

Organism	Histone Modification	Change
Mammals (mouse, rat, macaque) [166-169]	H4K20me3	↑
	H3K27me3	↑
	H3K79me1/2	↑
	H3K4me2	↑
	H4K20me1	↓
	H3K36me3	↓
Humans (HGPS*) [166, 170, 171]	H4K20me3	↑
	H3K9me3	↓
	H3K27me3	↓

* HGPS, Hutchinson-Guilford progeria syndrome

Histone acetylation is a foremost regulator of transcription. It is known to promote transcription by reducing electrostatic interactions between DNA and histones, and between neighboring nucleosomes.

[172] Histone acetylation is largely believed to function mostly via the cumulative charge effects of multiple acetylation event. [173-175] Recent studies suggest limited selectivity of acetylation-directed antibodies – thus, most acetylation antibodies exhibit a polyacetylation bias and most H3 and H4 acetylation antibodies only barely discriminate among single individual acetylation events. [164, 176]

Chromatin is a macromolecular complex of DNA and histone proteins that forms chromosomes within the nucleus of the eukaryotic cells. It is a dynamic structure existing either as a compact and transcriptionally inactive heterochromatin or a decondensed transcriptionally active euchromatin (Figure 4B). [177, 178] **Chromatin remodeling** refers to the reorganization of chromatin from a condensed state to a transcriptionally accessible state, permitting transcription factors or other DNA binding proteins to access DNA and manage gene expression. [179] The chromatin structure is reorganized by means of several mechanisms, including histone modification, histone tail separation, and ATP-dependent chromatin remodeling. [180, 181] The chromatin status can be modulated by environmental factors, which further modify the expression of genes related to aging and longevity. [182, 183]

Non-coding RNAs, including long non-coding RNAs (lncRNAs), microRNAs (miRNAs), and circular RNAs, are important regulators of transcriptional networks and chromatin states; they have appeared as epigenetic factors that affect aging. Thus, lncRNAs stimulate gene silencing through interactions with chromatin-modulating enzymes and are emerging as important factors in the progression of aging. [184, 185] Non-coding RNAs are able to modify healthspan and lifespan by post-transcriptional regulation of stem cell behavior. [186] Overall, studies suggest that RNAs may inherently impact aging and aging-related pathologies, and represent likely therapeutic targets for deferring or ameliorating these pathologies.

Epigenomic changes, including modifications in transcription factors, histone features, nucleosome placement, and DNA methylation, are interrelated with the other hallmarks of aging. [4, 56, 187] Epigenomic changes are able to activate the emergence of other hallmarks of aging and can also be influenced by them. [148]

Disorders in RNA processing

Robust alterations in expression pathways with advancing age have been reported, with a great part of these pathways controlling messenger RNA splicing. Furthermore, interventions that reverse senescent phenotypes help in restoring youthful patterns of splicing factor expression. [188] It is believed that alterations in RNA processing add an additional level of gene expression regulation over those of genome integrity, transcriptional efficiency and epigenetic regulation that have been already recognized to change during aging. Thus, dysregulation of RNA management regulation in aging human population has been identified as a characteristic aging feature. [189]

2.2. Loss of protein and organellar turnover

- Loss of proteostasis

Proteostasis denotes protein homeostasis, which includes the maintenance of stable functional proteins. Upon aging, proteostasis weakens. Aging cells accumulate misfolded and impaired proteins as a result of the functional decay in their protein homeostasis (proteostasis) mechanism, causing a lowered cellular viability and the development of protein misfolding diseases generically known as proteinopathies or protein conformational diseases, such as Alzheimer's and Huntington's diseases. [190]

The main participants in proteostasis preservation are the chaperones, the ubiquitin-proteasome, and the lysosome-autophagy proteolytic systems. They take care of misfolded proteins, whether being refolded into the original stable conformation or being eradicated from the cell through proteolysis. [191, 192] Chaperones help *de novo* synthesized proteins and unfolded proteins to achieve their stable folded status. If folding happens to be unachievable, chaperones target the unfolded/misfolded protein for degradation by the proteasome or in lysosomes. The elimination of the misfolded proteins from the cytosol takes place either by degradation in lysosomes through autophagy or expulsion outside the cell by means of exosomes. [192] All these systems function in a synchronized way to restore the structure of misfolded proteins or to remove and degrade them entirely, thus preventing the accumulation of damaged materials. However, multiple studies have revealed that proteostasis is changed with aging. [193]

Chaperones accompany and safeguard proteins through each of their conformational changes including *de novo* folding, assembly and disassembly, transport through membranes, and targeting for degradation. [194] Once targeting for degradation takes place, chaperones may decide which proteolytic pathway the misfolded protein will follow: through the proteasome – a multi-subunit protease accountable for the degradation of proteins often tagged with ubiquitin, or through autophagy in the lysosomes. Certain age-related cellular alterations can influence chaperoning activities. The loss of chaperone function and a decline in their availability further worsen the difficulties with protein quality control. Improper age-related modifications in the substrate protein can also obstruct the chaperones capability to recognize its target. Thus, accumulation of advanced glycation end-products via non-enzymatic alterations on long-lived proteins upon aging disturbs the normal chaperone activity (Table 2). [192]

Proteasome activity and autophagy have also been reported to decline with aging. [195, 196] Stimulating proteasome or autophagy activity by overexpressing proteasome subunits or essential autophagy genes enhances lifespan and imparts resistance to stress in S. cerevisiae, C. elegans, and D. melanogaster. [197, 198] Evidence of such interventions in mammals is also emerging. [192] Examples exist showing that genetic manipulations can improve proteostasis and delay aging in mammals. [199]

Table 2. Mutations in proteostasis systems and associated age-related diseases [192]

Proteostasis system mutations	**Age-related disease**
Chaperone mutations	
α-Crystallin	Early cataracts, desmin-associated myopathy, cardiomyopathy
DNAJB6	Hereditary myopathy
HSC70	Cardiovascular disease
HSJ1	Motor neuropathy (distal hereditary, dHMN)
HSP22, HSP27	Charcot-Marie-Tooth disorder
Sacsin	Spastic ataxia
Ubiquitin-proteasome system mutations	
Ataxin-3	Machado-Joseph disorder
PSMB8	Nakajo-Nishimura syndrome

Ubiquilin-2	Amyotrophic lateral sclerosis (ALS)
UCHL1	Parkinson's disease
VCP/p97 (ERAD)	Paget's disease, frontotemporal dementia
Autophagy system mutations	
ATG16L1	Crohn's disease
LAMP2A	Cardiovascular disease, myopathy
p62	ALS, Paget's disease
Parkin, PINK1 (mitophagy)	Parkinson's disease
Presenilin-1	Familial Alzheimer's disease

- Impaired autophagy

Autophagy is a fundamental intracellular catabolic process used by cells to degrade and recycle components through lysosomes to balance their sources of energy and building blocks in effort to maintain cellular homeostasis, differentiation, development and survival upon stress. [105, 200-202] It involves the sequestration and transport of macromolecules and subcellular elements such as nucleic acids, proteins, lipids, and organelles to lysosomes for subsequent degradation. [203] A major regulatory incident in autophagy instigation is exerted by the initiation complex interactions with the nutrient-sensing mTOR kinase, and the energy-sensing AMP-activated protein kinase (AMPK), both of which are recognized inducers of autophagy in response to stress. Thus, autophagy initiation is controlled by both nutrient- and energy-sensing mechanisms. [204]

A growing body of evidence indicates that autophagy activity deteriorates with age in various organisms. [205] Upon aging and neurodegeneration, flaws in certain steps of autophagy regulation have been observed, which result in accumulation of damaged organelles and protein aggregates. They are harmful for cell metabolism and homeostasis, which further worsens imperfect autophagy. [202, 206] Noteworthy, activation of autophagy has been reported to increase mouse lifespan [207], and even enhance immune response to vaccination in older individuals by defeating immunosenescence. [208] Autophagy is in a close correlation with numerous other hallmarks of aging, and is currently considered as an integrative aging feature. [209] It is critical for maintaining protein homeostasis (proteostasis). Autophagy work together with the ubiquitin proteasome system to destroy toxic proteins. [192] Upon aging, post-mitotic cells exhibit compromised proteostasis, which correlates with the functional decay of protein quality control tools, including autophagy. [192]

Autophagy plays a well-documented role in eliminating dysfunctional mitochondria, termed mitophagy. [210] It has been also demonstrated that autophagy controls cellular senescence, the process of steady proliferation suppression of mitotic cells initiated by diverse stresses, including telomere attrition, DNA impairment, mitochondrial dysfunction, and abnormal hyperproliferative stimuli. [211] Altogether, loss of autophagy generates various cellular malfunctions that exacerbate aging. [205] Since reduced autophagy is implicated in multiple age-related diseases, including neurodegeneration, sarcopenia and osteoarthritis, therapeutic autophagy upregulation has potential towards treating such age-related disorders.

2.3. Metabolic Alterations

Metabolic modifications are the most common symptom of aging in cells. They include deregulated nutrient sensing, mitochondrial dysfunction, as well as accumulation of sphingolipids and dysregulation of cholesterol metabolism. Such changes increase the risk of age-associated diseases such as type 2 diabetes, stroke, and hypertension. Insulin resistance is a foremost metabolic syndrome noticed in older adults.

- Deregulated nutrient sensing

Nutrients are substances needed by the body to sustain basic functions, in order to survive, grow, and reproduce, and are optimally obtained by eating a balanced diet. Thus, glucose and other carbohydrates, amino acids, and lipids are essential cellular nutrients, with certain mechanisms to sense their availability in mammalian cells. The capability to sense and respond to variations in environmental nutrient availability is a key requisite for survival. Thus, cells must be able to store nutrients when they are abundant and access them when nutrients are scarce. Moreover, nutrient levels in circulation need to stay within certain safe ranges. Therefore, cells must be able to sense nutrient levels in order to react appropriately. Various pathways that sense intracellular and extracellular levels of carbohydrates, amino acids, lipids, and different metabolites are integrated and coordinated at the organismic level via hormonal signals. Throughout food abundance, nutrient-sensing pathways employ anabolism and storage, whereas food scarcity activates homeostatic mechanisms. [212]

There are four nutrient sensing pathways:

- Insulin and IGF-1 signaling (IIS)
- Mechanistic target of rapamycin (mTOR)
- AMP-activated protein kinase (AMPk)
- Sirtuins

The IIS and mTOR pathways indicate nutrient abundance, so downregulating them prolongs lifespan by reducing cell growth and anabolic metabolism. On the other hand, the AMPk and sirtuin pathways imply nutrient scarcity, so their upregulation prolongs lifespan by reducing nutrient sensing, thus imitating dietary restriction. Some adverse effects caused by upregulating or deregulating these nutrient signaling pathways include compromised wound healing, insulin resistance, cataract formation, and testicular degeneration upon mTOR pathway downregulation by rapamycin administration. [213] Nutritional anti-aging strategy known as calorie restriction has been successfully examined in diverse eukaryotic species. [214] Research efforts have been focused on outlining the molecular mechanisms linking metabolic balance induced by calorie restriction and the biology of aging, thus revealing the key significance of nutrient sensing upon aging. [215]

Amino acids regulate multiple, interacting nutrient sensing pathways. The adequate sensing of amino-acid availability is significant for the effective regulation of protein synthesis and catabolism. An important way of amino acid control for nutrient sensing is via the amino acid sensing taste receptors. [212] Taste receptors are members of the T1R and T2R families of G-protein-coupled receptors. Amino-acid taste receptors in humans exhibit a high affinity to glutamate, yet other L-amino acids also operate as ligands, while D-amino acids do not. [216] In a similar way to amino-acid taste sensing by T1R1–T1R3, a T1R2–T1R3 heterodimer constitutes the glucose taste receptor, which is activated by millimolar concentration of glucose, fructose or sucrose. [217]

Deregulated nutrient sensing ability takes place upon aging. [218] The significance of nutrient sensing throughout the aging process has been first established in the prominent observation that decreased food intake in rats prolongs lifespan relative to ad libitum fed controls. [219]

One of the predominant nutrient sensing dysfunctions that takes place upon human aging is insulin resistance. Upon aging, factors including oxidative stress, inflammation, enzymatic activity disorders and fatty acids accumulation in cells can all contribute to a decline in insulin sensitivity. These changes can be driven by many of the other aging denominators. As a consequence, body gradually loses its capability to regulate blood sugar level, with the pancreas producing more insulin in an effort to compensate. Insulin resistance increases inflammation and oxidative damage, promotes glycation, and alters fat metabolism in liver, thereby advancing atherosclerosis and fatty liver disease. [220]

- Mitochondrial dysfunction

Mitochondria are rightly known as the cell's powerhouses, converting nutrients into energy to be used by the cell. Mitochondrial damage impairs its ability to fuel the cell. The main source of such damage is due to the free radicals, natural byproducts of energy production in the mitochondria.

Reactive oxygen species (ROS) is a group of species that includes hydrogen peroxide (H_2O_2), superoxide ion (O_2•-) and hydroxyl radical (•OH). They are outcomes of the oxidative metabolism in mitochondria, typically scavenged by the superoxide dismutase (SOD) enzyme. Upon mitochondrial malfunction, ROS are released producing oxidative damage to mitochondrial and cellular DNA. [221-224] These reactions signal a DNA damage response similar to that produced by telomere shortening, causing senescence. [223] Thus, changes in mitochondrial biology resulting in enhanced ROS concurrently alter epigenetic status at the DNA methylation level. Moreover, DNA methylation and histone acetylation vary upon aging and convey modifications in expression of mitochondrial genes, thus producing a feedback loop of failing mitochondrial function. [221, 225]

Senescent cells undergo significant changes in their mitochondrial function, dynamics, and morphology. [226] They exhibit decreased membrane potential, higher proton leak, intensified enzyme release, higher mass, and higher amount of tricarboxylic acid cycle metabolites. [227] The number of mitochondria in senescent cells is enhanced, due to the accumulation of old and dysfunctional mitochondria because of deficient mitophagy (mitochondrial removal). [228] Furthermore, mitophagy deficiency appears as a distinct mechanism for mitochondrial mass expansion. [226, 229]

Regardless of their abundance, mitochondria in senescent cells typically exhibit a lower ability to produce ATP. [230] Instead, senescent cells are characterized by a Warburg shift (a shift from oxidative phosphorylation to rapid aerobic glycolysis); this produces more ROS, thus causing protein and lipid damage, telomere shortening, and DNA damage response. [225, 226, 231]

- Accumulation of sphingolipids & dysregulation of cholesterol metabolism

Recent data points to a new mechanism of aging: the accumulation of sphingolipids. [106, 107] Ceramides, a common class of sphingolipids, build up in aging muscles, driving down their function and affecting the functional ability of older adults. Thus, it has been reported that inhibiting ceramide production in cells could prevent sarcopenia, or muscle loss associated with aging. Administration of

myriocin (a drug shown to inhibit the production of ceramides) to aging mice slowed down sarcopenia, maintaining their muscle strength. It has been reported that the effects were related to muscle stem cell operation – when ceramide production has been inhibited, the number of muscle stem cells and their operational ability has been better preserved. [106, 108] The study opens up a new research approach regarding the effect of ceramides on aging and stimulates the development of prospective therapeutic strategies involving sphingolipids in humans.

2.4. Cellular Senescence, Stem Cell Exhaustion and Altered Intercellular Communication

Cellular senescence, stem cell exhaustion, and altered intercellular communications are aging attributes that have effect mainly at the cellular level.

- Cellular senescence

Cellular senescence denote cells that have entered a status of arrested growth in reaction to cellular damage (Figure 4C). Thus, senescent cells lose productiveness and no longer divide; they also trigger growth in inflammation, which can aggravate aging. [221] Even though all cell types are able to undergo senescence upon aging, it mainly impact fibroblasts, endothelial cells, and immune cells. [232, 233] Even post-mitotic or slowly proliferating cells, such as the brain or the heart cells, may experience senescence. [234] Senescent cells exhibit modifications in their metabolic activity, undergo significant changes in gene expression, and develop a complex senescence-associated secretory phenotype (SASP), composed of proinflammatory cytokines, chemokines, growth factors, and matrix-remodeling enzymes able to alter their microenvironment. [235, 236] Cellular senescence can impair tissue repair and regeneration, thereby promoting aging. Cellular senescence has been associated with multiple age-initiated disorders, such as cancer, diabetes, osteoporosis, cardiovascular disorders, stroke, Alzheimer's disease and dementias, as well as osteoarthritis. [237] It has also been related to declines in eyesight, mobility, and cognitive capability.

It has been reported that continuous removal of senescent cells by genetic or pharmacological interventions extends the longevity and health of aged mice, verifying the key role of cellular senescence in aging. [238] Thus, removal of senescent cells can attenuate age-related tissue dysfunction and extend health span.

Senescence can be triggered by different kinds of stress. Cells can go through senescence in response to various stimuli, such as telomere shortening, alterations in telomeric structure, mitogenic indications, oncogenic stimulation, radiation, oxidative stress, epigenetic alterations, chromatin disorders, loss of proteostasis, mitochondrial dysfunction, inflammation, tissue damage, and nutrient deficiency. [239-244]

Senescence can also perform as an effective anti-tumor mechanism, by inhibiting proliferation of cancer cells during carcinogenesis. [245, 246] It is a cellular framework that exhibits both favorable and harmful effects on the health of an organism, a supposed instance of evolutionary antagonistic pleiotropy. Initiation of the p53/p21$^{WAF1/CIP1}$ and p16^{INK4A}/pRB tumor suppressing pathways, which is actuated in response to DNA damage produced by telomere attrition and oxidative or oncogenic stress, perform a key role in controlling senescence. Several other pathways have recently been associated

with mediating senescence and the senescent phenotype. [247] Better in-depth knowledge of the mechanisms regulating senescence may provide promising translational prospects to develop novel therapeutic strategies minimizing the harmful consequences of senescence. Targeting senescence by senolytic drugs to selectively eradicate senescent cells or control SASP using small molecules or antibodies will facilitate treatment of senescence related disorders and may contribute toward expanding healthspan.

- Stem cell exhaustion

Stem cells play a critical role in tissue repair and regeneration. [213] However, as an organism ages, their function declines, leading to a reduction in tissue regeneration capacity and an increased risk of age-related diseases. Stem-cell exhaustion indicates stem cells and progenitor cells accruing damage over time, and eventually becoming depleted upon aging. Thus, aging is accompanied by a continuous decrease in tissue renewal, as well as with compromised tissue repair upon injury. [248, 249]

This decline in stem cell function is due to a variety of factors, including increased cellular damage, changes in gene expression, and alterations in the microenvironment surrounding the stem cells. The suggested mechanisms of stem cell exhaustion include:

- telomere shortening
- DNA damage accumulated upon aging caused by a variety of factors, including oxidative stress, radiation, and chemical exposure
- epigenetic modifications, such as changes in DNA methylation or histone modifications, can alter gene expression and affect stem cell function. These changes can accumulate over time and contribute to stem cell exhaustion
- alterations in the stem cell microenvironment: the microenvironment, or niche, surrounding stem cells is critical for their function. Age-related changes in the niche, such as decreased nutrient and oxygen supply or the accumulation of toxic metabolites, can impair stem cell function. [249-251]

Studies have shown that stem cell exhaustion is a major contributing factor to age-related declines in tissue regeneration, including the loss of muscle mass, impaired bone healing, and decreased skin elasticity. Stem cell exhaustion also increases the risk of age-associated disorders such as Alzheimer's disease, cardiovascular disease, and cancer. Tissue repair is supposed to largely rely on injury-induced cellular de-differentiation and plasticity. Thus, in certain tissues, injury induces de-differentiation of multiple non-stem cells acquiring stem cell properties, attaining the plasticity necessary for tissue repair. [252]

There is ongoing research to investigate strategies to reverse stem cell exhaustion and restore their regenerative capacity. These strategies include genetic manipulation, cellular reprogramming, and the use of growth factors and other compounds that stimulate stem cell proliferation and differentiation. [253]

- Altered intercellular communication

Aging causes modifications in cell signaling at every level. Neuronal and hormonal signaling gets deregulated, causing enhanced inflammation (inflammaging), reduced immune performance (immunosenescence), and alterations in the extracellular surroundings. [221] Altered intercellular

communication implicates the change in signaling between cells possibly leading to certain diseases and disabilities of aging. The age-dependent alterations in intercellular communication integrate the effects of the other features of aging. Specifically, senescent cells initiate chronic inflammation, which can further damage aging tissues. Thus, multiple factors bring about the altered intercellular communication, one of which – the SASP – is directly triggered by the cellular senescence.

In addition to the above well-recognized hallmarks of aging, recently more distinctive features of that process have been identified. [103, 105, 106, 108, 254]

- Microbiome disorders – dysbiosis

The importance of gut microbiome in many aspects of human health is currently well recognized. [255] Recent progress in next generation sequencing tools have made possible the identification of prominent changes in the gut microbiome upon aging, indicating specifically certain shifts in microbial populations and loss of species diversity. [256] Such imbalance in the gut microbial community is referred to as dysbiosis. Along with age-related deficiency of structural integrity of the gut and other physiological barriers, such shift in microbial populations can trigger inflammation and other disorders. [257, 258]

Age-associated changes in the gut microbiota include a decrease in microbial diversity, an increase in the abundance of potentially harmful bacteria, and a decrease in the abundance of beneficial bacteria. In particular, there is often an increase in the abundance of potentially pathogenic bacteria, such as Proteobacteria, and a decrease in the abundance of beneficial bacteria, such as Bifidobacteria. [259] These changes can contribute to a variety of health issues that are more common in older adults, such as constipation, inflammation, and impaired immune function.

Aging is also associated with changes in the structure and function of the intestinal barrier, which can lead to increased intestinal permeability as well as translocation of bacteria and bacterial products into the systemic circulation. This can result in low-grade inflammation and immune activation, which are supposed to contribute to the development of age-related diseases. [260, 261]

Aging can also cause impaired immune function, including a decline in the function of innate immune cells such as macrophages, as well as a decrease in the diversity and function of T and B cells. These changes can lead to impaired immune surveillance of the gut microbiota, and the decreased ability to respond to pathogens. [262, 263]

Dysbiosis has been linked to a variety of age-related diseases, including metabolic disorders, cardiovascular disease, cognitive decline, and frailty. However, it is not yet clear whether dysbiosis is a cause or consequence of these conditions. There is growing interest in developing interventions to promote a healthy gut microbiota in older adults, with the goal of preventing or mitigating the effects of age-related dysbiosis. Potential interventions include prebiotics and probiotics, dietary interventions, fecal microbiota transplantation, and even microbial therapeutics such as bacteriophages. Maintaining a healthy gut microbiota through healthy lifestyle habits and interventions may help to promote healthy aging. [262-265]

- Chronic inflammation – Inflammaging

Chronic inflammation, also known as "inflammaging" is a low-grade, persistent, and systemic state of inflammation that occurs upon aging and is currently considered a key biological basis of the aging process. [266-268] It is believed to be caused by the accumulation of cellular damage and the failure of the immune system to clear damaged cells efficiently. This results in the release of certain inflammatory mediators in the blood, including IL-1, IL-6, C-reactive protein, and IFNα. [268]

Chronic inflammation has been linked to a wide range of age-related diseases, including cancer, diabetes, cardiovascular disease, and neurodegenerative diseases, as well as atherosclerosis, neuroinflammation, osteoarthritis, and intervertebral disc degeneration. [269] It is also associated with a decline in physical and cognitive function, as well as an increased risk of disability and mortality.

While the exact mechanisms behind inflammaging are not fully understood, researchers believe that a variety of factors can contribute to its development, including lifestyle choices such as poor diet, sedentary behavior, and smoking, as well as environmental exposures such as pollution and toxins. [270]

Inflammaging is related to other characteristic features of aging process such as cellular senescence and the disturbances in gut microbiota known as dysbiosis. [271] It might be triggered by ineffective/disabled autophagy and genomic instability. [103] Overexpression of pro-inflammatory mediators can be a result of epigenetic dysregulation or deficient proteostasis. [254] Inflammaging is aggravated by disturbances of the circadian rhythm as well as by gut barrier dysfunction. [272]

Reducing chronic inflammation may be an important strategy for improving health and preventing age-related diseases. Lifestyle interventions such as regular exercise, healthy diet, stress reduction, and adequate sleep have been shown to reduce inflammation and improve health outcomes. [273] Additionally, certain medications and supplements may also be effective in reducing inflammation. However, more research is needed to fully understand the complex mechanisms behind inflammaging and to develop effective interventions, reducing its impact on aging.

- Mechanical properties alterations

Cellular and extracellular mechanical properties alterations take place upon aging. Fibroblast senescence is associated with a change in actin – from a faction that can be polymerized and depolymerized upon cell motility, to f-actin fibers, which are likely to impact cell motility and cell-cell communication. [274] Motility changes are of significant importance for the innate immune system aging, in which neutrophils from aging individuals induce substantial tissue damage upon migration to sites of inflammatory signaling. [275] The nucleoskeleton also undergoes changes upon aging, with the nuclear lamina becoming destabilized and concomitant extrusion of chromatin into the cytoplasm triggering the SASP in senescence. [276] Lastly, extracellular matrix also changes with aging, which greatly affects cell performance. [277] Enhanced rigidity and loss of elasticity, as a result of glycation crosslinking between collagen molecules, can be in charge of multiple age-related disease conditions such as hypertension with related kidney and neurological disorders. The field of biomechanics is thus considered highly relevant to the physiology aging and anti-aging strategies. [105]

2.5. Hallmarks of aging are interrelated

Overall, the different hallmarks of aging are interconnected and can contribute to each other (Figure 5). [4, 56, 187] For example, cellular senescence can promote inflammation, which can further exacerbate mitochondrial dysfunction and genomic instability. Similarly, genomic instability can lead to epigenetic alterations, which can impact the function of stem cells and contribute to their exhaustion. [4, 102, 103]

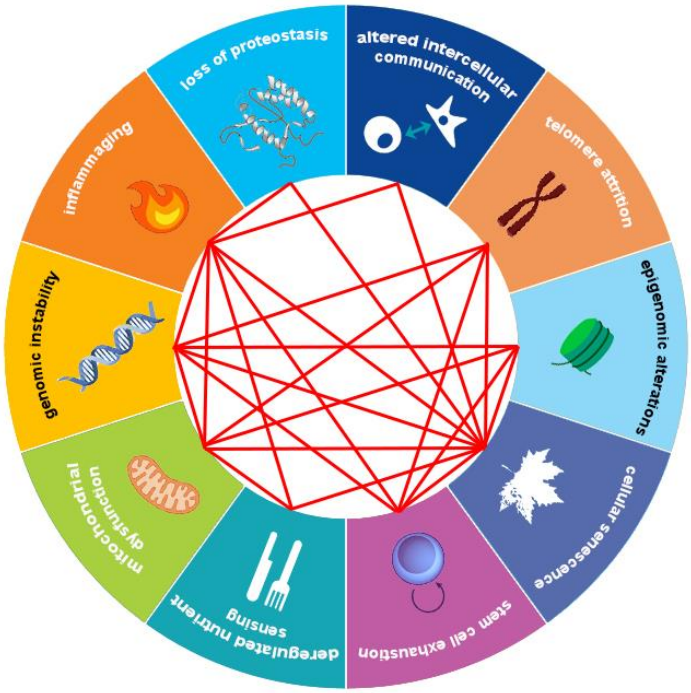


Figure 5. Interrelations between the hallmarks of aging

- Genomic instability can also contribute to telomere attrition and cellular senescence. Conversely, telomere attrition can also contribute to genomic instability, as shortened telomeres can lead to DNA damage and mutations. Epigenetic alterations can impact genomic instability and cellular senescence. Loss of proteostasis, deregulated nutrient sensing, mitochondrial dysfunction, altered intercellular communication, and stem cell exhaustion can all contribute to cellular senescence and inflammation. Cellular senescence can impact genomic instability and telomere attrition. [278-280]
- Epigenetic alterations can affect gene expression, including the expression of genes that regulate cell growth and senescence. [281, 282] For example, certain epigenetic changes can lead to the upregulation of p16 and p21, two proteins that promote cellular senescence. [283]
- One of the consequences of loss of proteostasis is the accumulation of misfolded and damaged proteins. This can trigger cellular senescence, as cells can activate senescence pathways in response to protein stress. [284]
- One of the pathways that regulates nutrient sensing is the mTOR pathway. Dysregulation of this pathway can lead to increased mitochondrial dysfunction, as mTOR can impact mitochondrial biogenesis and function. [285, 286]
- Senescent cells can secrete a variety of molecules, including cytokines and growth factors, that can impact the function of neighboring cells. This can contribute to altered intercellular communication. [287]

– Mitochondrial dysfunction resulting in increased ROS, concurrently brings about epigenetic alterations at the DNA methylation level. DNA methylation as well as histone modifications upon aging impart modifications in gene expression of mitochondrial genes, creating a feedback loop of declining mitochondrial function. [225]

Understanding the relationships between the different hallmarks of aging can help in developing effective interventions to prevent or treat age-related diseases.

An alternative approach to considering aging as a set of isolated processes in terms of discrete hallmarks, it has been suggested considering aging as involving **four layers**, each at a different biological scale. [187] From a general phenotype to a molecular mechanism, the suggested four layers of aging include: (i) a decline in physical function of the organism and increased susceptibility to diseases; (ii) systemic immune, metabolic, and endocrine malfunction; (iii) cellular dysfunction; and (iv) failure of biomolecule performance. [187] Failures within each layer and relations between them allegedly generate the aged phenotype and its associated susceptibility to disease.

2.6. Age-related diseases

Decline of bodily functions upon aging is a major risk factor for crucial human pathologies. Moreover, because advanced age is the common inherent cause, such chronic disorders frequently take place concurrently as comorbidities in the elderly population. [4, 288-290] Among these major pathologies are cancer and cardiovascular disorders. Age-associated diseases impacting the musculoskeletal system are common as well, particularly osteoarthritis, osteoporosis and sarcopenia. Metabolic disorders such as diabetes and hepatic steatosis are also common with age. Organ and tissue fibrosis, a pathological progression typified by excessive fibrous connective tissue production [291], also raises upon aging and is one of the main causes for age-related deterioration of human organs. Overall weakening of the immune system increases susceptibility to infectious diseases. [292] Neurodegenerative diseases, such as Alzheimer's, Parkinson's, and Huntington's diseases, and sensorial malfunctions such as auditory and macular degeneration all increase considerably upon aging. [20, 289, 293, 294]

- **Cardiovascular disease** is the most frequent cause of death in older adults. This disease class mainly includes coronary artery disease, congestive heart failure, and arrhythmia. Vascular stiffing and remodeling are known to take place throughout normal aging. [295, 296]
- **Atherosclerosis** progresses as cholesterol, fat, and other substances in blood form plaques, which cause narrowing of the arteries. This decreases the supply of oxygen-enriched blood to tissues and organs in the body. [296] Atherosclerosis triggers inflammation and further vascular changes thus enhancing risk for cardiac and cerebrovascular disorders, peripheral vascular disease, cognitive impairment, and other cardiovascular damage. [295, 297]
- **Cerebrovascular disease (stroke)** is another common age-related disease. Stroke happens when blood stops flowing in an area of the brain as a consequence of a disruption of a blood vessel. It is a very critical condition because brain cells deprived of oxygen die quickly, so it can cause death or serious disability. [298]

- **Hypertension,** the most common chronic disease of older adults, is the major promoter of atherosclerosis=. [299] However, the worth of intensive pharmacotherapy for hypertension in people over age of 75 remains controversial. [295] Current belief is that aggressive treatment needs to be offered and continued as long as it is well-tolerated. [299]
- **Cancer** is the second leading cause of death in older adults, most commonly lung, breast, prostate, and colorectal cancers. [300] Slow-growing tumors are common in this age group. Response to cancer treatment is better related to the physiological status rather than the age.
- **Osteoarthritis** is a very common chronic disorder among older adults and a frequent cause of chronic pain and disability. [301] The occurrence of osteoarthritis is higher among women than men. Obesity is a risk factor for osteoarthritis, with increasing rate of severe hip and knee arthritis. Osteoarthritis treatments include expensive joint replacement surgery, in addition to intensive rehabilitative treatments. Lower back pain is a common symptom and its cause is often multifactorial. [295]
- **Diabetes** rates are on the rise in the aging population. Diabetes is a strong risk factor for cardiovascular disease in older adults. [302] It is also related to peripheral arterial disease and peripheral neuropathy, causing diabetic foot ulcers and amputations.
- **Osteopenia/Osteoporosis.** Osteopenia is normal loss of bone density upon aging. Older adults frequently suffer from osteoporosis, a harsher deterioration of bone density. [303]Osteoporosis is associated with an increased rate of bone fractures. Calcium and vitamin D supplementation may be efficient in preventing osteoporosis and bone fractures.
- **Sarcopenia** is an age-related gradual loss of muscle mass and strength, a type of muscle atrophy primarily caused by the natural aging process. It is one of the most important causes of functional decline and loss of independence in older adults. Being physically inactive and eating an unhealthy diet can contribute to the disease. [304]
- **Chronic Obstructive Pulmonary Disease (COPD)** is a common age-related disease. It is typified by a reduction of airflow into the lungs due to the inflammation of airways, thickening of the lungs lining, and an over-production of mucus in the air tubes. [305]
- **Cognitive decline** producing mild short-term memory loss, difficulty finding words, and slower processing are all normal features of aging. Deviations from normal brain aging may lead to dementia, manifesting as memory loss, mood changes, confusion, communication difficulties, or deprived judgment. [306] Rates of dementia rise with age. Alzheimer's disease is the most common cause of dementia [307], but a number of other disorders such as vascular dementia, Lewy body dementia, frontotemporal disorders, Huntington's disease, and Parkinson's disease can trigger it as well.

2.7. Major biochemical processes related to aging

The three main biochemical processes that cause cellular damage and age-related diseases include: methylation, glycation, and oxidation.

Glycation

Glycation is a spontaneous non-enzymatic reaction of free reducing carbohydrates with free amino groups of proteins, nucleic acids, and lipids, which results in the formation of Amadori products (Figure 6). [308] Further, these Amadori products go through an assortment of irreversible dehydration and reorganization reactions leading to the development of advanced glycation end products (AGEs). [309] The

glycation reaction leads to protein function deficit and reduced elasticity of biological tissues such as blood vessels, skin, and tendons. [310, 311] The glycation process is augmented in the presence of hyperglycemia and oxidative stress. [312] Since there are no enzymes to eliminate glycated products from the organism, glycation comply with the theory that the accumulation of metabolic waste promotes aging. A set of exemplary Advanced Glycation End products (AGEs) are listed in Table 3, along with the number of related documents in the CAS Content Collection.

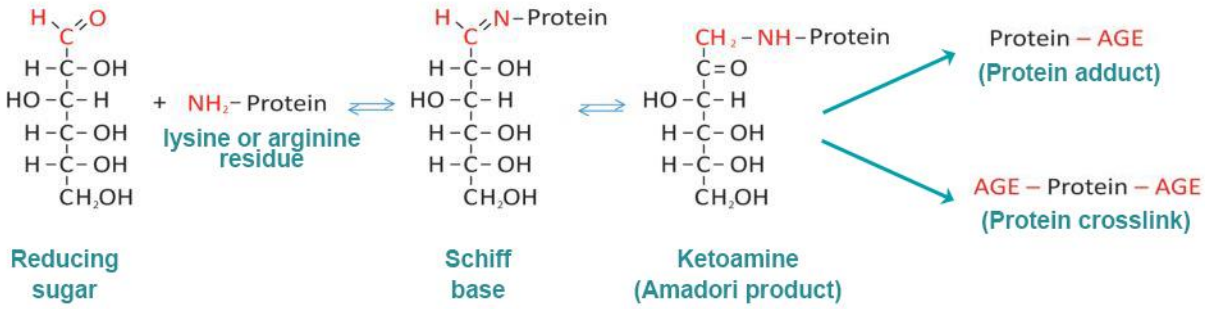
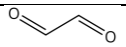
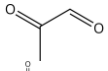
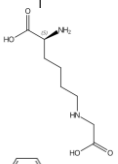
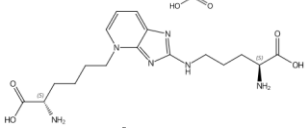
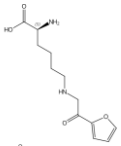
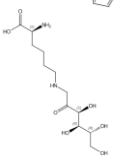
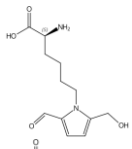
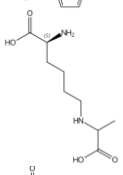
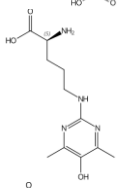
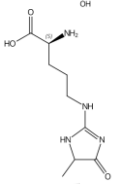
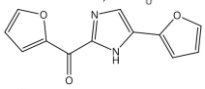
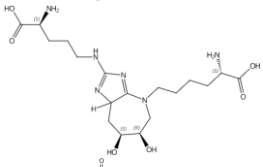
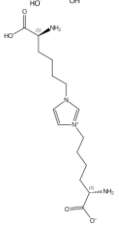
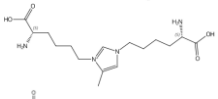
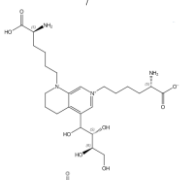
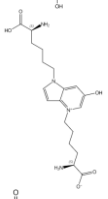
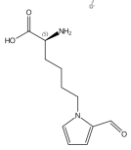
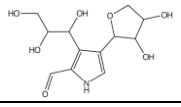


Figure 6. Scheme of the Maillard reaction: Reducing sugars reactive carbonyl groups react with the proteins amino groups to form a Schiff base, which further rearranges to a more stable Amadori products. These early glycation end products further form either protein adducts or protein crosslinks.

Table 3. Chemical structures of exemplary Advanced Glycation End products (AGEs) and a number of related documents in the CAS Content Collection

AGEs	CAS Reg #	Chemical structure	No. documents
Glyoxal (GO)	107-22-2		19222
Methylglyoxal (MGO)	78-98-8		8938
Nε-(Carboxymethyl)lysine (CML)	5746-04-3		1699
Pentosidine	124505-87-9		1269
Furosine	19746-33-9		696
Fructoselysine	21291-40-7		361

Pyrraline	74509-14-1		272
Nε-(Carboxyethyl)lysine (CEL)	5746-03-2		260
Argpyrimidine	195143-52-3		193
L-Arginine-methylglyoxal adduct (MG-H1)	149204-50-2		147
Furoyl-furanyl-imidazole (FFI)	91037-91-1		64
Glucosepane	257290-23-6		64
Glyoxal-lysine dimer (GOLD)	209267-39-0		36
Methylglyoxal-lysine dimer (MOLD)	209276-80-2		28
Crossline	857058-48-1		21
Vesperlysine A	188985-17-3		14
Formyline	1312365-68-6		11

AFGP	401574-77-4		9

Oxidation

Oxidative stress has been assumed to notably contribute to aging. [313-316] The oxidative stress theory of aging hypothesizes that age-related decline in physiological performance is caused by a slow continual accumulation of oxidative damage to biomolecules, which grows with age and is associated with life expectancy decline of organisms. [317] Oxidative damage contributes to multiple hallmarks of aging and drive multiple age-related diseases. Thus, telomeres are highly sensitive to oxidative damage. [318, 319] Therefore, oxidative damage may cause telomere attrition, which accelerates aging and augments the risk of age-related diseases. [320] Oxidative stress has been defined as an imbalance between the production of oxidants and their elimination by antioxidants, leading to disturbance of redox signaling and control, and/or molecular impairment. [315]

According to the oxidative stress theory of aging, damages caused by free radicals are the main reason of aging and a shorter lifespan. [27, 321, 322] ROS are highly reactive species, mainly including free radicals comprising at least one unpaired electron – superoxide radicals (O_2−•), hydroxyl radicals (•OH) and hydrogen peroxides (H_2O_2) – and have been believed to be the primary source of endogenous oxidative stress damage. [323] It is widely agreed that the largest part of ROS are produced by the electron transport chains of mitochondria during regular oxidative respiration in addition to numerous intracellular pathways. [324] The principal process of ROS production in mitochondria can be schematically described as $O_2 \rightarrow O_2^{-}\bullet \rightarrow H_2O_2 \rightarrow \bullet OH$. [324]

Furthermore, ROS are generally produced by mitochondria, throughout physiological and/or pathological processes. Thus, O2•− can be formed by cellular respiration, by lipoxygenases and cyclooxygenases via the arachidonic acid metabolic pathway, and by endothelial and inflammatory cells. [325] In the electron transport chain, oxygen molecules have been reduced into O_2-• with a leak of electrons, with the formation of superoxide being the initial step in a cascade reaction of other ROS generation. When generated, it can be catalyzed by the super oxidize dismutase (SOD) into H_2O_2. Next, in the presence of reduced form transition cation (Fe^{2+} or Cu^{+}, known as a Fenton reaction) or myeloid peroxide, H_2O_2 further converts to •OH. Meanwhile, H_2O_2 can also be reduced into H_2O by the enzymatic antioxidants such as catalase and glutathione peroxidase. Haptoglobin steadily binds hemoglobin with strong affinity, inhibiting the release of heme iron from hemolysis into systemic circulation, therefore terminating Fenton reaction and avoiding the production of •OH. Mitochondrial dysfunction upon aging results in increased ROS, thus causing enhanced oxidation of biomolecules (proteins, DBA, lipids) and opens a positive feedback loop of aging damage (Figure 7).

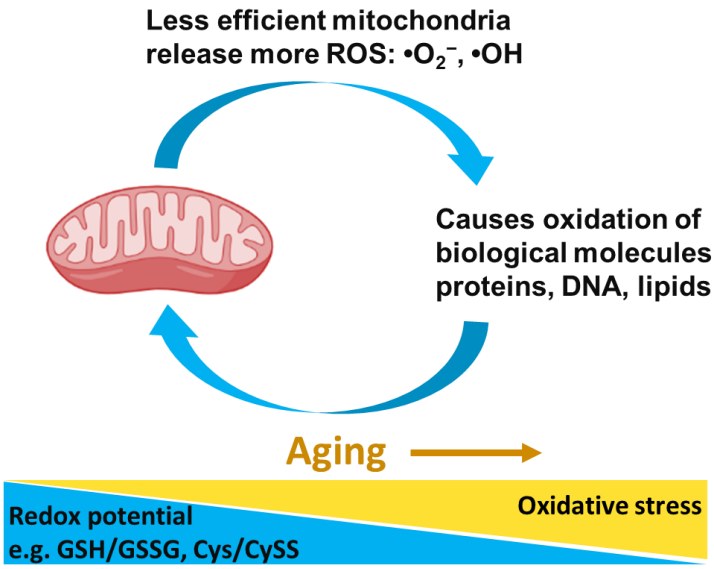


Figure 7. Schematic presentation of the positive feedback loop between mitochondria disfunction and oxidative stress upon aging

Noteworthy, examining the hypothesis of oxidative stress in aging and diseases have disclosed controversial results. There is sizeable evidence that macromolecular oxidative damage raises with age and seems to be related to life expectancy in multiple organisms. Yet, a direct relationship between oxidative damage and aging has not been conclusively established. [326] In fact, the role of ROS in the body is complex, and its effects on health vary largely along with changing ROS levels. Within physiological levels, ROS facilitate the preservation of cellular homeostasis and performance. [315] Therefore ROS levels can exhibit both favorable and detrimental effects, as suggested in the concept of mitohormesis. [327]

Oxidative stress may result in damage to various classes of biomolecules including lipids, proteins, and nucleic acids. Polyunsaturated fatty acid (PUFA), especially those with a higher number of double bonds, are highly susceptible to **lipid peroxidation** by an autocatalytic oxidative chain reaction. [328] Peroxidation of phospholipids in lipid membranes may result a decline in membrane fluidity and permeability, and thus inactivation of membrane receptors, resulting in cell apoptosis. Moreover, lipid radicals generated during oxidation can form a multitude of harmful end products, including reactive aldehydes, alkanes, and alkenes. [329]

Proteins are also key targets for ROS. **Protein oxidation** includes: (i) oxidative alteration of amino acid residues, particularly cysteine and methionine; (ii) fragmentation as a result from oxidative cleavage of the peptide backbone; (iii) production of protein carbonyl derivatives; (iv) protein crosslinks generation. [330-332] Protein oxidation may cause changes in their three-dimensional structures, alteration of their physiological features such as enzyme performances and signal transduction, and further proteolytic degradation or aggregation of proteins, partial unfolding and modified conformation. [331, 333, 334]

ROS, specifically the hydroxyl radicals, can incite oxidative damage to the **nuclear DNA**, including: base mutation, strand breaking, DNA-protein crosslinking, and DNA-adducts formation. [324] Overall,

hydroxyl radicals can react with DNA bases and sugar-phosphate backbone, leading to inaccurate base pairing and further common mutations. [335] Hydroxyl radicals can also react with the deoxyribose moiety resulting in loss of DNA bases and DNA breaks. Such breaks are documented risk factors of genome instability, cell cycle disruption, and cell death. [336-338] DNA-protein crosslinks involving thymine and tyrosine in the nucleoprotein complex of histones and DNA can also be activated by the hydroxyl radicals. [339]

Methylation

Recent research progress provides convincing evidence of genome-wide **DNA methylation** changes upon aging and age-associated disease. Methylation is a process in which a methyl group (-CH3) is attached to a cytosine base (C) of DNA. It initiates DNA condensation, a configuration in which genes have not been transcribed. Methylation levels change throughout life, but generally tend to decrease upon aging.

The methylation reaction is catalyzed by DNA methyltransferases (DNMTs), enzymes transferring a methyl group from the S-adenosyl-l-methionine (SAM) to the C5 of a cytosine. Such reaction includes SAM as a electrophile methyl donor and C5 as a weak nucleophile incapable to interact with SAM by itself. However, a nucleophile from a DNMT can bind covalently to the carbon-6 of cytosine, which activates the nucleophilic nature of C5, enabling the transfer of a methyl group from SAM. The enzyme nucleophile is consequently removed and deprotonation at C5 breaks up the nucleotide–DNMT complex. [340] DNA methylation typically leads to gene silencing. [341] There are various routes to gene silencing through methylation. Since the greater part of mammalian transcription factors exhibit DNA recognition elements containing motifs rich in CpG, as well as GC-rich binding sites, DNA methylation can block or abolish their capability to act on many significant regulatory sites. [342]

Histone methylation is a reaction in which methyl groups are relocated to amino acids of histone proteins. These proteins participate in the fundamental unit of chromatin, the nucleosome. The DNA double helix wraps around the nucleosomes to form chromosomes. Histone methylation is critical for the regulation of gene expression by controlling the chemical attractions between histone tails and DNA. [166, 181]

The question of whether alterations in methylation are results of aging and pathology or in fact one of its contributing factors has not been decisively solved yet. A wide variety of age-associated diseases exhibit abnormal methylation and many prospective treatments based on rejuvenating the methylome are yet unexplored. Future research will require a better understanding of alleged mechanisms surrounding DNMTs and their associated partners in DNA methylation. Advanced research into methylomic aging, associated disease, drug discovery, and regulatory mechanisms is essential to uncover the function of DNA methylation in aging, rejuvenation, and age-associated disease.

2.8. Brain aging

The brain is remarkably sensitive to the effects of aging, displaying as changes in structure and cognitive capacity, as well as increased risk for developing certain neurological disorders. [343, 344] Brain health refers to the maintenance of brain functions in several aspects: (i) cognitive health – the ability

to adequately think, learn, and remember; (ii) motor function — the ability to control movements and balance; (iii) emotional health — the ability to interpret and respond to emotions; (iv) tactile function — the ability to feel and respond to sensations of touch, including pressure, pain, and temperature. [345]

At the molecular level, brain aging, similarly to all other organ systems, is characterized by changes in gene expression, epigenetic modifications, and alterations in protein synthesis and turnover. It is also associated with the accumulation of toxic protein aggregates, such as beta-amyloid and tau, which can disrupt neuronal function and contribute to the development of neurodegenerative diseases. [14, 19] At the cellular level, brain aging is characterized by the accumulation of cell damage, including oxidative stress, DNA damage, and protein misfolding. This damage can lead to the dysfunction and death of brain cells, including neurons and glia. Studies have shown that dendritic arbors and spines decrease in size and/or number in cortex as a result of aging. [346, 347] Aging also sets off a decline in the regenerative capacity of brain cells, such as decreased neurogenesis and oligodendrogenesis. [16, 17]

At the system level, brain aging includes changes in brain connectivity and function, such as alterations in neural activity, neurotransmitter function, and white matter integrity. Aging is associated with a decline in the function of essential neurotransmitter systems, such as dopamine and acetylcholine, which can lead to cognitive impairment. Brain aging is associated also with changes in brain structure, such as the loss of grey matter volume and changes in white matter microstructure. [12, 348-350] At the organismal level, brain aging is associated with declines in cognitive function, sensory function, and motor function. Age-related changes in the cardiovascular system, immune system, and endocrine system can also impact brain function and contribute to age-related neurodegenerative diseases. [19, 351]

Hallmarks of aging, including mitophagy, cellular senescence, genomic instability, and protein aggregation, have been related to the age-associated neurodegenerative and cerebrovascular disorders. [20] Furthermore, the most frequent neurodegenerative diseases share the common attribute of protein aggregation. The aggregation of senile plaques containing amyloid-β peptide and the formation of intraneuronal tau containing neurofibrillary tangles in Alzheimer's disease and the accumulation of misfolded α-synuclein in Parkinson's disease are major pathogenic aspects of these diseases. [352] Protein aggregation is also a feature of amyotrophic lateral sclerosis and frontotemporal lobar dementia. [353]

Brain tissues comprise primarily postmitotic cells, including neurons and oligodendrocytes, which are sensitive to age-related alterations, such as DNA damage or methylation. Indeed, Parkinson's disease patients have been reported to consistently exhibit DNA methylation patterns associated with advanced aging. [354] Advanced aging has been also related to enhanced mitochondrial dysfunction and damage, thus promoting neurodegeneration via the production of ROS and the advancing neuroinflammation. [19]

In addition to the most common age-associated neurodegenerative diseases such as Alzheimer's and Parkinson's diseases and stroke, others included are, age-related macular degeneration associated with blurred or distorted vision; multiple sclerosis associated with myelin damage, which disturbs the information flow within brain, and between brain and body; amyotrophic lateral sclerosis (Lou Gehrig's disease) affecting motor neurons thus causing loss of muscle control; Huntington's disease associated with involuntary movements, difficulty with coordination, and changes in mood and behavior; and various kinds of dementias including Lewy bodies dementia characterized by the presence of abnormal protein deposits in the brain, which causes changes in attention and alertness, visual hallucinations, and movement disorders, and vascular dementia associated with damage to the blood vessels that supply

blood to the brain, which causes memory loss, difficulty with decision-making, and changes in mood and behavior. [19, 348, 355, 356]

There is a steady, nearly exponential growth of the number of journal publications related to brain aging in the CAS Content Collection over time, remarkably intense in the last two years (Figure 7), a sign of the enhanced scientific interest in this area. At the same time, patenting activity is low, probably awaiting the knowledge accumulation reaching critical level.

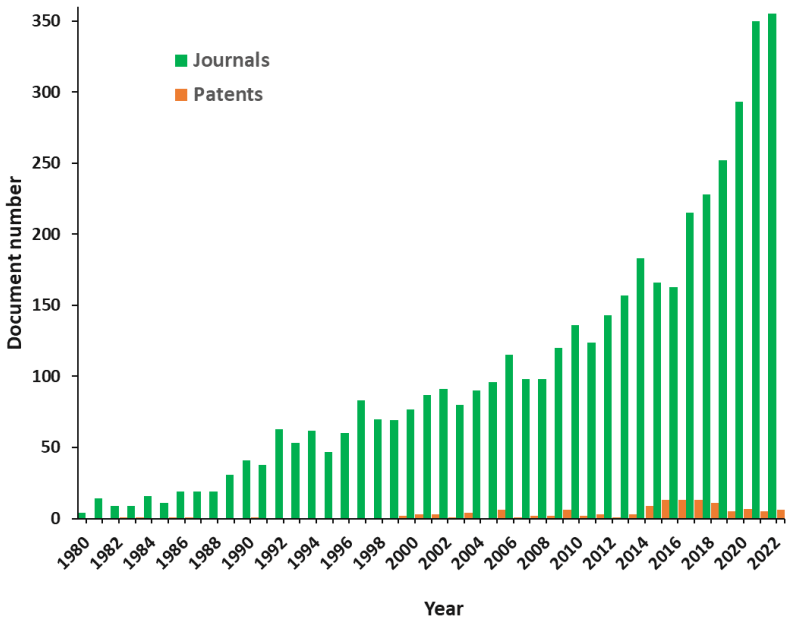


Figure 7. Yearly growth of the number of documents related to brain aging in CAS Content Collection.

2.9. Skin aging

Skin aging is one of the most studied aspects of aging because it is visible and can affect a person's appearance, which can have significant social and psychological effects. Aging of the skin can lead to changes in skin texture, color, and elasticity, which can affect how a person looks and feels about themselves. Furthermore, the skin plays an important role in protecting the body from environmental factors such as UV radiation and pollution. It also prevents excessive water loss and the entry of toxic substances and pathogens from the environment. Upon aging, the skin's ability to perform these functions can decrease, which can have negative effects on overall health.

As the largest organ of the body exposed to the external environment, the skin endures both intrinsic and extrinsic aging factors, with extrinsic aging prompted by environmental impacts and overlaying the effects of temporal aging. Intrinsic aging is a physiological process that results in several phenotypes such as, but not limited to, wrinkling, pigmentation, telangiectasis, and gradual dermal atrophy [357-363], while extrinsic aging is provoked by exterior environment and behavioral factors such as air pollution, tobacco smoking, inadequate nutrition, and sun exposure, causing wrinkles, elasticity loss, as well as rough-textured appearance. [357, 358] Particularly, long-term exposure to solar UV radiation is the prime factor of extrinsic skin aging referred to as photoaging. [358]

Skin aging is accompanied by phenotypic changes in cutaneous cells along with structural and functional alterations in extracellular matrix components such collagen, elastin and proteoglycans, which are required to afford tensile strength, elasticity, and moisture to the skin. [364, 365] This can result in the appearance of fine lines and wrinkles, sagging skin, and loss of facial volume. In addition, skin aging is characterized by a decrease in the production of hyaluronic acid, a substance that helps to maintain skin hydration and suppleness. Other intrinsic factors that contribute to skin aging include genetic inheritance, slower cell turnover, hormonal changes, including estrogen, progesterone, and testosterone decrease, which can affect the skin structure. which can lead to a loss of skin elasticity, and changes in skin cell metabolism. Additionally, changes in skin microbiota, the collection of microorganisms that live on our skin, can contribute to skin aging and the development of aging-associated skin diseases. [364]

Extrinsic factors that can contribute to skin aging include exposure to ultraviolet (UV) radiation, cigarette smoke, pollution, and poor diet. UV radiation from the sun is a major contributor to skin aging, causing damage to the skin cells and breaking down collagen and elastin fibers. This can result in the development of age spots, rough texture, and uneven skin tone. Additionally, exposure to cigarette smoke and pollution can cause oxidative stress, leading to inflammation and damage to the skin cells. A diet that is high in sugar, processed foods, and unhealthy fats can lead to inflammation, which can also accelerate the aging process. [366, 367]

Macrophages are the most abundant immune cell type in the skin and are vital for skin homeostasis and host defense. [368] However, they have also been associated with chronic inflammation upon aging. It has been suggested that age-modified skin macrophages may promote adaptive immunity exacerbation and exhaustion, facilitating the development of proinflammatory pathologies, including skin cancer. [368]

While the intrinsic and extrinsic aging factors are both related to phenotypic changes in dermal cells, the most significant structural changes take place in the extracellular matrix (ECM) of dermis, in which collagens, elastin, and proteoglycans impart tensile strength and hydration. The utmost longevity of these biomolecules, relative to the intracellular proteins exposes them to accumulated damage, which in turn affects their capability to provide mechanical properties and to manage tissue homeostasis. [369-373] Thus, at variance with the intracellular proteins, the half-lives of which are measured in hours or at most days [371] , many ECM proteins exhibit half-lives measured in years. For instance, human skin and cartilage collagens types I and II have half-lives of about 15 and 95 years [374], while the half-lives of elastin fibers is equal to [369] or many times longer than average human life. [375, 376] Therefore, in humans, ECM proteins are required to function for long years, during which time they are at risk of accumulating damage via glycation [377], calcium and lipid accumulation [378, 379], and alterations of aspartic acid residues. [370, 380] In turn these events have a profound effect on the mechanical properties of ECM proteins. [381]

Various molecular models are proposed to rationalize the molecular basis of skin aging, mostly including the overall recognized aging mechanisms such as cellular senescence, telomere shortening, decrease in cellular DNA repair capacity and point mutations of extranuclear mitochondrial DNA, oxidative stress, chromosomal abnormalities, gene mutations, and chronic inflammation (inflammaging). [381]

While skin aging is a natural process that cannot be completely prevented, there are steps that can be taken to slow down the process and maintain healthy skin. These include protecting the skin from UV radiation by wearing protective clothing and using sunscreen, avoiding smoking and exposure to

pollution, and maintaining a healthy diet and lifestyle. Additionally, skincare products that contain ingredients such as retinoids, antioxidants, and hyaluronic acid can help decreasing the appearance of fine lines and wrinkles, improve skin texture and tone, and enhance hydration. Generally, the strategies for treating skin aging include the common anti-aging approaches: stem cell therapy, hormone replacement therapy, telomere modification, diet restriction, antioxidant and retinoid treatment, anti-inflammaging. [381]

In addition to the social and health-related implications, skin aging is also an area of interest for the cosmetics and skincare industries. There is a large market for anti-aging skincare products, and research into the underlying mechanisms of skin aging can help to develop new and more effective products.

3. Anti-aging strategies

The modern concept of anti-aging strategy is focused on promoting healthy aging and extending healthy lifespan. Rather than trying to reverse the aging process, anti-aging strategies aim to reduce the risk of age-related diseases and disabilities, maintain physical and cognitive function, and enhance overall well-being in later life. [382] Key features of the modern concept of anti-aging strategy include:

- A focus on prevention: Anti-aging strategies are centered on prevention, with an emphasis on reducing the risk of age-related diseases and disabilities before they occur. This may involve lifestyle changes such as healthy eating, exercise, and stress management, as well as preventive medical interventions such as vaccines and screening tests.

- An integrated approach: Anti-aging strategies take an integrated approach, recognizing that aging is a multidimensional process that affects many aspects of our lives. This may involve interventions that target multiple aspects of aging, such as physical activity to maintain muscle mass and cognitive training to maintain mental function.

- A personalized approach: Anti-aging strategies recognize that there is no "one size fits all" approach to promoting healthy aging, and that interventions may need to be tailored to individual needs and circumstances. This may involve the use of personalized medicine, genomics, and other technologies to identify individuals at higher risk of age-related diseases and tailor interventions accordingly.

- An emphasis on quality of life: Anti-aging strategies are focused on promoting not just longevity, but also quality of life in later years. This may involve interventions that promote physical and cognitive function, as well as social and emotional well-being.

- A multidisciplinary approach: Anti-aging strategies involve a wide range of disciplines, including medicine, biology, psychology, sociology, and public health. Researchers and practitioners from these different fields work together to identify effective interventions and promote healthy aging at the individual, community, and societal levels.

Numerous age-related attributes have been identified and potential mechanisms of the aging process are extensively examined. At the molecular level, aging features include DNA damage,

epigenetic modifications, telomere shortening, protein aggregation, and accumulation of aberrant mitochondria and lysosomes. [4, 103, 383] At the cellular and organismic level, aging features comprise cellular senescence, stem cell exhaustion, impaired nutrient sensing and chronic low-level inflammation. [4, 103, 383] A variety of anti-aging strategies targeting these hallmarks have been explored largely including blood factors, metabolic manipulations, senescent cell elimination and cellular reprogramming. Most of them are targeted to multiple aging hallmarks (Figure 8).

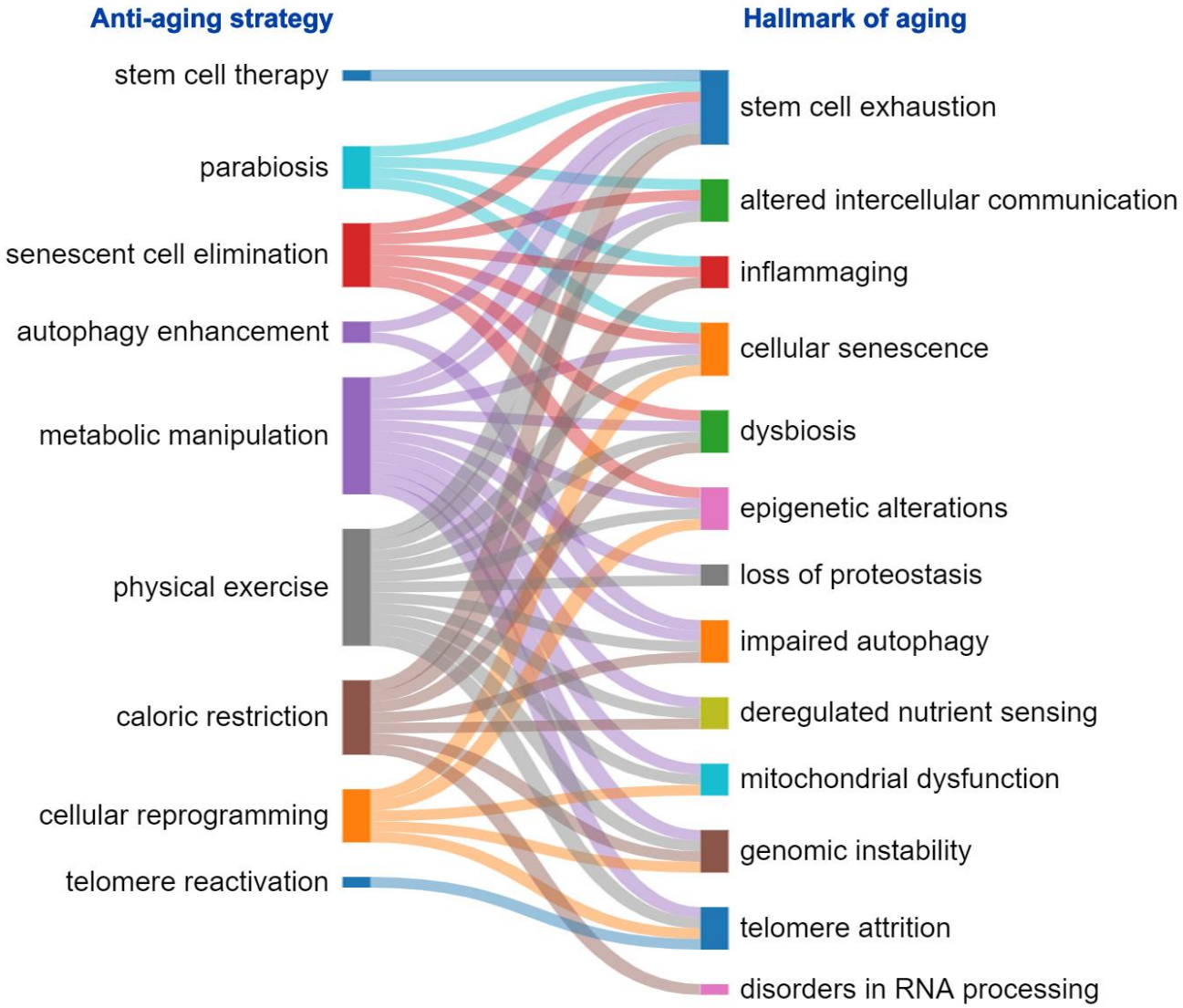


Figure 8. Relationship between anti-aging strategies and the hallmarks of aging they counteract.

3.1. Parabiosis (blood exchange)

Blood transfusion/exchange has been believed to exhibit rejuvenation effect since ancient times. Parabiosis is a procedure of placing young blood into an old animal (heterochronic parabiosis) by joining the circulatory systems of the two animals so that they share their blood circulations. [101, 384] The

procedure has been reported to bring about an anti-aging effect by specifically activating molecular signaling pathways in liver, muscle, or neural stem cells of the old parabiont resulting in increased tissue regeneration. [385, 386] In search for the physiological background of such rejuvenating effects, certain soluble blood factors have been identified as in part responsible, including the chemokine CCL11, the growth differentiation factor 11 (GDF11), a member of the TGF-β superfamily, and oxytocin. [386-389] It has been implied that parabiosis reverses age-related decline by targeting several aging hallmarks including stem cell exhaustion, cellular senescence, altered intercellular communication, and inflammaging (Figure 7). [101] Still, the mechanisms of action of the factors identified as responsible for the rejuvenating effects in parabiosis animal studies remain largely unclear, with many unresolved issues. Further, a study of the lifespans of old and young rat pairs demonstrated that older partners lived for 4-5 months longer than controls, indicating that circulation of young blood might affect longevity. [390] A start-up company, Alkahestin (Menlo Park, CA, USA), initiated a clinical trial assessing the safety, tolerability, and feasibility of infusion of plasma from young donors to treat patients with mild-to-moderate Alzheimer's disease. [391] The trials implied that the treatment with young fresh frozen plasma has been safe, well tolerated, and feasible. [392] Certain limitations of the study were the small sample size, short duration, and variation in study design, but the conclusion was that the results demand further examination in larger, double-blinded placebo-controlled clinical trials.

3.2. Metabolic manipulation (mTOR inhibitors)

The mammalian target of rapamycin (mTOR) signaling pathway has been identified as significant participant of cellular metabolism that associates nutrient sensing with critical cellular processes that energize cell growth and proliferation. [393] mTOR belongs to a family of phosphatidylinositol kinase-related kinases, which are known to mediate cellular responses to stress factors such as DNA impairment and nutrient deficiency. In model studies on organisms such as *Saccharomyces cerevisiae*, *Caenorhabditis elegans*, and *Drosophila melanogaster*, mTOR has been identified as a negative regulator of life span. [393] The mTOR is involved in various distinctive aging pathways, such as nutrient sensing deregulation, proteostasis loss, autophagy impairment, mitochondrial dysfunction, cellular senescence, and stem cell function decline. [394, 395] It has been reported able to manipulate gut microbiota and its metabolites. [396]

One of the foremost pharmacological actions prolonging life span in certain model organisms is rapamycin. [393] Rapamycin is a natural product isolated from *Streptomyces hygroscopicus*, which exhibits antifungal, immunosuppressant, and antitumor proprieties, mediated by the inhibition of its target, the mammalian (mechanistic) target of rapamycin (mTOR). [397, 398]

Consistent with its central role in cellular metabolism, in synchronizing protein synthesis, energy metabolism, and autophagy, mTOR has been identified as an attractive target to ameliorate age-related pathologies. Inhibition of the mTOR pathway has profound effects on longevity and age-associated phenotypes across a wide variety of organisms. [393] However, the inherent mechanisms are still largely unclear and extensive research is still needed to fill many deficiencies in the available knowledge related to the mTOR function in the context of aging.

3.3. Senotherapy

Senotherapy involves development of potential therapeutic agents and approaches to explicitly target cellular senescence, a cell condition associated with aging and age-associated pathologies. [399] Such senotherapeutic strategies include:

- Geroprotection refers to strategies preventing or reversing the cell senescence by preventing DNA damage, oxidative stress, proteotoxicity, telomere shortening, and other senescence promoters.
- Senescence-associated secretory phenotype (SASP) inhibition can be achieved by applying agents restricting pro-inflammatory SASP production [400, 401], such as glucocorticoids [402], statins such as simvastatin [403], JAK1/2 inhibitors such as ruxolitinib [404, 405], NF-κB and p38 inhibitors, and IL-1α blockers.
- Senolytics are small molecule drugs that specifically provoke senescent cell elimination by aiming survival pathways and anti-apoptotic systems, antibodies and antibody-mediated pharmaceuticals. [69, 406, 407] Unlike SASP inhibitors, senolytics can be successful following intermittent rather than continuous administration. [408]
- Senomorphics are a wide range of agents, which suppress senescent phenotypes without killing cells. [409]
- Gene therapy approaches edit the cells' genes in order to enhance their resistance to aging, senile diseases, and to prolong the lifespan of the organism. [410, 411]

3.4. Cellular reprogramming

Cellular reprogramming includes the conversion of terminally differentiated mature cells into induced pluripotent stem cells (IPSCs). This process involves complete dedifferentiation, i.e. somatic cell identity has been erased as cells are converted to a pluripotent state. Reprogramming-induced rejuvenation strategy termed epigenetic rejuvenation implicates using a cocktail of four factors known as Yamanaka factors, a finding for which a Nobel prize was awarded in 2012. [64] Indeed, it was shown that overexpression of four transcription factors (Oct3/4, Sox2, Klf4 and c-Myc, referred to as "OSKM" factors or the "Yamanaka factors") reorganizes the epigenetic landscape and converts somatic cells to a IPSCs. [412, 413] The process of rejuvenation by cellular reprogramming results in the amelioration of certain aging hallmarks such as mitochondrial dysfunction, telomere attrition, changes in epigenetic alterations, genomic instability, and senescence. [101]

Furthermore, it has been recognized that cellular identity is determined by epigenetic changes, rather than by genomic DNA alterations. [414] The process of generating iPSCs has been improved with time, and has also been accomplished via chemical induction, rather than gene expression. [415-417] IPSCs have effectively unlimited regenerative ability and offer the potential for tissue replacement to counteract age-induced decline. Thus, iPSCs induction offer the potential of directed, personalized regenerative therapy for currently incurable diseases, such as certain neurodegenerative diseases, heart infarction, diabetes, and others. [418] Partial cellular reprogramming in mice has demonstrated promising results in alleviating age-associated symptoms without increasing the risk of cancer.

3.5. Telomere reactivation

As mentioned, telomere shortening takes place during aging. It is also related to certain age-associated diseases, including osteoarthritis, atherosclerosis, coronary heart disease, and atrial fibrillation. [419-421] It has been reported that aging can be impeded by telomerase overexpression, however, that can stimulate tumorigenesis. [422-424] Recently, that adverse effect has been eliminated by developing anti-aging strategies based on a telomerase activation, telomerase expression activation, and telomerase gene therapy. Thus, it has been found that an extract of the plant *Astragalus membranaceus*, TA-65, is a telomerase activator, which can restore telomere length without provoking cancer, and enhance age-related markers, including glucose tolerance, bone health, and skin quality. [425] Further, TERT transcription activator and sex hormones have been reported to be directly engaged in telomerase activation, which avoided telomere shortening and enhanced lifespan. [426, 427] Reactivation of telomerase expression by using a gene therapy strategy has been reported as successful approach for aging delay and lifespan extension of mice without cancer incidence. [428]

3.6. Hormesis

Hormesis is defined as a dose-responsive phenomenon typified by low-dose stimulation and high-dose inhibition. [429] It has been identified as an overcompensation response for mild environmental stress related to the body's intrinsic ability for self-maintenance and repair. In fact, the beneficial effects of mild stress on aging and longevity have been examined for long time. [430]

Thus, beneficial hormetic effects of repeated mild heat stress on aging human cell cultures in terms of their structural and functional integrity have been demonstrated. [431] Other mild stresses have also been reported to defer aging and stimulate longevity in experimental animals. These include mild hyperthermia, irradiation, hyper-gravity, exercise and caloric restriction, as well as chemical agents such as heavy metals, pro-oxidants, acetaldehyde, alcohols, and resveratrol. [432-435] In experimental animals, mild dietary stress without malnutrition postpones the majority of age-related physiological changes, prevents aging disease, and extends lifespan. [429]

3.7. Hormonal replacement

Hormone levels decline with age, as a result of the reduced secretion from the gland. [436] Diminished hormone levels are related to decline in bone mineral density, muscle mass, sexual appeal, erectile activity, and intellectual potential. Therefore, hormone supplementation has been widely applied to help improve the quality of life in the elderly. [437]

Perimenopause women suffer from discomforting symptoms such as hot flashes or vaginal dryness, so hormone replacement has been applied to lessen or eliminate them. Estrogens and progesterone have been beneficial in osteoporosis treatment as well. However, adverse effects of combined treatment of estrogens and progestin such as higher risk for cardiovascular disease, thromboembolic event, stroke, and breast cancer have been reported [438], so guidelines recommended lower dose for the shortest time for the hormone supplements administration. [439]

Elderly men typically exhibit low testosterone levels which have been associated with certain age-related pathologies. [440] Sarcopenia and osteoporosis are more common in older men. Thus, low plasma testosterone levels have been associated with sarcopenia and osteoporosis, as well as mild cognitive impairment and Alzheimer's disease. [441, 442] Therefore, testosterone replacement therapy has been found advantageous as it can augment muscle mass, physical strength, and body-mass index in elderly men. [439, 443] However, adverse effect of the testosterone therapy such as polycythemia and risk of aggravating prostate cancer have been reported as well. [437, 444]

Dehydroepiandrosterone (DHEA) is a precursor for sex hormones and is transformed into androgen or estrogen respectively. The decrease of plasma DHEA levels in elderly has been associated with certain age-related pathologies. Thus, DHEA supplementation has been reported beneficial for muscle mass, muscle strength, physical performance, and body mass index, as well as for mood and sexual function. [445-448] The adverse effects of DHEA are minimal, with no significant effect on hormone-dependent tumors. [449, 450]

3.8. Prebiotic/probiotics and fecal transplantation

Microbiota plays an essential role in human physiology and pathology. Recent reports on the involvement of microbiota in regulating health status and lifespan [451] have triggered significant growth in the field of life sciences research and industry. [452] Dysbiosis is a state characterized by distinct alterations in the microbiome resulting in an imbalance in the microbiota, modifications in their functional composition and metabolic performance, or a change in their allocation. The effect of the microbiota on human physiology and pathology is so widespread that it has been considered as an essential organ of the human body. [453-455]

Numerous studies have reported proof that microbiota-targeted interventions can have a therapeutic power not only for age-related diseases, but also for delaying aging and promoting longevity. Thus, longevity has been correlated to the *Firmicutes* bacteria rearrangement and *Proteobacteria* enrichment, substantial decrease in *Faecalibacterium prausnitzii* and elevation of *Eubacterium limosum* level. [456] Noteworthy, microbiota composition can be strongly modulated by certain factors including diet, probiotics/prebiotics/synbiotics, physical activity, drugs, and psychological stress. Thus, microbiota-aimed interventions comprise probiotics mostly containing *Bifidobacterium* and *Lactobacillus*. [457, 458]

Fecal microbiota transplantation is a procedure used to restore the intestinal ecosystem through transferring of feces filtrate from a healthy donor into the gastrointestinal tract of the recipient. [459] Recently, Its potential benefit and safety in pathologies commonly associated with aging, type 2 diabetes mellitus, metabolic syndrome, atherosclerosis, and neuro-degenerative diseases have been recently demonstrated. [460, 461]

3.9. Caloric restriction / intermittent fasting

One of the first antiaging approaches has followed from the observation that caloric intake control can increase lifespan – first demonstrated in mice and rats. [30] Indeed, the reduction of caloric

intake by some 10-30% as compared to ad libitum food intake has been demonstrated to extend the longevity of various species. Furthermore, among all anti-aging interventions, dietary interventions have demonstrated the greatest promise. Recently, two related dietary interventions, caloric restriction and intermittent fasting, have been reported to effectively prolong the healthy lifetime of the nervous system by affecting basic metabolic and cellular signaling pathways that regulate longevity. [462] Multiple interactive pathways and molecular mechanisms exist by which caloric restriction and intermittent fasting benefit neurons. Indications exist that the advantages of caloric restriction are associated with modification of the metabolic rate. Thus, the key physiological routes that have been inferred as potential mechanisms by which caloric restriction stimulates longevity include: (i) activation of AMP protein kinase [463]; (ii) sirtuins activation [464, 465]; (iii) insulin-like growth factor-1 signaling inhibition [466]; and (iv) mammalian target of rapamycin inhibition.[467, 468] These pathways promote the protein chaperones, neurotrophic factors and antioxidant enzymes production, which facilitate cells managing stress and fight disease.

Although the calorie restriction has exhibited the greatest efficiency of all anti-aging interventions, it is a difficult strategy to successfully apply in humans, since it requires a high level of determination and self-control. As an alternate route, compounds that emulate the outcome of caloric restriction on health and longevity without an actual restriction named 'calorie restriction mimetics' have been discovered (reviewed further in the paper). [469]

3.10. Physical exercise

Physical exercise has been well validated as an effective antiaging intervention. Regular physical activity of the elderly plays a vital role at a multisystem level, avoiding muscle atrophy, mending or sustaining cardiorespiratory health and cognitive performance, and enhancing metabolic activity [470-472], specifically including:

- enhancing neurogenesis and reducing neurodegeneration and cognitive decline;
- reducing blood pressure and augmenting various cardiovascular activities, e.g., maximum cardiac output, blood flow, endothelial performance, vagal tone and heart rate adaptability, and heart preconditioning;
- advancing respiratory activity by enhancing ventilation and gas exchange;
- augmenting metabolic activity in enhancing the resting metabolic rate, protein synthesis in muscles, and lipid oxidation;
- boosting muscle performance and body composition by enhancing muscle strength and stamina, maintaining or restoring balance, motor control, and joint flexibility and mobility, as well as decreasing weight and local adiposity, and enhancing muscle mass and bone density. [471, 472]

Furthermore, physical exercise exhibits a significant antiaging impact at a cellular level, related to each and every aging hallmark. [470] Thus, exercise plays a role in maintaining genomic stability. Data analysis comprising hundreds of genetic elements from a large number of exercising elderly individuals found a reduction in DNA methylation percentage in dominating number of genes, specifically in genes associated with a cancer- defeating miRNA gene network. [473] Exercise exhibits a beneficial impact on telomere length as well, antagonizing the regular age-provoked telomere attrition. Possible mechanisms have been debated correlating exercise and telomere length declines to alterations in telomerase

activity, inflammation, oxidative stress, and reduced skeletal muscle satellite cell content. [474] It has been documented that acute exercise protocols are correlated with increased heat-shock proteins transcription, which indicates potential positive impact of physical activity on proteostasis. [475]

Recommendations predicated on the most recent American College of Sports Medicine Guidelines advise that physical exercises for elderly need to involve aerobic exercise, muscle strengthening and endurance training, as well as flexibility and neuromotor exercises. [476]

3.11. Stem cell therapy

Stem cell therapies are widely used in the regenerative medicine thanks to their intrinsic biological characteristics, such as plasticity, self-renewal, and multiway differentiation ability. Stem cell treatment includes human autograft or allograft cultured stem cells locally injected into specific parts of the body or administered by intravenous infusion. [477, 478] Thus, bringing active stem cells into the body can rejuvenate existing cells and allow the body to age more gently and even reverse some impacts of the aging. Currently, neural stem cells, bone marrow mesenchymal stem cells, umbilical cord mesenchymal stem cells, adipose stem cells, embryonic stem cells, and human induced pluripotent stem cells are the most closely related anti-aging agents and exhibit direct (cellular replacement) and indirect (paracrine) effects. [478]

Pluripotent stem cells naturally differentiate when culture conditions no longer support their pluripotency. [289] Thus, pluripotent stem cells can be directed towards preferred cell identities when specific stimuli are supplemented, such as those available during embryonic differentiation. There are many examples of pluripotent stem cells differentiation. The differentiation of pluripotent stem cells into renal podocytes, hematopoietic progenitor cells, neurons, endothelial cells, cardiac muscle cells, retinal progenitor cells, pancreatic β islet cells or ciliated epithelial cells, infers no limits to human tissue modeling *in vitro*. [479-486] The reported progresses in organoids development also demonstrates the advanced knowledge in cell manipulations. Three-dimensional cultures of pluripotent cells let them organize and differentiate into spheroid structures, with several cohabitant cell types. The most sophisticated current organoid models relate to brain, intestine, kidney, heart, or retina. [487-496] With the emergence of cell 3D-printing technologies using pluripotent stem cells or differentiated cells as inks, impressive progress has been successful in the formation of heterogeneous tissues. The development of ear cartilage regeneration utilizes this technology. [497-499]

Thus, stem cells may be capable to defer aging in several ways:

- Tissue regeneration: Stem cells can differentiate into various cell types and can thus replace damaged tissue, potentially reversing the impacts of aging.
- Augmenting body's repair mechanisms: Stem cells stimulate production of growth factors and other signaling molecules thus enhancing the repair mechanisms of the body, allowing to maintain healthy tissue and postpone age-related alterations.
- Immune modulation: Stem cells may act as immunomodulators, affording to maintain a healthful immune system and avoid age-related immune dysfunction.
- Lowering inflammation: Stem cells may have anti-inflammatory properties, thus reducing the chronic aging-related inflammation.

- Oxidation protection: Stem cells may safeguard against oxidative stress, a process that can result into cell damage throughout aging.
- Mitochondrial support: Stem cells can preserve mitochondrial health by intercellular communication via tunneling nanotubes physically transferring mitochondria from stem cells to unhealthy aging cells.

3.12. Dietary supplementation

Dietary supplements, such as vitamins, minerals, amino acids, essential fatty acids, flavonoids, plants and herbs, and accessory food ingredients, are considered valuable and safe materials for prevention and treatment of chronic and acute diseases. [500]

Interest in the therapeutic capacity of vitamin supplements, specifically vitamin D, for stimulating human longevity and reducing risk of aging-related pathologies is currently a hot topic in clinical studies. [501] Indeed, it has been reported that vitamin D and its metabolites can delay age-associated diseases by lowering oxidative stress, strengthening innate immunity, preventing DNA damage and supporting DNA repair pathways, controlling mitochondrial and glucose metabolism, defeating cellular senescence, enhancing telomerase activity. [500, 502-504] These research findings imply that vitamin D consumption exhibits anti-aging properties. Recent systematic review have also reported that vitamin D intake notably lowers the risk of acute respiratory infections. [505] Other vitamins such as B vitamins, K vitamins, and others, have been examined as dietary supplements that assist healthy aging. [500] Thus, vitamin B12, in combinations with bacopa, lycopene, and astaxanthin successfully alleviated cognitive decay associated with brain aging. [506] Improving vitamin interventions by combining them with other health-supporting supplements might result in successful clinical utilization.

Minerals are vital for health and well-being and are typically obtained from diet. Deficiencies in some minerals have been associated to age-related diseases. Thus, dysregulated calcium levels are correlated to accelerated cellular aging. Calcium-related changes upon aging are regarded as a contributing factor for neurological degenerative disorders such as Parkinson's disease. There is evidence that aging individuals are at risk of calcium deficit, so appropriate calcium dietary intake can lower the risk of osteoporosis and bone fractures. [507, 508] Zinc is another mineral that promotes healthy aging and supports healthspan. Zinc has numerous biological roles, such as antioxidant, anti-inflammatory, immune modulation, DNA damage prevention, and others. Zinc insufficiency is often reported upon aging, and contributes to age-associated disorders. Thus, zinc supplementation is considered an efficient choice for controlling age-related health pathologies, including neurological disorders, infectious diseases, age-related macular degeneration. [509, 510]

Long-chain polyunsaturated fatty acid supplementation is believed to protect human health by affecting biological activities, notably aging processes. [511, 512] Recent reports suggest that higher levels of ω3 fatty acids in circulation correlate with lower risk of premature death from age-associated diseases such as cardiovascular disease and cancer. [513] Dietary ω3 fatty acid consumption have been related to cognitive improvements in aging populations. [514] The ω3 fatty acids have been shown to exhibit anti-inflammatory, antiapoptotic, antioxidant, and endothelial vasodilator effects. [515]

3.13. Autophagy enhancement

Autophagy is a natural degradation process for removing unnecessary or dysfunctional cellular components via a lysosome-dependent mechanism. [382, 516, 517] Dysfunctional autophagy is known to contribute to neurodegeneration [518] while improvement of autophagy has been believed to be useful for treating a variety of disorders, including metabolic disorders, neurodegenerative and infectious diseases, and cancers. [516] An unharmed autophagy performance in neurons exhibits a neuroprotective effect since autophagy expedites the elimination of pathogenic kinds of proteins such as α-synuclein involved in Parkinson's disease [519] or tau-protein in Alzheimer's disease. [520, 521] Therapy involving autophagy enhancers such as rapamycin and lithium have been reported to engender favorable effects in animal models of Parkinson's disease, such as reduced α-synuclein aggregation, oxidative stress, mitochondrial dysfunction, and neurodegeneration. [519] Delaying aging and stimulating longevity achieved through food deprivation and caloric restriction are facilitated through upregulation of autophagy because reducing autophagy diminishes the anti-aging outcomes of caloric restriction. [198, 516, 522] Furthermore, studies have indicated that enhancing autophagy could reinstate the regenerative capacity of aging stem cells. [523, 524] Thus, autophagy enhancing interventions would likely facilitate successful aging and increased longevity.

4. Landscape view of aging physiology and anti-aging strategies research – insights the from CAS Content Collection

The CAS Content Collection[100] is the largest human-compiled collection of published scientific information, which represents a valuable resource to access and keep up to date on the scientific literature all over the world across disciplines including chemistry, biomedical sciences, engineering, materials science, agricultural science, and many more, thus allowing quantitative analysis of global research publications against various parameters including time, geography, scientific area, medical application, disease, and chemical composition. Currently, there are over 500,000 scientific publications (mainly journal articles and patents) in the CAS Content Collection related to aging physiology and anti-aging strategies. There is a steady growth of these documents over time, especially intense in the last decade (Figure 2).

China, United States, Japan, and Korea are the leaders with respect to the number of published journal articles (Figure 9A) and patents (Figure 10) related to aging physiology and anti-aging strategies. University of California and the Chinese Academy of Sciences are the leaders with the largest number of published articles in scientific journals (Figure 9B). The journal PLoS One is the distinct leader publishing the highest number of articles related to the physiological mechanisms of aging and the anti-aging strategies (Figure 9C).

Patenting activity is dominated by corporate players as compared to academics. L'Oreal and Amorepacific have the highest number of patents, and most of the companies offer products targeted to

defy skin aging (Table 4). Figure 11 summarizes the most frequent concepts in the patents filed by four of the top patent assignee companies: L'Oreal, Amorepacific, Noevir, and Maruzen Pharmaceuticals.

A

Country	Journal Papers No.
China	108,578
United States	86,548
Japan	28,903
Korea	16,826
Germany	13,458
United Kingdom	13,264
France	12,362
Italy	12,260
India	8,605
Spain	7,525
Canada	7,418
Netherlands	5,696
Australia	5,094
Russia	4,018
Brazil	3,930
Switzerland	3,398

B

Organization	Journal Papers No.
University of California	2,063
Chinese Academy of Sciences	1,231
Harvard Medical School	927
Central South University	691
University of Michigan	661
University of Washington	644
Huazhong University Sci. & Technol.	592
Sichuan University	570
Zhejiang University	549
National Institutes of Health	539
Fudan University	524
University College London	524
Mayo Clinic	521
University of Southern California	520
University of Florida	512
University of Cambridge	497
Russian Academy of Sciences	492
McGill University	484
Baylor College of Medicine	466
Albert Einstein College of Medicine	420

C

Journal	No. of papers
PLoS One	8,109
Scientific Reports	3,658
Experimental Gerontology	2,674
Mechanisms of Ageing and Development	2,371
Neurobiology of Aging	2,336
International Journal of Molecular Sciences	2,193
American Journal of Physiology	2,042
Journal of Clinical Endocrinology & Metabolism	1,711
Journals of Gerontology	1,680
PNAS USA	1,663
International Journal of Environmental Research and Public Health	1,565
Journal of Alzheimer's Disease	1,492
Aging	1,489
Nutrients	1,440
Frontiers in Aging Neuroscience	1,361
Journal of the American Geriatrics Society	1,359
Aging Cell	1,351
Brain Research	1,183
Annals of the New York Academy of Sciences	1,116

Figure 9. Top countries (A), organizations (B), and scientific journals (C) publishing articles related to aging mechanisms and anti-aging strategies.

Figure 10. Top countries with respect to the number of patents related to aging mechanisms and anti-aging strategies.

Table 4. Top patent assignee companies with their anti-aging area of expertise.

Top Patent Assignees	No. Patents	Area
L'Oreal	656	Skin
Amorepacific	450	Skin
Noevir	251	Skin & Healthy food
Shiseido	213	Skin
Guangzhou Saliai Stemcell Sci. Technol.	199	Skin
Coreana Cosmetics	169	Skin
Maruzen Pharmaceuticals	145	Skin & Obesity
Avon	129	Skin
LG Household & Health Care	128	Skin
Pola Chemical Industries	104	Skin
Procter & Gamble	100	Skin & Anti-microbial
Nestec	95	Dietary Supplement

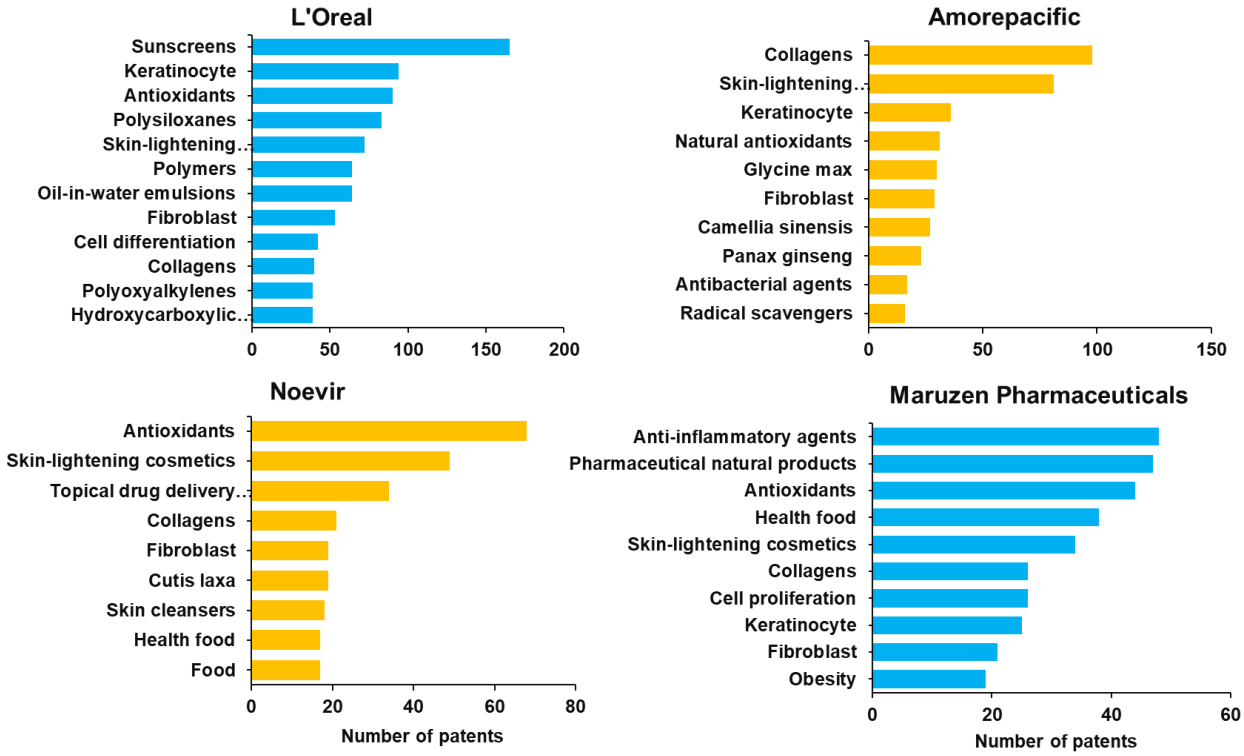


Figure 11. Major concepts in patents of four of the top patent assignee companies

Figure 12 presents the most popular concepts in the overall patent landscape of the CAS Content Collection. The antiaging cosmetics and antioxidants the distinct leaders.

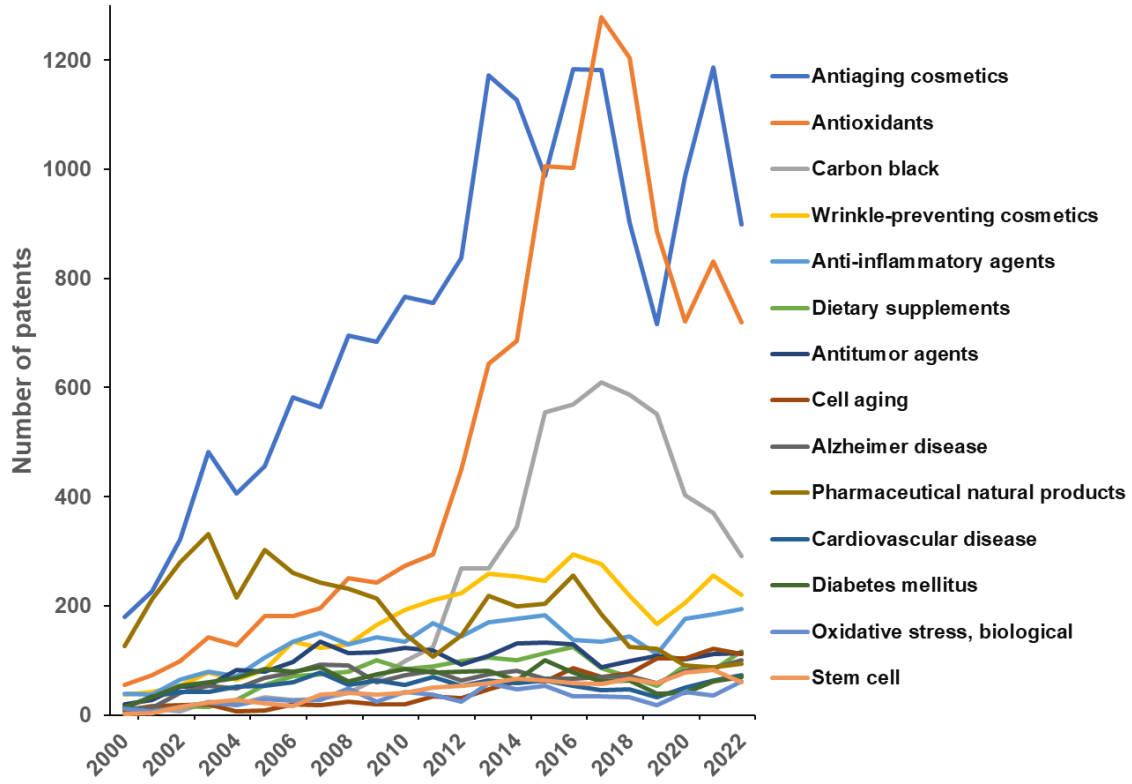


Figure 12. Popular concepts in patents related to aging mechanisms and anti-aging strategies according to the CAS Content Collection.

Figure 12A presents the distribution of patents related to the aging mechanisms and anti-aging strategies with respect to the patent office. The China patent office clearly dominates with account of the patent applications, followed by the Korean patent office, the World Intellectual Property Organization (WIPO), Japan, and US patent offices.

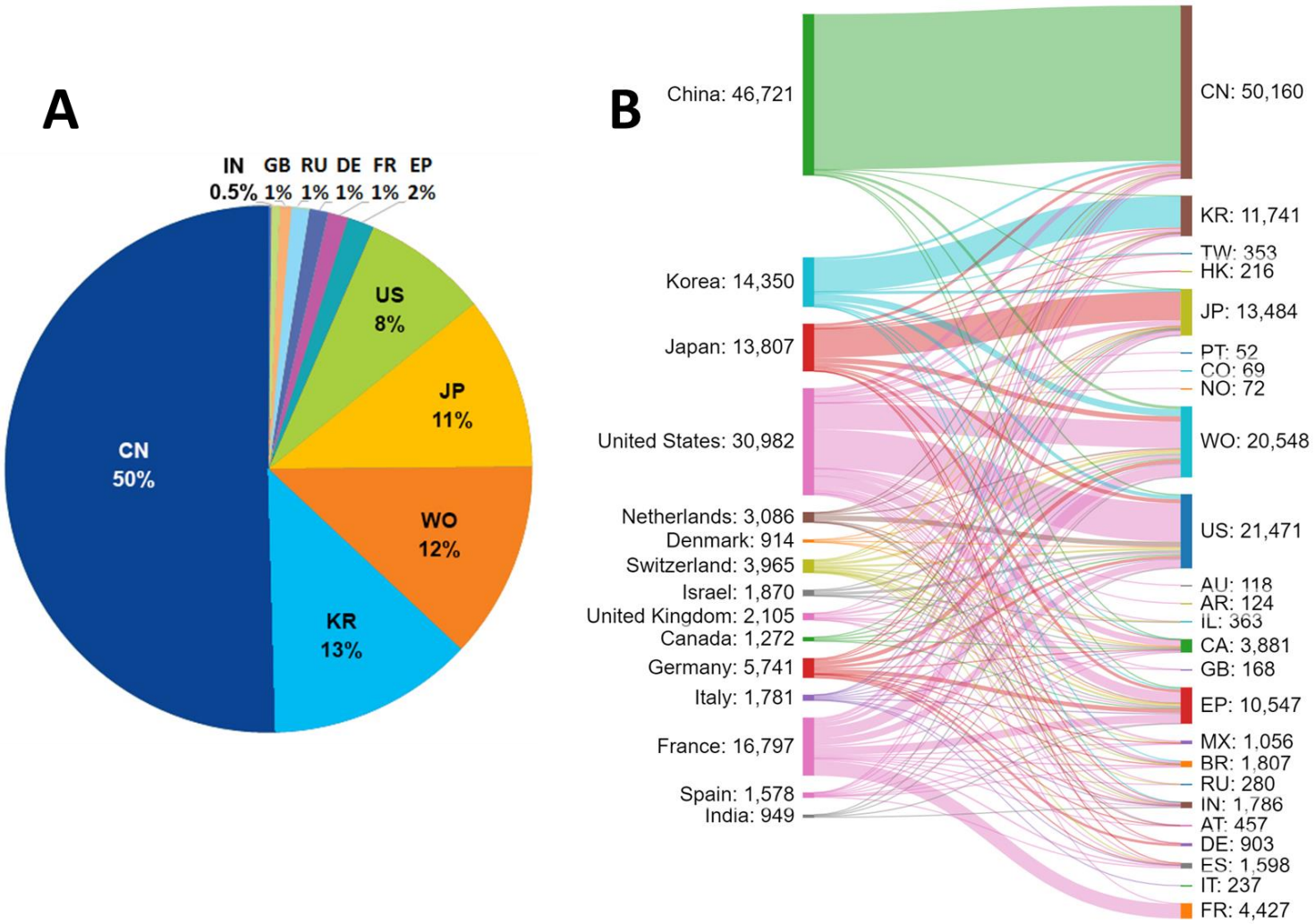


Figure 12. (A) Top patent offices receiving patent applications related to aging mechanisms and anti-aging strategies. (B) Flow of patent filings related to aging mechanisms and anti-aging strategies from different patent assignee locations (left) to various patent offices of filing (right). The abbreviations on the right indicate the patent offices of China (CN), Korea (KR), Israel (IL), Hong Kong (HK), Argentina (AR), Australia (AU), Russian Federation (RU), United States (US), World Intellectual Property Organization

(WO), Taiwan (TW), Mexico (MX), Japan (JP), Canada (CA), Brazil (BR), Austria (AT), European Patent Office (EP), India (IN), Great Britain (GB), Spain (ES), France (FR), Germany (DE), and Italy (IT).

Patent protection is territorial and therefore the same invention can be filed for patent protection in several jurisdictions. We thus searched for all related filings on aging mechanisms and anti-aging strategies. Certain patent family might be counted multiple times when they filed in multiple patent offices. Figure 12B presents the flow of patent filings from various applicant locations to a variety of patent offices of filing. There are various patent filing strategies: some patent assignees, such as those from China, for example, file exclusively in their home country patent office (CN), with only a small portion filing through the World International Patent Office WIPO (WO), or other jurisdictions. Others, for instance United States and France-based applicants, have a comparable number of their home country and WO filings, and a sizable number of filings at other patent offices such as the European Patent Office (EP).

We further explored the distribution and trends in the published documents dealing with the various hallmarks of aging (Figure 13). The cellular senescence is clearly the aging attribute attracting most attention (Figure 13A). This should come as no surprise since cellular senescence is the aging denominator closely related to all other aging features (Figure 5). It has been also connected to multiple age-related disorders, including cancer, diabetes, osteoporosis and osteoarthritis, cardiovascular disease, stroke, Alzheimer's disease and other dementias; furthermore, it has also been linked to deteriorations in eyesight, mobility, and cognitive capability. [237] With respect to the annual trends in the aging hallmarks related publications, steady annual growth has been seen in those related to the stem cell exhaustion, altered intercellular communication, impaired autophagy, and especially dysbiosis (Figure 13B). Indeed, the extreme significance of gut microbiome in multiple aspects of human health is recently becoming well renowned and a hot topic in scientific research. [525] Substantial data suggest the gut microbiome plays a role in virtually all physiological processes in the human body, including metabolism and immune homeostasis. Alterations to these processes can disturb the balance in the microbiome – a process termed dysbiosis – and trigger a range of pathological processes fundamental to mental health, metabolism, including multiple age-related diseases. [526]

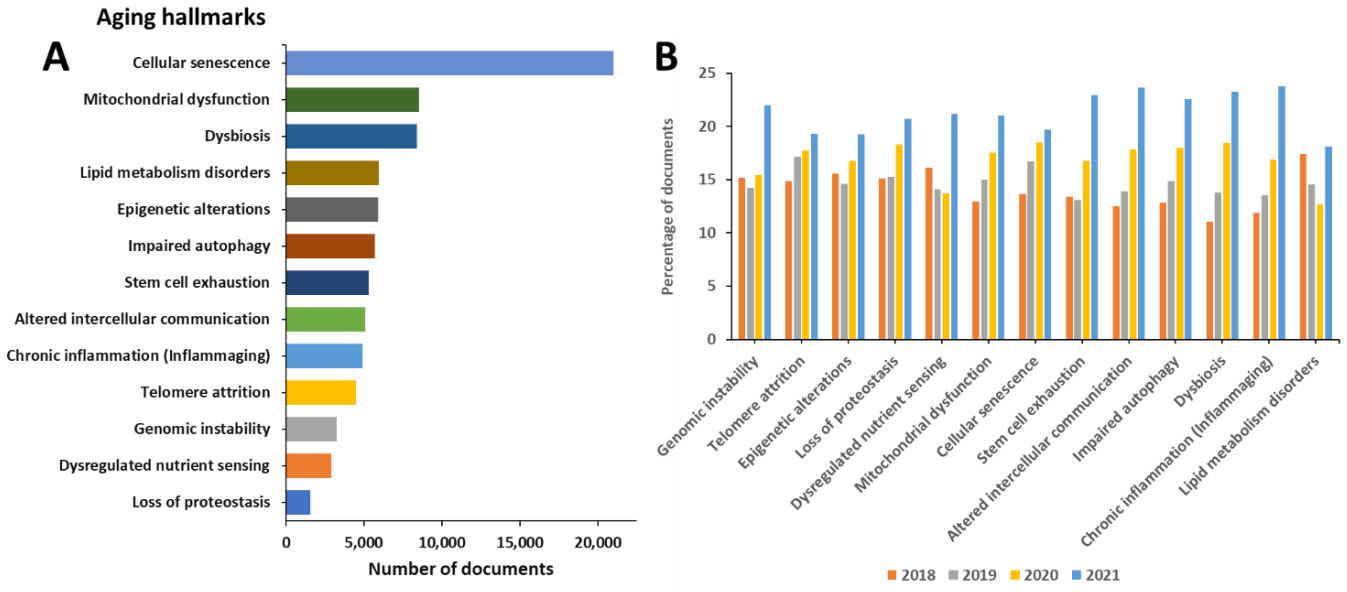


Figure 13. Hallmarks of aging explored in the scientific publications: (A) Number of publications exploring hallmarks of aging; (B) Trends in number of publications exploring hallmarks of aging during the years 2018−2021.

We examined also the distribution and trends in the published documents dealing with prominent anti-aging strategies (Figure 14). The physical exercise comes up as the most studied approach, along with the metabolic manipulation (Figure 14A). This can be well anticipated considering the fact that these strategies are the ones related with the highest number of aging attributes (Figure 8). Indeed, physical exercise has been well justified as one of the particularly effective and highly recommended anti-aging practice. Regular physical activity of the elderly has a proven vital role at a multisystem level, avoiding muscle atrophy, mending or maintaining cardiorespiratory health and cognitive performance, and boosting metabolic activity. Next, the inhibition of the mTOR pathway has been shown to exhibit profound effects on longevity and age-related phenotypes across a wide variety of organisms. [393]

With respect to the annual trends in the anti-aging strategies related publications, parabiosis (blood exchange) attracts substantial recent attention (Figure 14B). It has been suggested that blood exchange reverses the age-associated decline by targeting multiple attributes of aging including stem cell exhaustion, cellular senescence, altered intercellular communication, and chronic inflammation (Figure 8). A steady growth in the number of recent publications have been documented with respect to the prebiotic/probiotics and fecal transplantation as well (Figure 14B). Multiple studies have conveyed evidence that microbiota-targeted interventions can have a strong therapeutic power not only for age-associated diseases, but also for delaying aging and stimulating longevity. Indeed, longevity has been correlated to certain bacteria phyla alterations: *Firmicutes* rearrangement and *Proteobacteria* enrichment, decrease in *Faecalibacterium prausnitzii* and elevation of *Eubacterium limosum*. [456] Furthermore, microbiota composition can be intensely modulated by activities and interventions including diet, probiotics/prebiotics/synbiotics, physical activity, drugs, and psychological stress. [457, 458] Fecal transplantation is another recently emerging intervention being looked at for a wide range of conditions including pathologies commonly associated with aging such as diabetes, metabolic

syndrome, atherosclerosis, and neurodegenerative diseases. [460, 461] Remarkably, recent animal studies showed that, similarly to blood exchange, transfer of young donor microbiota into older animals can reverse age-related central nervous system and retinal inflammations, and cytokine signaling, effects which are coincident with increased intestinal barrier permeability. [527] Thus, microbial modulation emerges as therapeutically beneficial in preventing inflammation-associated tissue decay in later life. [527]

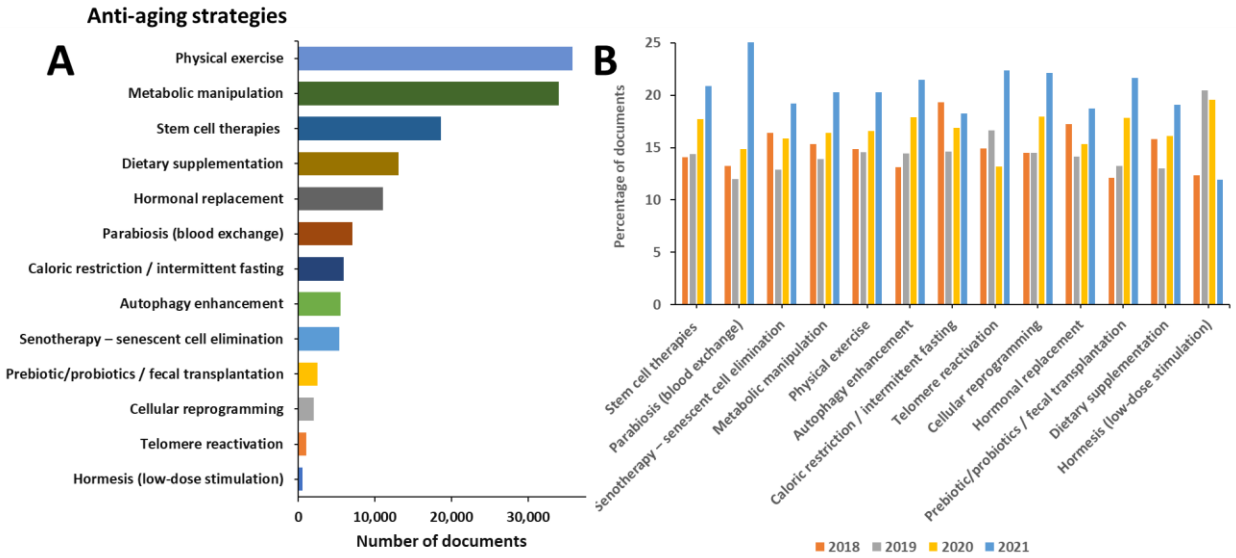


Figure 14. Anti-aging strategies explored in the scientific publications: (A) Number of publications exploring anti-aging strategies; (B) Trends in number of publications exploring anti-aging strategies during the years 2018–2021.

Next, we explored the correlations between the hallmarks of aging and the anti-aging strategies as reflected by the number of documents in the CAS Content Collection (Figure 15).

	Stem cell therapies	Parabiosis (blood exchange)	Senotherapy – senescent cell elimination	Metabolic manipulation	Physical exercise	Autophagy enhancement	Caloric restriction / intermittent fasting	Telomere reactivation	Cellular reprogramming	Hormonal replacement	Prebiotic/probiotics / fecal transplantation	Dietary supplementation	Hormesis (low-dose stimulation)
Genomic instability	166	14	47	113	42	52	43	55	53	20	8	111	8
Telomere attrition	250	11	46	131	130	33	65	341	46	42	6	242	3
Epigenetic alterations	285	22	33	377	203	60	92	22	209	53	10	599	8
Loss of proteostasis	49	5	27	174	39	190	52	3	14	3	2	107	16
Dysregulated nutrient sensing	94	40	27	468	238	93	258	5	27	95	71	1641	11
Mitochondrial dysfunction	230	38	95	1412	500	503	181	25	90	83	15	900	29
Cellular senescence	1377	67	784	741	242	873	260	502	366	168	19	752	43
Stem cell exhaustion	3127	143	75	357	161	140	52	46	127	263	9	293	5
Altered Intercellular Communication	246	46	47	298	191	45	45	9	51	63	28	283	12
Impaired autophagy	234	31	153	702	208	3056	169	18	75	81	9	516	29
Dysbiosis	56	25	12	1048	270	27	79	3	20	47	1196	2595	4
Chronic inflammation (Inflammaging)	159	57	67	407	264	98	79	19	38	127	84	621	8
Lipid metabolism disorders	72	93	43	2139	485	54	64	4	15	354	29	987	1

Figure 15. Correlation of the number of documents related to the hallmarks of aging with the anti-aging strategies.

Along with some foreseeable strong correlations such as stem cell exhaustion – stem cell therapies, lipid metabolic disorders – metabolic manipulations, impaired autophagy – autophagy enhancement, cellular senescence – senotherapy, and dysbiosis – prebiotic/probiotic/fecal transplantation, there are some less anticipated and instructive:

- Metabolic manipulations appear as closely aligned with the mitochondrial dysfunction and dysbiosis.

Indeed, metabolic manipulation have been recognized as noteworthy strategy in mitochondrial medicine. [528] Cellular alterations triggered by mitochondrial dysfunction include enhanced reactive oxygen species production, enhanced lipid peroxidation, and modified cellular calcium homeostasis. Thus, metabolic manipulation is aimed to prevention of oxidative damage by ROS, adjustment of lipid peroxidation, amendment of altered membrane potential, and restoration of calcium homeostasis. [528]

- Autophagy enhancement associates with cellular senescence.

Autophagy has been initially reported to inhibit mesenchymal stem cells senescence by eliminating damaged cytoplasmic organelles and macromolecules, yet recent studies found that autophagy can in fact promote mesenchymal stem cells senescence by triggering the production of senescence-associated secretory proteins (SASP). [529]

- Epigenetic alterations and mitochondrial dysfunction are well linked to dietary supplementation.

Nutritional epigenetics is a novel subfield of epigenetics dealing with the specific effects of bioactive food constituents on epigenetics and their relations to phenotypes. Elucidating the epigenetic features prompted by bioactive food components might set the stage for personalized nutritional therapeutics and enhance the understanding of how organisms respond to specific diets or nutrients. [530] Thus, a recent study reported that fruit and juice epigenetic effects as assessed by DNA methylation are related to independent immunoregulatory routes, indicating the distinct health benefits of fruits and juices. The discovery of such differences amongst foods is the first step toward personalized nutrition. [531] Further, adequate nutrient levels are important for mitochondrial function as certain particular micronutrients play essential roles in energy metabolism and ATP production. [532]

- Stem cell therapies strongly affect cellular senescence.

One of the advantages of mesenchymal stem cell-based therapies is that they have demonstrated effectiveness by targeting multiple pathological pathways. [533] Thus, a recent study has indicated that mesenchymal stem cells could alleviate renal cellular senescence. [534]

- Lipid metabolism disorders appear impacted by dietary supplementation.

Recently, there is remarkable interest in the health benefits of food constituents against chronic diseases in which elevated lipids are a major issue. [535] Thus, the nutritional regulation of lipid metabolism has become an essential tool to prevent or reverse the development of lipid metabolism disorders.

- Dietary supplementation emerges as well correlated with virtually all aging attributes, and especially with dysbiosis.

A wide collection of diseases are associated with aging. Figure 16 illustrates the distribution of documents in the CAS Content Collection related to such age-associated pathologies. Among these major diseases are cancer, diabetes, and hypertension. Inflammation, cardiovascular diseases, and cognitive disorders are also highly represented (Figure 16).

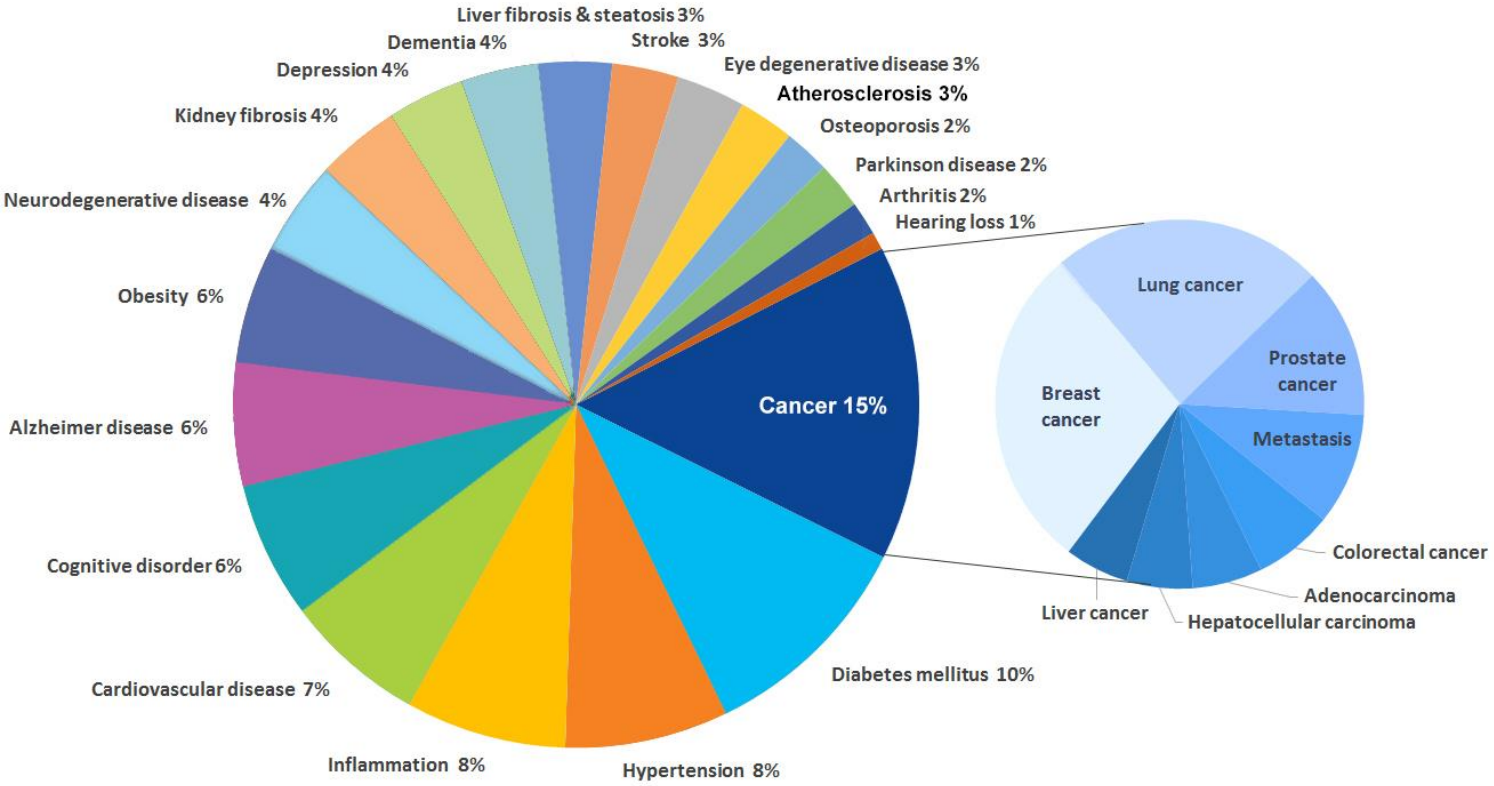


Figure 16. Distribution of documents in the CAS Content Collection related to the age-associated diseases.

Figure 17 presents the annual trend in the number of documents related to the age-associated diseases. A steady growth in the number of recent publications have been documented with respect to inflammation and neurodegenerative disease including dementia and depression. Indeed, chronic low-grade inflammation (inflammaging) has been identified as playing an increasingly important role in the rate of aging and has recently emerged as a challenging and promising new domain of aging-related research. [536] Neurodegenerative diseases, although originating from different primary causes, all share a hallmark of neuroinflammation. [537]

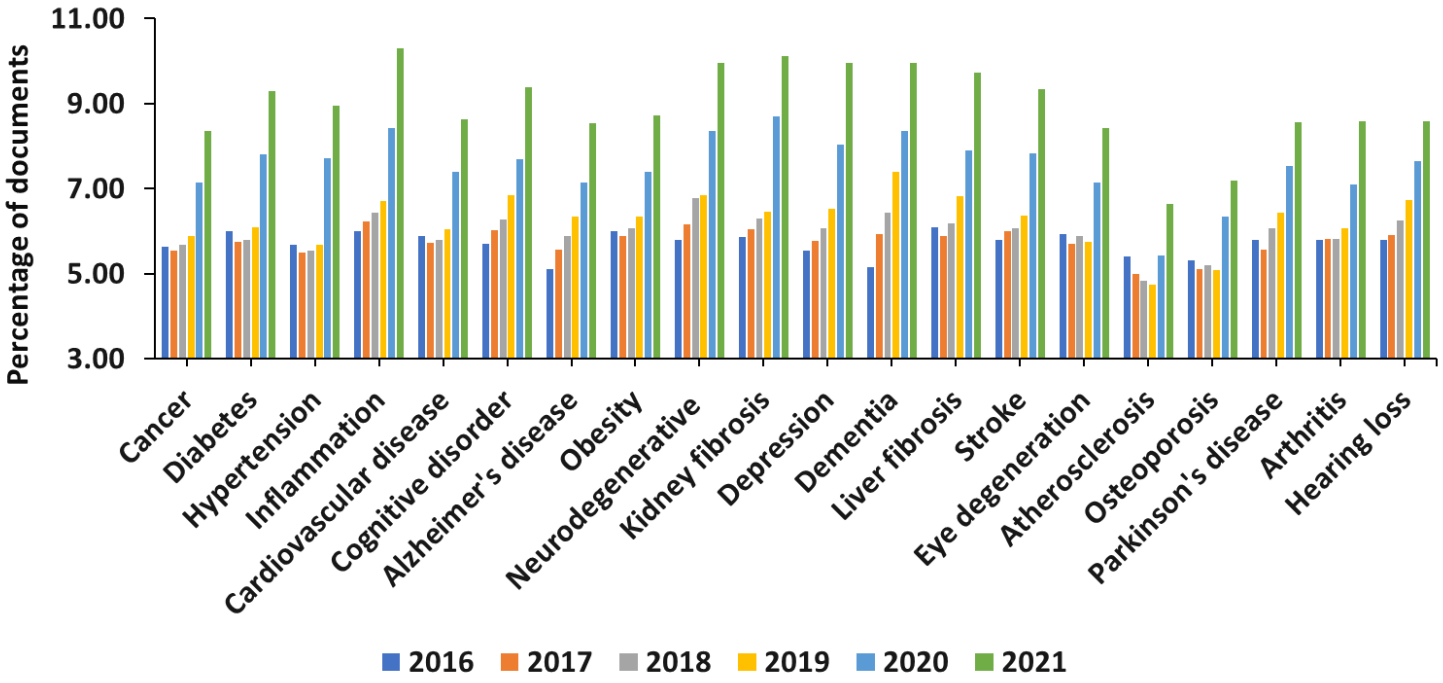


Figure 17. Annual growth of number of documents related to age-associated diseases

We explored the correlations between the age-related diseases and the anti-aging strategies as reflected in the number of documents in the CAS Content Collection (Figure 18). Generally, metabolic manipulations, physical exercise, hormonal replacement, dietary supplementation, as well as stem cell therapies, appear as well exploited approaches against multiple pathologies.

	Stem cell therapies	Parabiosis (blood exchange)	Senotherapy – senescent cell elimination	Metabolic manipulation	Physical exercise	Autophagy enhancement	Caloric restriction / intermittent fasting	Telomere reactivation	Cellular reprogramming	Hormonal replacement	Prebiotic/probiotics / fecal transplantation	Dietary supplementation	Hormesis (low-dose stimulation)
Cancer	6371	2453	934	3945	2333	1362	689	432	600	2408	204	1954	68
Diabetes mellitus	866	982	346	8699	4185	410	616	37	85	2028	213	2031	39
Hypertension	424	710	247	4850	3715	111	253	16	31	1219	88	1357	8
Inflammation	1384	731	431	4340	2363	893	593	110	244	1101	529	2138	46
Cardiovascular disease	783	520	298	4484	4129	442	560	41	80	1815	110	1917	37
Cognitive disorder	495	328	147	1847	3016	257	223	7	40	841	131	1243	18
Alzheimer's disease	569	200	242	2005	1159	456	219	21	73	904	96	879	43
Obesity	372	466	197	5382	4047	233	1269	16	68	1194	241	1945	16
Neurodegenerative disease	838	187	235	1824	769	863	365	15	168	559	89	788	70
Kidney fibrosis	705	607	336	2537	738	217	195	13	41	1336	57	601	8
Depression	154	149	69	1042	1625	28	42	13	10	618	59	430	3
Dementia	229	158	85	1042	1260	134	64	6	23	598	58	485	11
Liver fibrosis & steatosis	883	582	276	2637	754	303	291	43	70	830	119	874	10
Stroke	415	442	89	1366	1423	83	80	6	18	698	32	408	18
Eye degenerative disease	826	337	138	1045	366	390	74	16	96	592	37	670	10
Atherosclerosis	268	276	160	1700	631	129	113	39	24	832	25	483	7
Osteoporosis	709	218	86	1966	1020	77	79	9	21	6842	47	903	6
Parkinson's disease	394	148	110	1146	439	474	98	4	50	529	33	313	38
Arthritis	414	304	108	1008	424	161	42	18	19	1132	34	343	4
Hearing loss	139	56	28	227	181	40	29	1	14	171	7	92	0

Figure 18. Correlation of the number of documents related to the age-related diseases with the anti-aging strategies.

Some particular correlation are noteworthy:

- Stem cell therapies exhibit strong correlation with cancer.

Stem cell transplants are procedures, which restore the hematopoietic stem cells in patients receiving high doses of chemotherapy or radiotherapy used to treat certain cancers. [538-540] Stem cell transplants do not typically work against cancer directly; they rather help recover body's ability to produce stem cells after such high-dose interventions. Still, in multiple myeloma and some leukemias, a stem cell transplant may work against cancer directly, due to the graft-versus-tumor effect taking place after allogeneic transplants, when leukocytes from the donor attack the remained cancer cells after high-dose treatments. This effect enhances the success of the treatments. [538] Growing evidence is also indicating that cancer stem cells can differentiate into various cell types, including noncancerous cells. Scientists are taking advantage of this observation through a treatment called differentiation therapy. [541]

- Metabolic manipulation approach is well correlated with virtually all age-associated diseases, but specifically with diabetes and obesity.

Indeed, mTOR dysregulation is known to result in a number of metabolic pathologies, including obesity and type 2 diabetes. [542]

- Physical exercise seems like another potent approach against multitude of age-related diseases, but especially against diabetes, cardiovascular disease, and obesity.

There is undeniable evidence of the efficacy of regular physical activity in the prevention of multiple chronic diseases, including age-associated disorders, e.g., cardiovascular disease, diabetes, cancer,

hypertension, obesity, depression, and osteoporosis. [543] Physical exercise can enhance the cognitive power, prevent cognitive impairment, and attenuate the development and progression of Alzheimer's disease. [544, 545] Physical activity interventions successfully reduce the incidence of type 2 diabetes. [546]

- Dietary supplementation is also a prominent approach against multiple age-related diseases, especially diabetes, inflammation, cancer, cardiovascular disease, obesity, hypertension, and cognitive disorders.

- Hormonal replacement exhibits strong correlation with osteoporosis

Estrogen deficiency is known as the major factor in the pathogenesis of postmenopausal osteoporosis. [547] Hormone replacement therapy – either estrogen alone or a combination of estrogen and progestin – has been approved for the prophylactics and therapy of osteoporosis in women and has been reported to rapidly normalize turnover, preserves bone mineral density at all skeletal sites. [548, 549]

5. Anti-aging drugs

The search for remedies that can prevent, retard, or reverse aging has a long history (Figure 1) and is currently attracting a lot of attention. There is a steady growth of the number of journal articles over time, rather explosive in the last three years (Figure 19). The number of patents rapidly grew until 2016-2017, possibly correlating with the initial accumulation of knowledge and its transfer into patentable applications. Later on, the patent number is at a steady level, after a peak in 2017, perhaps awaiting the forthcoming breakthroughs in the anti-aging drug awareness.

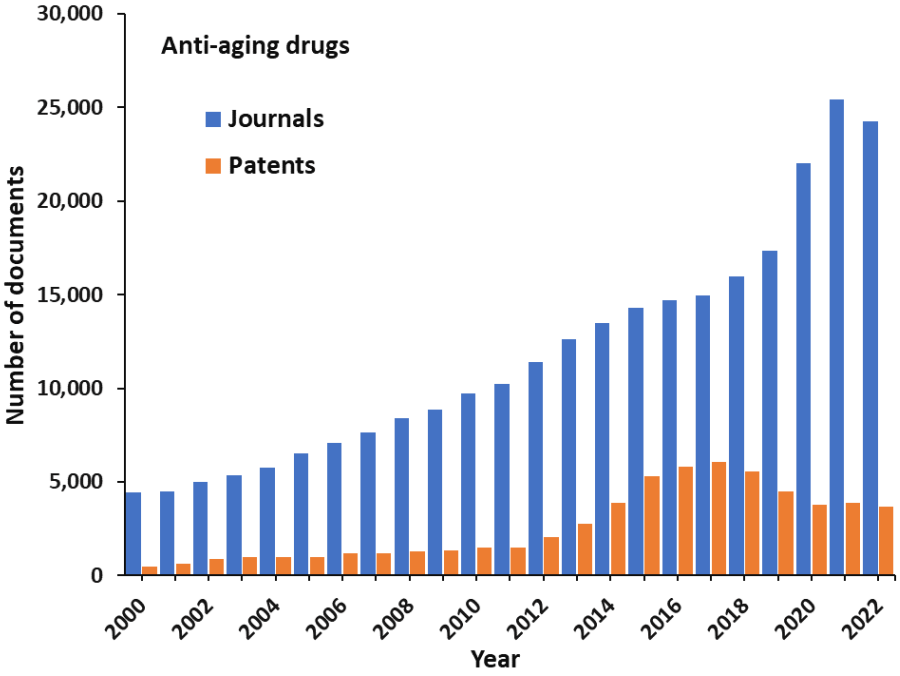


Figure 19. Yearly distribution of journal articles and patents related to anti-aging drugs in CAS Content Collection

A new domain of geriatric medicine termed geroscience has recently emerged aiming to develop new tools to enhance health span. [550] The key claim of geroscience is that aging can be controlled to delay or prevent the beginning of aging-related disorders by targeting the global aging process rather than treating aging disorders once they occur. Applying geroscience-based strategies in healthcare and clinical practice could open an opportunity to increase the proportion of healthy individuals and reduce morbidity to a restricted period near the end of life, which would bring significant economic and social benefits.

The extreme complexity of interactions amongst factors and pathways implicated in the process of aging, looking for pharmacological strategies targeting aging is certainly a very difficult task. Still, substantial progress has been made in this research field recently. Significant anti-aging capacity has been revealed in some natural compounds as well as in certain classes of chemically synthesized substances. These include calorie restriction mimetics such as metformin, rapamycin, and resveratrol. [551] Using several model organisms, such as yeast, worms, flies, and rodents, these potential drugs have been reported to extend life expectancy by up to 25%–30%. [552] Great expectations are currently related to the development of senolytics – drugs that can selectively eliminate senescent cells. [553] Another hopeful class of pharmacological agents involve substances targeting the epigenetic control of gene expression such as histone deacetylase inhibitors, including sodium butyrate, suberoylanilide hydroxamic acid, and trichostatin A. [554]

Another strategy of searching for anti-aging agents involves assessing the health span-promoting ability of drugs that have been already approved by the regulatory authorities to treat certain chronic pathological conditions. These include common medications such as aspirin, metformin, melatonin, certain statins, vitamins, and antioxidants. [555] A benefit of repurposing such drugs is that their long-term safety has been repeatedly examined and approved, their possible side effects have been well known through various clinical trials and examinations. Evidence has been accumulated that such common drugs may indeed enhance health and well-being in elderly individuals suffering from a variety of age-related chronic pathologies. [550, 555]

The collection of currently explored anti-aging agents is dominated by natural compounds – either pure substances or extracts. Vital nutrients such as certain vitamins, minerals (as micronutrients), amino acids, polyunsaturated fatty acids, probiotics, and plant metabolites, such as polyphenols and terpenoids, are recognized for their ability to prevent aging and promote healthy aging. Natural anti-aging sources are present in a wide variety of foods including meat, fish, poultry, fruits, vegetables, herbs, cereals, nuts, grains, legumes, dairy products, cocoa/chocolate, as well as in beverages such as juice, tea, coffee, and wine. Natural extracts from plants and herbs such as green tea, turmeric, yerba mate, thyme, licorice, mulberry, and grape are also known for their anti-aging, mainly antioxidant potencies. [556-560]

5.1. Calorie restriction mimetics

Drug candidates and dietary supplements categorized as caloric restriction mimetics delay aging and extend healthspan and lifespan by modifying aging-associated pathways similarly to caloric restriction and intermittent fasting strategies. The term has been coined in a pivotal 1998 paper. [561] Because caloric restriction requires continuous efforts and firm discipline, identifying active agents that

produce similar but effortless effect have attracted significant attention. Such compounds would open the possibility of enhancing physiological functions, expanding longevity and lowering risk of chronic diseases. [551] Various organic compounds have been shown to modulate anti-aging pathways in a manner similar to caloric restriction and intermittent fasting. Examples of the most widely examined caloric restriction mimetics include rapamycin, metformin, resveratrol, acarbose, aspirin, glucosamine, nicotinamide riboside, and spermidine. [469, 551, 562]

Rapamycin (CAS # 53123-88-9) represents one of the best-known caloric restriction mimetics. [551] It is a macrolide compound isolated from *Streptomyces* bacteria. Rapamycin is an mTOR inhibitor and historically used to avoid immunosuppressive organ transplant rejection. [563] Inhibition of mTOR is known to activate autophagy, a cellular process recognized as a powerful anti-aging approach, believed to also mediate the rapamycin effect. [564] Rapamycin treatment has been reported to extend lifespan and to enhance health markers in model organisms. [563] Certain reported adverse side effects such as cataract risks, infections, as well as insulin resistance restrain the use of rapamycin to delay aging and stimulate active searches for analogous mTOR inhibitors, so called rapalogs, in effort of finding potential drugs with a better safety profile. [565] One of the first-generation rapalogs, everolimus (CAS # 159351-69-6), found within the 95% similarity limit of rapamycin by CAS SciFinder^n [566], has been approved to prevent organ rejection and for cancer treatment. Other compounds found within the 95% similarity limit of rapamycin by CAS SciFinder [566] are listed in Table 4. Later generation rapalog compounds are currently being examined in preclinical and clinical studies. [565]

Table 4. Top compounds found within the 95% similarity limit of rapamycin by using CAS SciFinder^n

Substance	CAS #
7-epi-Rapamycin	157182-37-1
32-Desmethoxyrapamycin	83482-58-0
7-Demethoxyrapamycin	157054-88-1
40-O-(3-Hydroxy)propylrapamycin	159351-72-1
Rapamycin, 42-O-(2-methoxyethyl)-	169288-19-1
32-Desmethylrapamycin	141392-23-6
(27R)-27-Deoxo-27-ethoxyrapamycin	2250063-46-6
(27R)-27-Deoxo-27-methoxyrapamycin	2250062-73-6
SAR 943	186752-78-3
(31S)-Rapamycin	253431-35-5
Rapamycin, 27-deoxo-27-hydroxy-, (27R)-	221895-97-2
31-O-Methylrapamycin	159351-88-9
Novolimus	151519-50-5

Metformin (dimethylbiguanide hydrochloride, CAS # 657-24-9), an antidiabetic drug widely used for treatment of type-2 diabetes, is also considered a caloric restriction mimetic providing anti-aging benefits, believed to be mediated by the activation of AMPK in *C. elegans* and rats. [562, 567] It has been demonstrated that metformin administration prolongs the lifespan in animal models, including mammals. [65] In humans, metformin is shown beneficial against certain age-associated diseases, such as

cancer, metabolic syndrome, as well as cognitive deficits and cardiovascular disorders. [568-570] Metformin may cause adverse side effects, such as vitamin B deficiency and cognitive decline in older adults, as well as lowered testosterone levels, possibly resulting into erectile dysfunction. [571, 572]

Resveratrol (3,5,40-trihydroxystilbene, CAS # 501-36-0), a SIRT activator, which is a natural polyphenolic phytoalexin, mostly abundant in red wine and grape skins, but also in berries and peanuts, has been widely investigated. [573] Resveratrol has been reported to prolong lifespan and delay the onset of aging-related markers in model organisms. [574, 575] It also demonstrates ability to protect against an assortment of age-related disorders including type 2 diabetes, Alzheimer's disease, and cancer. [576] A widespread opinion is that anti-aging and longevity effects of resveratrol are mediated by sirtuins activation. [577] Indeed, resveratrol has been shown to target certain stress-related cellular mechanisms, such as AMPK and SIRT1, with both targets, AMPK and SIRT1, required for resveratrol-induced health promotion. Stimulation of SIRT1 brings about protein deacetylation and autophagy induction. [578-582] Other similar small-molecule SIRT1 activators have been examined including **SRT-1720** (CAS # 925434-55-5) and **SRT2104** (CAS # 1093403-33-8), and have been demonstrated to prolong lifespan, reducing inflammation and protecting from neurodegeneration in model organisms. [57]

Spermidine (CAS # 124-20-9) is a polyamine known to induce autophagy in various model organisms, which is considered causal for some of the reported beneficial effects. [583] The autophagy induction is believed to be result of the inhibition of certain acetyltransferase activity by spermidine. [584] Furthermore, spermidine is able to promote mitophagy. [585]

Aspirin (acetylsalicylic acid, CAS # 50-78-2), a non-steroidal anti-inflammatory drug, has been in widespread medical use for long time. It is known to quickly metabolize into salicylate *in vivo*, which inhibits EP300 by competing with acetyl-CoA, thus activating autophagy and exhibiting lifespan-prolonging effect in model organisms. [586]

Acarbose (CAS # 56180-94-0) is a widely used anti-diabetic drug known for its ability to decrease plasma glucose and cholesterol levels by inhibiting intestinal α-glucosidase and pancreatic α-amylase. [587] It was recently reported to exhibit promise as an anti-aging drug by increasing lifespan in mice [588, 589] and relieve certain age-related pathologies. [590] Acarbose has been hypothesized to extend life span by controlling gut microbiota, thus reducing inflammatory reactions, and therefore diminishing the risk of mitochondrial disorders and telomere attrition. [591]

5.2. Senolytic drugs

The small-molecule drugs known as senolytics selectively eliminate senescent cells. Senescent cells accumulate upon aging and are causative of multiple age-associated disorders. Thus, senescent cells are known to develop a senescence-associated secretory phenotype (SASP) to provoke immune clearance, which boosts chronic inflammation and plays a key role in aging and age-related diseases. [553, 592] It is believed that chronic inflammation activated by senescent cells is among the main grounds of aging-associated pathologies. [552] Senolytic drugs are intended to delay, prevent, relieve, or treat such age-related diseases. As expected for therapeutics targeting one of the very fundamental aging mechanisms, the prospective uses of senolytics are variable, hopefully alleviating multiple conditions, opening a new route for curing age-related dysfunction and diseases.

The initially identified potential senolytic drugs have been discovered using a hypothesis-driven approach [553], based on the observation that senescent cells resist apoptosis. [593] The basic drug discovery hypotheses suggested that: (i) senescent cells resist apoptotic stimuli, and (ii) senescent cells are in certain aspects similar to cancer cells that do not divide. [553] Further senolytic drug identification was bioinformatics-based [594] and initially included the natural flavonoid **quercetin** (CAS # 117-39-5) targeting senescent umbilical vein endothelial cells (HUVECs) and the tyrosine kinase inhibitor **dasatinib** (CAS # 302962-49-8) targeting senescent primary adipocyte progenitor cells. [553] Other members of this first generation senolytics class included **fisetin, luteolin, curcumin, navitoclax**, and others. [246, 552, 553, 592] Figure 20 illustrates the use of ChemScape tool within SciFinder^n [566] to search for compounds of similar chemical structure to quercetin as potential anti-aging drugs.

	Compound CAS #	Structure	Number of patents
1	Quercetin 117-39-5		425
2	Kaempferol 520-18-3		129
3	Luteolin 491-70-3		107
4	Myricetin 529-44-2		84
5	Fisetin 528-48-3		72
6	Morin 480-16-0		24
7	Isorhamnetin 480-19-3		22
8	Galangin 548-83-4		17
9	Tricetin 520-31-0		11
10	Gossypetin 489-35-0		11

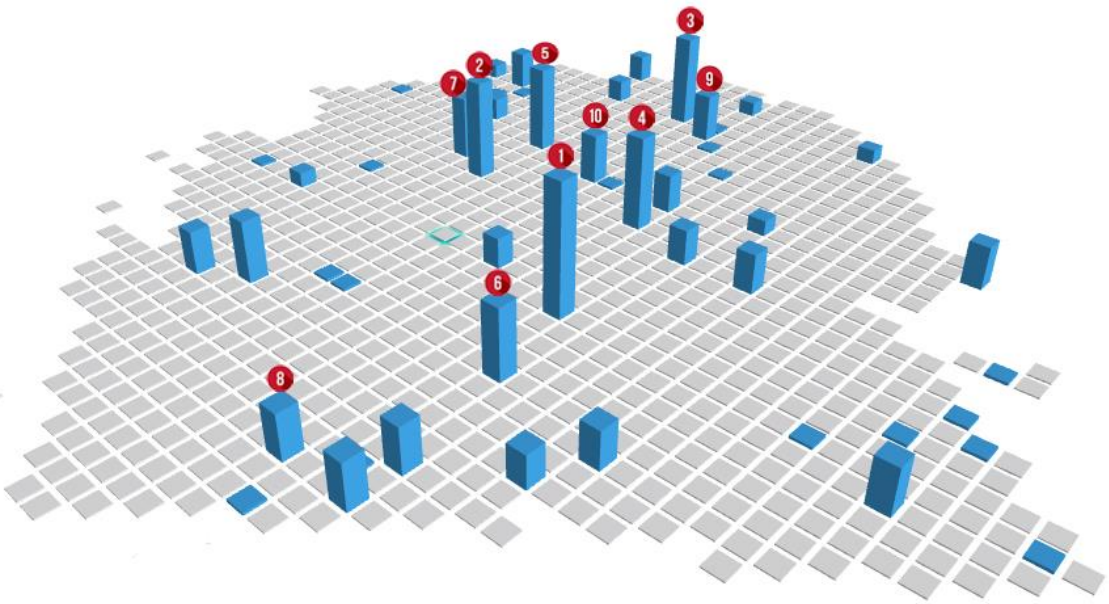


Figure 20. A search in SciFinder^n for compounds within the 90% similarity limit to quercetin used as anti-aging drug gave 51 compounds. Each column on the figure reflects a single compound, with the top 10 indicated by numbers. The distance between two columns reflects the similarity between these two compounds. The bar height reflects the number of patents related to a given compound. The figure was created using the ChemScape analysis tool of SciFinder^n. The table on the left lists the top 10 compounds of this collection.

The efficacy of the suggested senolytic drugs have been demonstrated in model organisms for age- and cellular senescence-associated physical disabilities, insulin resistance, cognitive decline, osteoporosis, osteoarthritis, and cancers. [553] Senolytic agents have the potential to delay, prevent, or treat senescence- and age-related disorders, based on successful forthcoming human clinical trials. Other promising senolytic drugs include **acarbose, 17-α-estradiol, and nordihydroguaiaretic acid** (NGDA). [588] Recently, new structurally diverse compounds with promising senolytic activity have been identified using deep learning neural networks simulations. [595]

5.3. Telomerase activators

Therapeutic targeting of telomerase activity is another prospective anti-aging approach. Since age-associated telomere shortening is known to play a key role in senescence, proper maintenance of telomeres is decisive for genome stability. [596, 597] Telomerase is the reverse transcriptase enzyme, which is able to sustain telomere length via telomeric repeat addition onto the chromosomes ends. [598] Telomerase activation has been proposed to be an anti-aging moderator, which can play a role in the aging-associated diseases therapy.

Several telomerase activator formulations have been examined as anti-aging agents. **TA-65** (cycloastragenol, CAS # 78574-94-4), an extract of *Astragalus membranaceus* roots, has been reported to perform as telomerase activator able to restore telomere length without cancer incidence and enhance age-related markers, such as glucose tolerance, bone health, and skin quality. [425] Another telomerase activator, **metadichol** (CAS # 1627854-29-8), has been used to defeat organ failure by enriching cells with telomerase. [599] Another study reported that natural formulations including *Centella asiatica* extract (08AGTLF) comprising >95% triterpenes, Astragalus extract, TA-65, oleanolic acid (CAS # 508-02-1), maslinic acid (CAS # 4373-41-5), and certain multi-nutrient formulations trigger significant increase in telomerase activity. [600]

5.4. Epigenetic drugs

Epigenetic dysregulations associated with aging implicate intense health concerns for numerous pathologies including metabolic and cardiovascular diseases, cancer, psychiatric and neurodegenerative disorders. Epigenetic drugs-based therapy has emerged as a potential strategy for treating certain aging-associated diseases. [601] Epigenetic modifications are known to be reversible, which makes them suitable targets for pharmacological intervention.

An assortment of therapeutics have been developed recently targeting epigenetic regulation, including DNA methyltransferase modulators, histone deacetylase modulators, histone acetyltransferase modulators, and noncoding miRNAs, exhibiting possible effects against age-related disorders. [602, 603] Among DNA methyltransferase modulators, **5-azacytidine** (azacytidine, CAS # 320-67-2) and **5-aza-2'-deoxycytidine** (decitabine, CAS # 2353-33-5) are the most thoroughly examined and demonstrate therapeutic potential against certain leukemias. [604, 605] Histone deacetylase inhibitors include several chemical groups: cyclic peptides, hydroxamic acids, short chain fatty acids, and benzamides. [606] Experimental evidence shows significant life-extending potential of the histone deacetylase inhibitors such as 4-phenylbutyrate (PBA), trichostatin A, sodium butyrate, and suberoylanilide hydroxamic acid (SAHA). [607, 608] A wide range of histone deacetylase inhibitors are emerging as potential anticancer medications, including belinostat, panobinostat, SAHA and FK228 [609], trichostatin A, sodium butyrate, vorinostat, valproic acid, and romidepsinor [610] demonstrating considerable activity in hematological and solid tumors.

5.5. Antioxidants

According to the oxidative damage theory of aging [27], free radicals and other reactive oxygen species (ROS), developed throughout mitochondrial metabolism, may end up producing impaired molecules including carbonylated proteins, lipid peroxides, and oxidized DNA [611-613], the accumulation of which is supposedly the primary cause of cellular senescence, age-related telomere attrition and diseases. [614] Accumulated ROS excess can be allegedly destroyed by the endogenous physiological antioxidative systems including the enzymes superoxide dismutase (SOD), glutathione reductase, catalase and glutathione peroxidase, which help to keep the balance between oxidative and anti-oxidative processes. In terms of chronic oxidative stress, however, the endogenous antioxidant systems happen to be insufficiently effective, and it has been believed that administration of certain exogenous antioxidants such as vitamins E and C, curcumin, melatonin, β-carotene, lipoic acid, coenzyme Q10, glutathione, polyphenols, phytoestrogens, as well as certain minerals including zinc, manganese and selenium, can be a factor in maintaining homeostasis. [552]

Vitamin E (CAS # 1406-18-4) comprises a group of several fat-soluble compounds including tocopherols and tocotrienols, of which **α-tocopherol** is the most potent antioxidant form of vitamin E. Due to their lipophilic features, they can be found in lipoproteins, cellular membranes, and fat deposits. Vitamin E is the key defender against free radicals effects. It is stored in the liver and fat cells and protects cellular components and especially cellular membranes from damage. [615, 616] Vegetable oils such as sunflower, soybean, and safflower oils are some of the best sources of vitamin E.

Vitamin C (L-ascorbic acid, CAS # 50-81-7), a water-soluble vitamin naturally present in citrus and other fruits and vegetables, is an electron donor and a versatile free radical scavenger. It has been found to regenerate other antioxidants, including α-tocopherol (vitamin E) from the tocopheryl radical. [617] It is a particularly efficient antioxidant because of its high electron-donation power and ready conversion back to the active reduced form. Vitamin C is necessary for the biosynthesis of collagen, L-carnitine, and some neurotransmitters, it is also a participant in protein metabolism [618, 619], and plays an significant role in immune function. [617]

Curcumin (CAS # 458-37-7) is the key active component of turmeric (*Curcuma longa*) root. It has been shown to exhibit antioxidant, anti-inflammatory, anti-neurodegenerative, and antitumor activities. [620, 621] Recently it has been implied that curcumin upregulates SIRT3 expression in skeletal muscle tissues [622], as well as in the brain after stroke. [623] Numerous other dietary **polyphenols** such as **resveratrol**, **(−)-epigallocatechin-3-gallate** (EGCG) have been shown able to mitigate age-produced cellular damage via metabolic formation of ROS, via specific cell-signaling actions that may stimulate SIRT1 activity. Furthermore, polyphenolic compounds have proven inhibitory activity against chronic vascular inflammation related to atherosclerosis. [624]

Melatonin (N-acetyl-5-methoxy tryptamine, CAS # 73-31-4) is a hormone secreted by the pineal gland and regulates sleep/wake cycles. It is a well-recognized antioxidant able to support healthy aging. The endogenous melatonin levels have been found to decrease upon aging and even stronger in certain neurodegenerative disorders, especially Alzheimer's disease, as well as in type 2 diabetes. [625] It has been reported relevant to the attenuation of inflammaging in the brain. [625] Studies have documented the ability of melatonin to increase SIRT levels against brain aging. [626, 627]

β-Carotene (CAS # 7235-40-7) is a carotenoid, a micronutrient with numerous physiological functions. It is an antioxidant, which has been shown to inhibit the incidence and development of cancer. It also has an anti-inflammatory effect in various animal and cell models. [628] Its anti-aging efficacy has been documented *in vitro*, using mesenchymal stem cells, as well as *in vivo*, in model animals. [629] It was implied to inhibit aging by regulating the KAT7-P15 signaling axis. [629] Other carotenoids, including α- and γ-carotenes, lycopene, lutein, β-cryptoxanthin, and zeaxanthin, are also known as effective natural antioxidants. Synergistic action of vitamins C and E, and carotenoids, has been reported to successfully prevent lipid peroxidation. [630]

α-Lipoic acid (6,8-thioctic acid, CAS # 1200-22-2) is a dithiol, which acts as a coenzyme factor for certain redox reactions. It is synthesized in animals naturally and is essential for aerobic metabolism. Its efficiency in diseases associated with aging-provoked oxidative stress has been documented, so its dietary supplementation has been found beneficial. [631-633] Lipoic acid has been also shown to activate SIRT1 and SIRT3 in peripheral tissues thus improving mitochondrial function and protecting against cardiac hypertrophy. [634, 635]

Coenzyme Q10 (CoQ10, CAS # 303-98-0), a powerful antioxidant known to offer a variety of benefits to support healthy aging, is naturally produced in the body and is participating in energy production. It has attracted large-scale interest due to its crucial role in mitochondrial bioenergy, antioxidation, anti-aging, and immune system regulation. Upon aging, the production of CoQ10 declines, so it is necessary to supply additional amounts through foods such as meats, fatty fish (e.g., trout) and nuts (e.g., pistachios), or through dietary supplements. It has been reported to exert positive effects in enhancing age-produced deterioration of oocyte quality. [636] CoQ10 and selenium supplementation have been reported to increase serum Sirtuin1 levels [637] and to improve heart function in elderly. [638]

L-Glutathione (GSH, CAS # 70-18-8) is an abundant antioxidant playing an essential role in protecting cells against oxidative stress-caused cellular damage. Declined glutathione levels are related to the typical characteristics of aging, as well as of a wide variety of pathological conditions, including neurodegenerative disorders. Thus, glutathione depletion seems to be important for the onset of Parkinson's disease. [639] Furthermore, glutathione is important for body detoxification, it is also a successful immunostimulant and skin rejuvenator. [640]

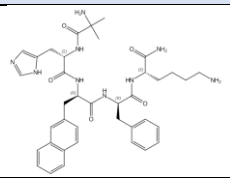
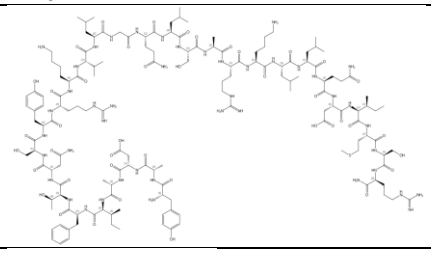
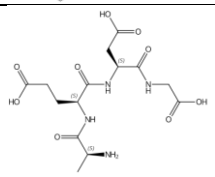
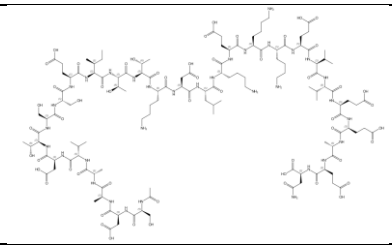
Antioxidant intake controversy. The reported inverse correlation between systemic levels of antioxidants and certain age-associated diseases has resulted in the perception that antioxidant supplementation is an effective prophylactic and therapeutic intervention for such aging pathologies. However, the therapeutic results of this strategy in clinical trials have been frequently disappointing. [641] The interplay of both endogenous and exogenous antioxidants is complex and still not well understood. To successfully maintain the redox homeostasis, exogenous and endogenous antioxidants need to act synergistically. [642] A fine-tuned equilibrium between the oxidative and antioxidative processes in the organism is vital in preserving homeostatic stability. Thus, excessive antioxidant supplementation might turn out to be damaging for the delicate homeostatic control mechanisms resulting in health decline. [643] Similarly to the hormesis-causing agents, antioxidants perform beneficially in certain concentration range, and their higher concentrations are typically toxic to organisms. The excessive levels of exogenous antioxidants may perturb the endogenous signaling pathways and thereby be harmful. [644] In view of these contradictions, it is not surprising that conflicting data have been reported on the health

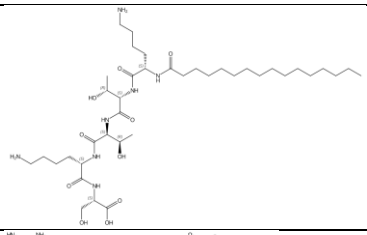
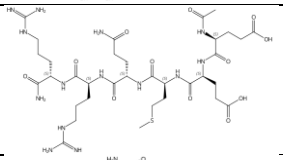
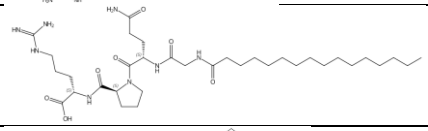
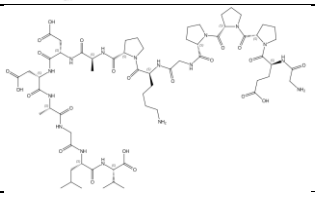
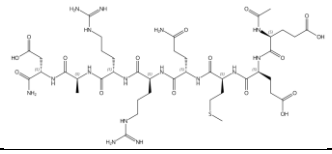
outcomes of long-term antioxidant intake. This ambiguity is often referred to as 'antioxidant paradox'. [552] While benefits of dietary antioxidant supplementation appears to be clear in cases of high oxidative stress and endogenous antioxidant insufficiency, further research is necessary to clarify the potential risks and advantages linked to the supplement of antioxidants by healthy people.

5.6. Anti-aging peptides

Peptides have been mainly used in anti-aging cosmetics and cosmeceuticals, to repair skin aging signs like wrinkles and sagging. They have been found effective also at hair regrowth stimulation as well as weight loss. [645-647] Furthermore, peptides were reported useful for treating rheumatoid arthritis [648] and as analgesics [649]. Peptide supplementation modified energy metabolism and oxidative stress, improved endurance and decreases fatigue in experimental animals. [650] Because of their hydrophilicity, peptides for use in skin cosmetics are commonly lipidated by esterification with an alkyl (most often palmitoyl) chain, to enhance their penetration through the highly lipophilic stratum corneum. Exemplary anti-aging peptides are shown in Table 5.

Table 5. Exemplary anti-aging peptides

Peptide	CAS #	Structure
Ipamorelin	170851-70-4	
Sermorelin (GHRH)	86168-78-7	
Epitalon	307297-39-8	
Thymosin alpha 1	62304-98-7	

Matrixyl	214047-00-4	
Argireline	616204-22-9	
Palmitoyl Tetrapeptide-7	221227-05-0	
BPC 157	137525-51-0	
SNAP 8	868844-74-0	

The mechanism of action of many anti-agent agents is often related to multiple aging hallmarks and refers to several anti-aging strategies. In Figure 21 we have exemplified certain correlations between some anti-aging drugs, hallmarks of aging (A), and anti-aging strategies (B).

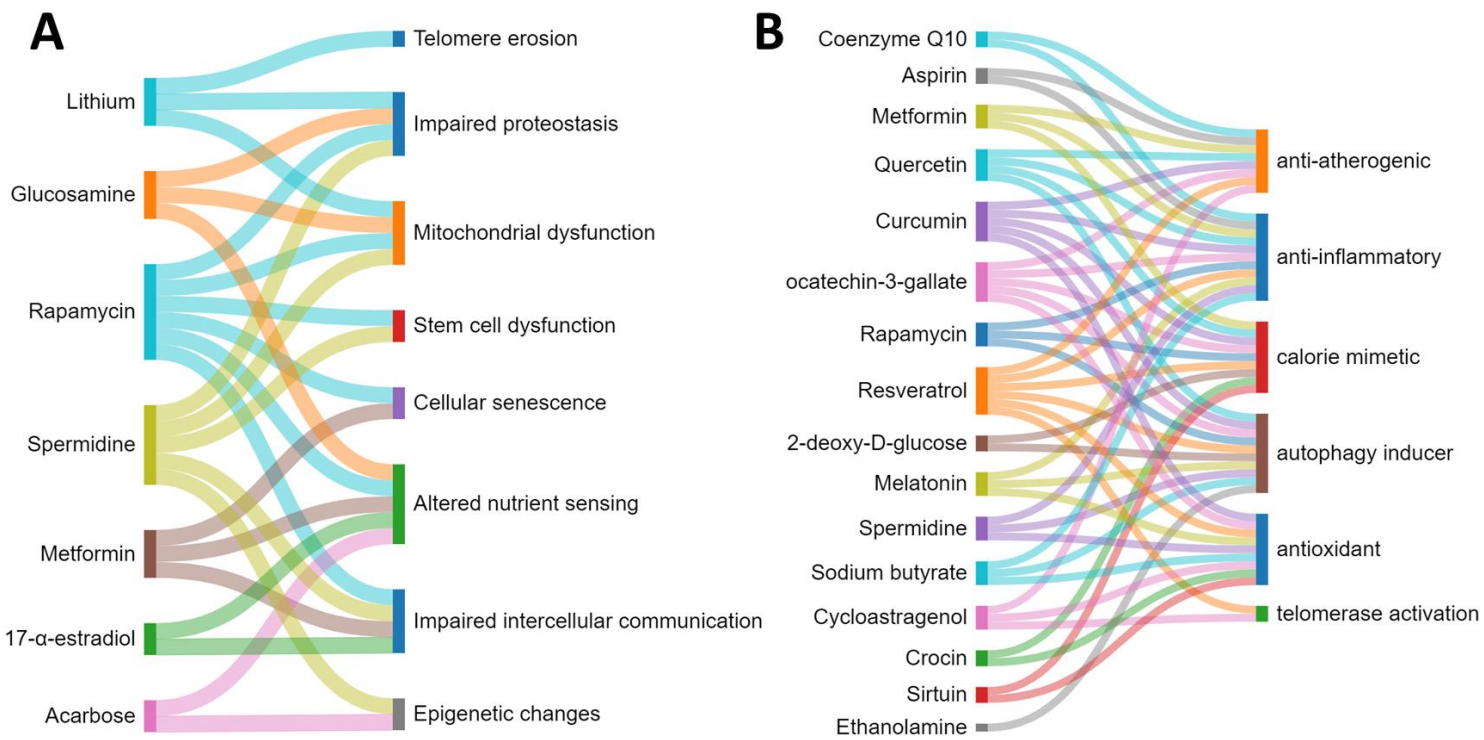
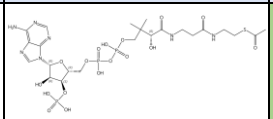
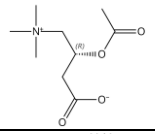
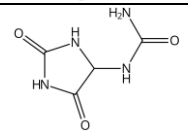
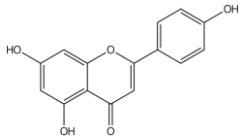
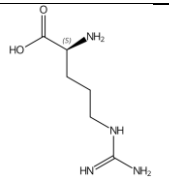
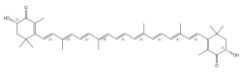
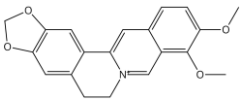
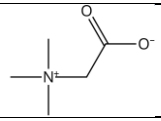
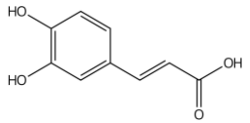
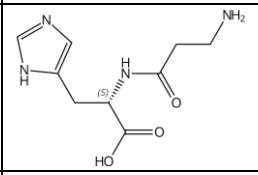
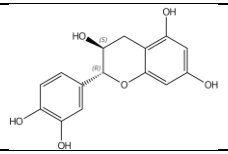
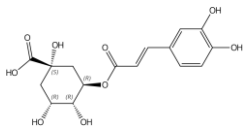
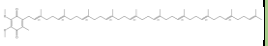


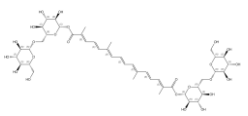
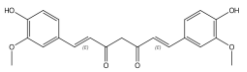
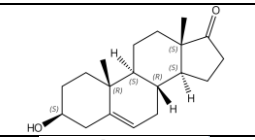
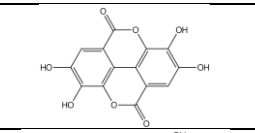
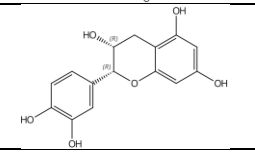
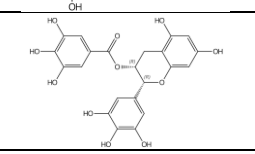
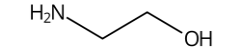
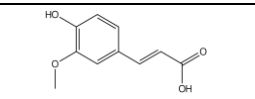
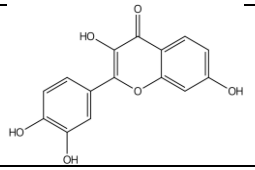
Figure 21. Correlations between exemplary anti-aging drugs and (A) hallmarks of aging, and (B) anti-aging strategies

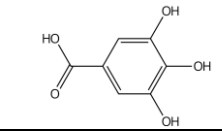
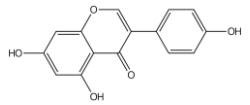
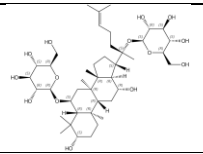
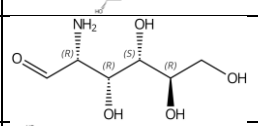
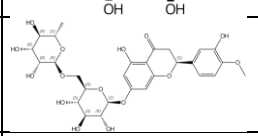
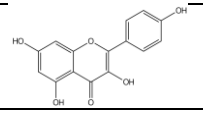
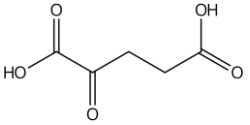
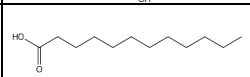
In Tables 6 and 7 we have summarized the natural (Table 6) and synthetic (Table 7) anti-aging agents most widely represented in the CAS Content Collection [6, 66, 552, 557-560, 651-661], complete with their chemical structures and the number of documents (journal articles and patents) in the CAS Content Collection, in which their anti-aging performance has been documented and discussed. A more extensive collection of anti-aging compounds represented in CAS Content Collection is provided in the Supporting Information, Table S1.

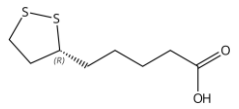
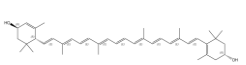
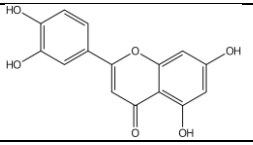
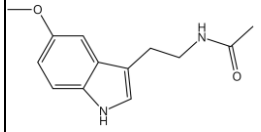
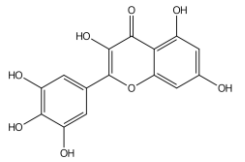
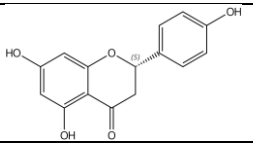
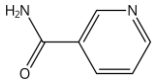
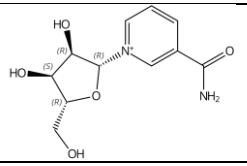
Table 6. Natural anti-aging agents most widely represented in the CAS Content Collection [6, 66, 552, 557-560, 651-661]

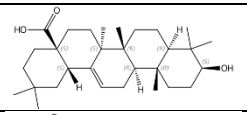
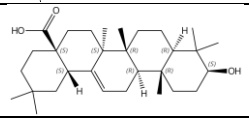
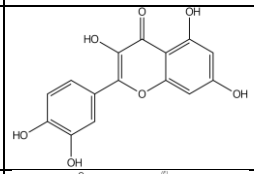
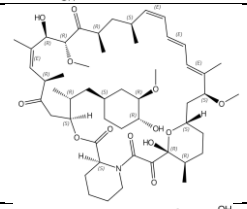
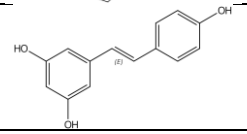
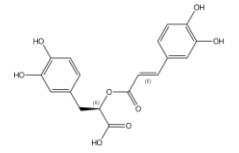
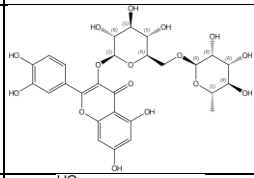
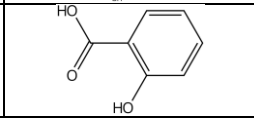
Natural anti-aging compounds	CAS REG #	Sources	Mechanism of action / Anti-aging strategy	Application / Benefits	Structure	Number of journal articles	Number of patents
Acetyl-coenzyme A (Acetyl CoA)	72-89-9	Oily fish (salmon, tuna), organ meat (liver), grains	Inhibits oxidation of proteins, lipids, and DNA	heart conditions, muscular dystrophy		147	11
Acetyl-L-carnitine	3040-38-8	Red meat, poultry, fish, dairy foods	Decline in interfibrillar mitochondria carnitine palmitoyltransferase 1 activity	Improves cognitive and neurological function, reduces mental fatigue, improve mood, alertness		722	596
Allantoin	97-59-6	Chamomile, wheat sprouts, sugar beet, comfrey	Helps in shedding of dead skin cells & cell turnover	Skin moisturizing and soothing, exfoliation, wound healing		45	652
Apigenin	520-36-5	Chamomile, celeriac, parsley	Inhibits skin inflammation by down-regulating transcription factors including AP-1, NF-κB, STAT; CD38 inhibitor	Antidiabetic, chemoprevention		132	102
Arginine, L-	74-79-3	Meat, fish, nuts & seeds, legumes, whole grains, dairy	Reduces oxidative stress and inflammation resulting in decreased NF-κB level and activity; vascular smooth muscle cell relaxation	Reduces systolic and diastolic blood pressure in hypertensive patients		726	615
Astaxanthin	472-61-7	Phaffia Rhodozyma	Protects cell membranes against reactive oxygen and nitrogen species and oxidative damage	Antioxidant, anti-inflammatory		136	436
Berberine	2086-83-1	Coptidis rhizome, Barberry plants, Chinese goldthread	AMPK activator, enhancing UCP2 expression, inhibits oxidative stress	Agent against dyslipidemia, antidiabetic, antioxidant, antiobesity, antiangiogenic		80	34

Betaine	107-43-7	Sugar beets	Lowers levels of homocysteine in circulating blood	Osmoregulator, ergogenic, supporting heart health		113	571
Caffeic acid	331-39-5	Coffee, red wine, berries, apples, olives, artichokes, pears	Regulates proteostasis; alleviates neuroinflammation and neurodegeneration	Antioxidant; liver damage prevention, cognitive function improvement; psychoactive drug		226	129
L-Carnosine	305-84-0	Meats: turkey, chicken, beef, pork	Reacts with methyl-glyoxal and scavenges ROS thus protective toward aging and ischemia; increase verbal episodic memory; increase of cerebral blood flow	Anti-aging, antioxidant		177	379
β-Carotene	7235-40-7	Fungi, plants, fruits	Photoprotecting agent, reduces rate of mitochondrial mutation	Antioxidant agent, promotes healthy skin, supports immune system, eye health and vision		687	327
Catechin	154-23-4	Tea polyphenols	Rich in oligomeric proantho-cyanidins, thus prevents premature aging	Anti-inflammatory agent		512	288
Chlorogenic acid	327-97-9	Coffee, tea, bamboo	Glucose regulation; induce continuous phosphorylation of ERK1/2	Antioxidant, neuroprotective, anti-inflammatory, gastro-protective, antirheumatic, antihypertensive, anti-atherothrombotic		221	122
Coenzyme Q10	303-98-0	Meat, fish, nuts	Increases production of key antioxidants such as superoxide dismutase; reduces levels of lipid peroxidation; intensify blood flow, protect blood vessels by upholding nitric oxide	Anti-inflammatory, anti-atherogenic		242	541

Crocin	42553-65-1	Flowers of crocus and gardenia, saffron	Reduces oxidative stress and ROS through enhancement of gene expression of Nrf2, HO-1, and antioxidant enzymes CAT, GSH, and SOD; counteract oxidative stress, mitochondrial dysfunction and neuroinflammation	Degenerative disease, metabolic syndrome; antioxidant, neuroprotective agent		228	64
Curcumin	458-37-7	Curcuma longa, turmeric	Inhibits TOR pathway; autophagy inducing	Anti-inflammatory, antioxidant		444	210
Dehydroepiandro-sterone (DHEA)	53-43-0	Wild yam, soy	Activates PPARα and constitutive androstane receptor (CAR)	Raises androgen and estrogen levels; improves bone & cardiovascular health, insulin sensitivity, and mood		388	132
Ellagic acid	476-66-4	Fruits: grapes, strawberries, pomegranate	Decreases amount of inflammatory cytokines, regulates the activities of antioxidant enzymes	Anti-inflammatory, antioxidant; treats viral and bacterial infections		115	156
(-)-Epicatechin	490-46-0	Cocoa	Stimulates mitochondrial respiration and biogenesis	Enhances nitric oxide production for improved vascularity, circulation, and resilience		202	119
Epigallocatechin-3-gallate (EGCG)	989-51-5	Green tea	Regulates cytokine secretion, autophagy inducing	Antioxidant, anti-inflammatory		292	180
Ethanolamine	141-43-5	Daikon radish, caraway, muscadine grape, lemon grass	Increases amount of cellular phosphatidylethanolamine, thus stimulating cytoprotective autophagy and anti-aging protection	Anti-aging, inducing autophagy		40	132
Ferulic acid	1135-24-6	Beet roots, oranges, carrots	Restrains radiation-induced oxidative stress by ceasing free radical chain reaction	Anti-aging, anticancer		184	248
Fisetin	528-48-3	Nuts, strawberries, apples, mangoes, persimmons	Inhibits NF-κB activation, promotes Nrf2 activity to prevent neurodegeneration; lipoxygenase inhibitor	Neuroprotective agent; anti-aging, anticancer, anti-inflammatory		59	47

Gallic acid	149-91-7	Rose flower	Anti-inflammatory mechanisms involve MAPK and NF-κB signaling pathways; reducing release of inflammatory cytokines, chemokines	Improves cognitive function, motor function; anti-inflammatory		345	144
Genistein	446-72-0	Soy products	Enhancing skin collagen by stimulating subcutaneous VEGF expression and increasing TGF-β in skin	Maintains arterial elasticity, blood glucose control, prevents hypertension, prostate & breast cancer		216	150
Ginsenoside Rg1	22427-39-0	Ginseng	Improves cognitive function; stimulates glucose uptake, relieves oxidative stress; possible neuroprotective role	Suppressive effects in neurodegenerative conditions; neuroprotectant, anti-inflammatory		98	30
D-Glucosamine	3416-24-8	Chitin from hard outer shells of shrimp, lobster, crab	Prevents collagen degeneration in chondrocytes; slow cartilage deterioration in the joints	Cartilage-protecting, anti-inflammatory		121	190
Hesperidin	520-26-3	Citrus genus	Inhibition of signaling pathway related to MMP-9 activated by UVB radiation	Antioxidant, anti-inflammatory, anti-aging		72	99
Hyaluronic acid	9004-61-9	Rooster combs (the red part on a rooster's neck)	Major component of extracellular matrix, key role in tissue regeneration, inflammation, angiogenesis, wound repair	Reduces facial skin wrinkles	N/A	584	2081
Kaempferol	520-18-3	Spinach, kale, tarragon	Prevents the activation of p38 mitogen-activated protein kinase C-JNK	Antiapoptotic, antiangiogenic		204	111
α-Ketoglutarate	328-50-7	Intermediate of the tricarboxylic acid cycle	Nitrogen scavenger, glutamate and glutamine source, promotes protein synthesis, prevents protein degradation in muscles	Improves amino acid metabolism		81	31
Lactic acid	50-21-5	Pickled vegetables, yogurt	Stimulates collagen renewal	Skin moisturizing and antiwrinkle properties		414	555
Lauric acid	143-07-7	Papaya	Rich in saturated fatty acids, hydrates skin	Anti-aging, antimicrobial, anti-bronchitis; soothes inflamed skin, inhibits acne bacteria		92	236

α-Lipoic acid	1200-22-2	Spinach, broccoli, potatoes, yeast, tomatoes, carrots	Suppression of p38 and p53 at gene level	Antioxidant, anti-aging		209	346
Lithium	7439-93-2	Ore mining, salt water from underground lakes	Modulates the release of dopamine or serotonin in brain	Mood stabilizer, helps to treat bipolar episodes	Li	266	145
Lutein	127-40-2	Green vegetables	Preserves visual function by preventing degradation of rhodopsin and synaptophysin	Prevents skin aging, age-related macular degeneration; antioxidant		385	189
Luteolin	491-70-3	Vegetables, tea	Reduces neuroinflammation and improves learning and memory; inhibits vascular inflammation	Anti-inflammatory, antioxidant		146	100
Melatonin	73-31-4	Tart cherries, tomatoes, corn, asparagus, olives, pomegranate, nuts, sunflower, mustard, flax seeds	Restores mitochondrial membrane permeability, promotes antioxidant enzymes including glutathione peroxidase, superoxide dismutase, glutathione reductase, catalase	Antioxidant, anti-inflammatory, autophagy inducing, anti-aging		637	176
Myricetin	529-44-2	Berries, red wine	Reduces epidermal thickening provoked by UVB and suppress MMP-9 protein expression and enzyme activity	Anti-aging; inhibits hyper-glycemia, decreases hepatic triglyceride, reduces oxidative stress and cholesterol contents, protects liver injury		87	60
Naringenin	480-41-1	Grapefruit, bergamot, orange, cherries, tomatoes, cocoa, oregano, mint	Enhances antioxidant ability by activating Nrf2 causing HO-1 expression; inhibit NF-κB activation in macrophages	Anti-inflammatory, antioxidant		88	63
Nicotinamide	98-92-0	Meat, milk, eggs, green vegetables	DAC activator of SIRT1; manages the NF-κB-mediated transcription and inhibits mast cells degranulation; calorie mimetic	Cell proliferation and improvement in skin texture		575	884
Nicotinamide riboside	1341-23-7	Cow's milk	Improves glucose tolerance, reduces age-related weight gain, exhibits neuroprotective effects	Anti-aging; neuroprotection and vascular protection		62	41

Oleanolic acid	508-02-1	Olea europaea, Viscum album, Aralia chinensis	Regulates macrophage polarization in adipose tissue	Antioxidant, anti-inflammatory, antiviral, anti-obesity, antidiabetic		77	69
Oleuropein	32619-42-4	Olives	Avoids the reduction of proteasome activities upon senescence	Antiatherogenic, antioxidant, anticancer		60	40
Phosphatidylserine	1446756-47-3	Soybeans, egg yolks, liver	Restores acetylcholine release, increases endogenous choline for de novo acetylcholine synthesis	Antioxidant; reduces stress, anxiety, and depression	N/A	208	84
Quercetin	117-39-5	Apples, honey, raspberries, onion, red grape, cherries, citrus, green leafy vegetables	Prevents the production of tumor necrosis factor α in macrophages and IL-8 in lung A549 cells, as induced by lipopolysaccharides	Calorie mimetics, anti-inflammatory, anti-atherogenic		654	303
Rapamycin (Sirolimus)	53123-88-9	Soil bacterium: Streptomyces hygroscopicus	mTORC1 inhibitor; autophagy inducing, dietary restriction, calorie mimetic	Natural anti-fungal antibiotic		1411	99
Resveratrol	501-36-0	Grapes, berries, Polygonum cuspidatum	Sirtuin activator; calorie mimetics; telomerase activation	Neuroprotective, cardio-protective; antioxidant, anti-inflammatory, antidiabetic		1270	602
Rosmarinic acid	20283-92-5	Rosemary	Prevents production of ROS and activation of abnormal mPTP provoked by high glucose, cytC release and caspase-3	Antibacterial, antiviral, antioxidant; anti-spasmodic, choleretic, hepatoprotective, antitumorigenic		83	69
Rutin	153-18-4	Buckwheat, orange, black tea	HAT inhibitor, p300 inhibitor, PCAF inhibitor, NFκB inhibitor; chelate metal ions such as iron, inhibits Fenton's reaction	Strong antioxidant, antiangiogenic		245	183
Salicylic acid	69-72-7	Broccoli, spinach, cauliflower, cucumber, mushroom	Decreases production of inflammatory prostaglandins	Anti-aging, anti-inflammatory		201	711

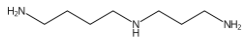
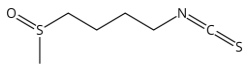
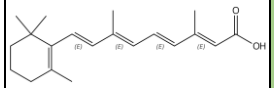
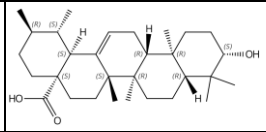
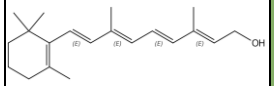
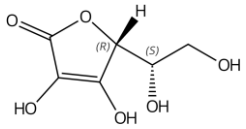
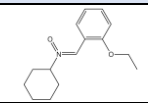
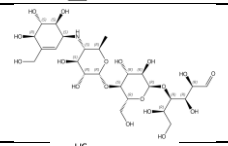
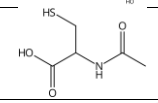
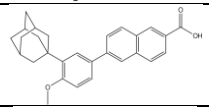
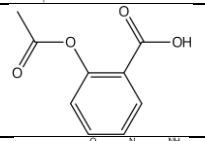
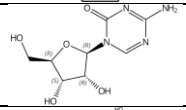
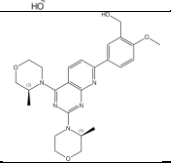
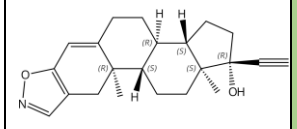
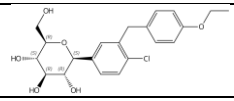
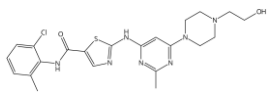
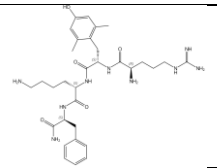
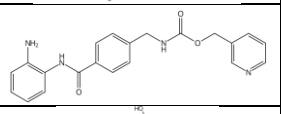
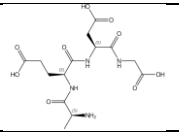
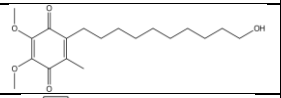
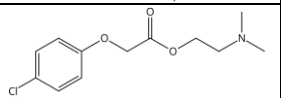
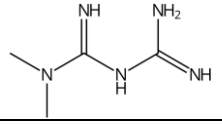
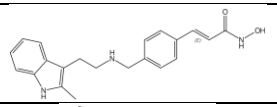
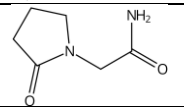
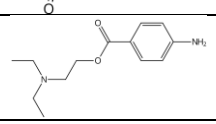
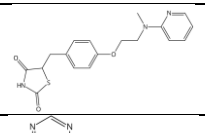
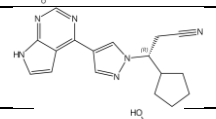
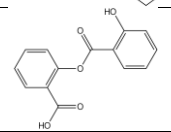
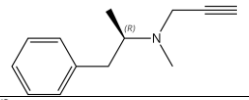
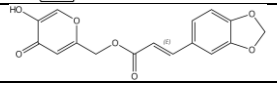
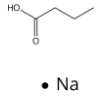
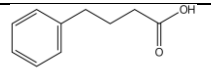
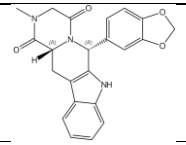
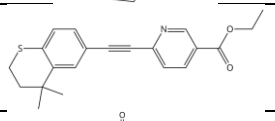
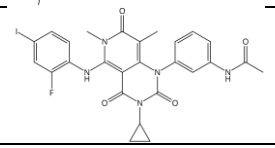
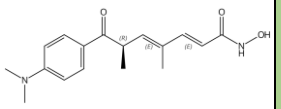
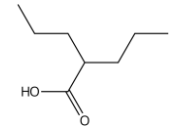
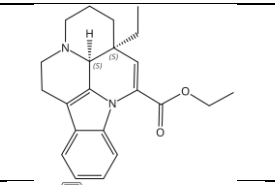
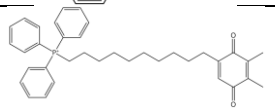
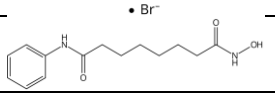
Sirtuin	438496-81-2	Kale, olives, red wine, strawberries	Maintains regular chromatin condensation; repair DNA damage; modulate oxidative stress; repress insulin resistance	Anti-aging, calorie mimetic; anti-inflammatory, stress resistance, fat & glucose metabolism, cardiac rhythm, mitochondria biogenesis	N/A	2235	180
Spermidine	124-20-9	Fresh green pepper, wheat germ, cauliflower, broccoli, mushrooms, cheeses	Autophagy inducing; reduces histone acetylation, lipid metabolism and regulates cell growth and signaling pathways	Antioxidant, anti-inflammatory		235	52
Sulforaphane	4478-93-7	Cruciferous vegetables: kale, bok choy, cabbage	Detoxifies carcinogens and pro-oxidants by blocking phase I metabolic enzymes; arrests cell cycle to impede cell proliferation; BACE1 inhibitor	Antioxidant, anti-inflammatory, protect DNA		73	27
Tretinoin	302-79-4	Natural retinol	Stimulates mitotic activity, enhances loosely adherent corneocyte turnover	Anti-aging agent		229	319
Ursolic acid	77-52-1	Apple peel, cranberry juice, grape skin, basil, rosemary, thyme, oregano, sage	Insulin secretagogue and insulinomimetic; regulates glucose uptake	Antihyperglycemic; insulin sensitivity increase; antioxidant, anti-inflammatory, antibacterial, antifungal		69	91
Vitamin A (Retinol)	68-26-8	Egg yolk, beef liver, carrots, pumpkin, sweet potatoes, mangos, papaya	Important to vision, growth, cell division, reproduction and immunity	Antioxidant		1141	934
Vitamin C (L-ascorbic acid)	50-81-7	Citrus, blackcurrant, rose hip, guava, chili pepper, parsley	Enhances collagen synthesis, slow down aging	Vital for immune system, heart, blood vessels; antioxidant, anti-hypertensive, immune stimulant; reduces heart disease and dementia risk		2030	2482
Vitamin E	1406-18-4	Nuts, seeds, vegetable oils: corn and soybean	Prevents peroxidation of unsaturated fatty acids; important to vision, reproduction, and blood, brain and skin health	Antioxidant	N/A	1593	1879

Table 7. Synthetic anti-aging agents most widely represented in the CAS Content Collection [6, 66, 552, 557-560, 651-661]

Synthetic compounds	CAS Reg #	Mechanism of action / Anti-aging strategy	Application / Benefits	Structure	Number of journal articles	Number of patents
4-hydroxy phenyl N-tert-butylnitrone, CPI-1429	223649-80-7	Blocks signal transduction associated with neuroinflammation enhanced in neurodegenerative disorders	anti-aging, neuroprotectant		2	2
Acarbose	56180-94-0	Synthesized by soil bacteria Actinoplanes sp through its precursor valienamine	Inhibits alpha glucosidase; anti-diabetic for type 2 diabetes		87	19
N-Acetylcysteine	7218-04-4	Increases cell protection to oxidative stress	Flu, dry eye, cough, and other lung conditions		48	9
Adapalene	106685-40-9	Targets abnormal desquamation of skin and anti-inflammatory properties	Treatment of acne vulgaris		7	52
Aspirin	50-78-2	Inhibits the activity of cyclooxygenase (COX) which leads to formation of prostaglandins causing inflammation; regulating AMPK and insulin-like signaling pathway	Protects telomeres upon cell reproduction, restrains their shortening and related cell aging and death		3569	162
Azacitidine	320-67-2	DNA methyltransferase inhibition by covalent bonding, resulting in DNA hypomethylation	Helps bone marrow grow normal blood cells; anti-atherogenic		47	21
AZD8055	1009298-09-2	Blocks mTORC1 and mTORC2 signaling in AML	Antitumor effect used against neuroblastoma cells		3	4
Danazol	17230-88-5	Androgen receptor agonist; ovarian steroidogenesis inhibitor; lowers gonadotropin levels in postmenopausal women	Treats endometriosis and fibrocystic breast disease by shrinking displaced tissue of uterus		15	5
Dapagliflozin (Dapagliflozon, Farxiga)	461432-26-8	Inhibits SGLT2 thereby controlling hyperglycemic activity	Treatment of type 2 diabetes		45	9

Dasatinib	302962-49-8	Inhibits proliferation, adhesion, migration and invasion of HCC cells by inhibiting Src tyrosine kinase and modifying SFK/FAK and PI3K/PTEN/Akt	Treatment of chronic myeloid leukemia		39	21
Elamipretide	736992-21-5	Targets mitochondrial inner membrane by its enrichment in cardiolipin	Anti-aging		4	7
Entinostat (SNDX-275; MS-275)	209783-80-2	Class I and IV HDAC inhibitor (HDAC1, 2, 3)	Antitumor agent; histone deacetylase inhibitor; memory promoter		3	11
Epitalon	307297-39-8	Pineal gland, retina, brain function regulator, induces neuronal cell differentiation in stem cells	Anti-aging		31	1
Ergoloid mesylate (Hydergine)	8067-24-1	Stimulates dopaminergic and serotonergic receptors and blocks alpha-adrenoreceptors	Treats dementia and age-related cognitive impairment; recovery after stroke	N/A	6	2
Human growth hormone (hGH)	12629-01-5	Regulates fat, muscle, tissue, and bone; stimulates the synthesis of chondroitin sulfate and collagen; promotes somatic growth	Maintains, builds, and repairs healthy tissue in brain and other organs; speeds up healing after injury and repair muscle tissue after exercise	N/A	200	18
Idebenone	58186-27-9	Blocks free radicals damage and sustains normal ATP levels	Alzheimer's disease, cognitive defects		15	49
Isotretinoin	4759-48-2	Inhibits sebaceous gland function and keratinization	Acne, cutaneous conditions		60	61
Meclofenoxate (Centrophenoxine, Lucidril)	51-68-3	Diminution of lipofuscin content of nerve cell; enhances activity of succinic and lactic dehydrogenase	Memory-boosting		34	9
Metformin	657-24-9	Inhibits mTORC1 activity; growth hormone suppression	Type 2 diabetes, PCOS; dietary restriction, anti-atherogenic		936	105

Panobinostat	404950-80-7	Inhibitor of class I HDAC; class IIa and IIb HDAC, class IV HDAC; histone deacetylase inhibitor	Antitumor agent		5	9
Piracetam	7491-74-9	Improves the function of neurotransmitter acetylcholine via muscarinic cholinergic receptors	Neuroprotective, anticonvulsant, improves neural plasticity in cognitive disorders and dementia		43	10
Procaine (Gerovital H3, GH3, KH-3)	59-46-1	Inhibits Na influx across voltage gated Na-channels in neuronal cell membrane of peripheral nerves	Anti-inflammatory, analgesic, vasodilatation, antioxidant; nervous system balance		60	35
Pyridopyrimidines	-	High affinity DHFR inhibitor, thus decreasing tetrahydrofolate quantity required for pyrimidine and purine synthesis	KRAS inhibitors; anticancer	N/A	1	0
Rosiglitazone	122320-73-4	Inhibits PPAR-γ activity	Indicated for treatment of type 2 diabetes		57	30
Ruxolitinib	941678-49-5	Inhibits JAK1 and JAK2, block dysregulated cell signaling, prevent abnormal blood cell proliferation	Treatment of myelofibrosis in adults		23	8
Salsalate	552-94-3	Decreases formation of prostaglandins involved in pain, fever, inflammation	Anti-inflammatory, antirheumatic		4	13
Selegiline (L-deprenyl, Eldepryl, Emsam)	14611-51-9	Selective inhibitor of MAO-B	Treats symptoms of Parkinson's disease and major depressive disorder		109	28
Seletinoid G	637357-50-7	Type I procollagen, tropoelastin, and fibrillin-1 expressions stimulation, reduces MMP-1	Anti-aging		4	2
Sodium butyrate	156-54-7	Suppresses NFkB activation, inhibits interferon γ production and upregulation of PPARγ	Antioxidant, anti-inflammatory, autophagy inducing	 • Na	37	17
Sodium phenylbutyrate (Buphenyl)	1716-12-7	Inhibitor of class I HDAC, class IIa HDAC and class IIb HDAC	Antitumor agent; memory enhancement; amyloid burden reduction	 • Na	7	8

Tadalafil	171596-29-5	PDE 5 inhibitor with potent anti-aging activity	Treats erective dysfunction and benign prostatic hyperplasia		7	11
Tazarotene	118292-40-3	Binds to retinoic acceptor and modify gene expression	Used for psoriasis, acne, and psoriatic arthritis		7	34
Trametinib	871700-17-3	ERK phosphorylation inhibitor, Ki67 suppression, tumor growth inhibition with mutant BRAF or RAS decreasing, G1 cell cycle arrest, apoptosis induction	Applied alone or in combination with dabrafenib to treat skin cancer (melanoma), thyroid cancer, and non-small cell lung cancer (NSCLC)		7	4
Trichostatin A	58880-19-6	Inhibitor of class I HDAC, class IIa HDAC, class IIb HDAC6	Antifungal, antibacterial, histone deacetylase inhibitor; protein synthesis inhibitor; antitumor, memory enhancement		28	4
Valproic acid	99-66-1	Inhibitor of class I HDAC	Anticonvulsant, mood stabilizer; histone deacetylase inhibitor; GABA modulator; memory enhancement; CDK5 inactivation		148	23
Vinpocetine	42971-09-5	Selective inhibitor of Ca(2+)-calmodulin dependent cGMP-PDE	Memory enhancer; against dementia, stroke, hearing loss		10	8
Visomitin	934826-68-3	Penetrates cellular membrane, accumulates in mitochondrial membrane inner leaflet where it is reduced or recharged	Treats inflammation associated with ophthalmic disease: dry eye, corneal wounds		13	3
Vorinostat	149647-78-9	Inhibitor of class I HDAC, class IIb HDAC	Antitumor, memory enhancement		22	21

6. Private Investment

Examining the overall global private investment activities of the anti-aging field provides insight to the commercial interest into this area. Performing a search of anti-aging within PitchBook [662], an online source for investment data, reveals the overall venture capital activities. Anti-aging field refers to companies, which perform research and development of restorative treatments to prevent or treat the effects of aging and enhance lifespan. Research areas include genomic instability, telomere attrition, epigenetic alteration, loss of proteostasis, impaired nutrient sensing, mitochondrial dysfunction, cellular senescence, stem cell exhaustion, and altered intercellular communication. The search revealed capital steadily raised within this industry (Figure 22A). [662] From 2012 through 2018, the total venture capital raised increased from $1.4B to over $12.2B. 2019 revealed the overall venture capital raised fell to just $7.0B. Capital raised continued to grow for 2019 to 2022 totaling over $30.6B in 2022 in total venture capital investment (Figure 22A). In the years 2013-2015 and 2020-2022 the investments were dominated by the United States, while in 2016-2019 investments from Asia were dominating. The venture capital investment data in this area clearly shows a recent and increasing commercial interest surrounding anti-aging agents, revealing its potential promise for therapeutic applications.

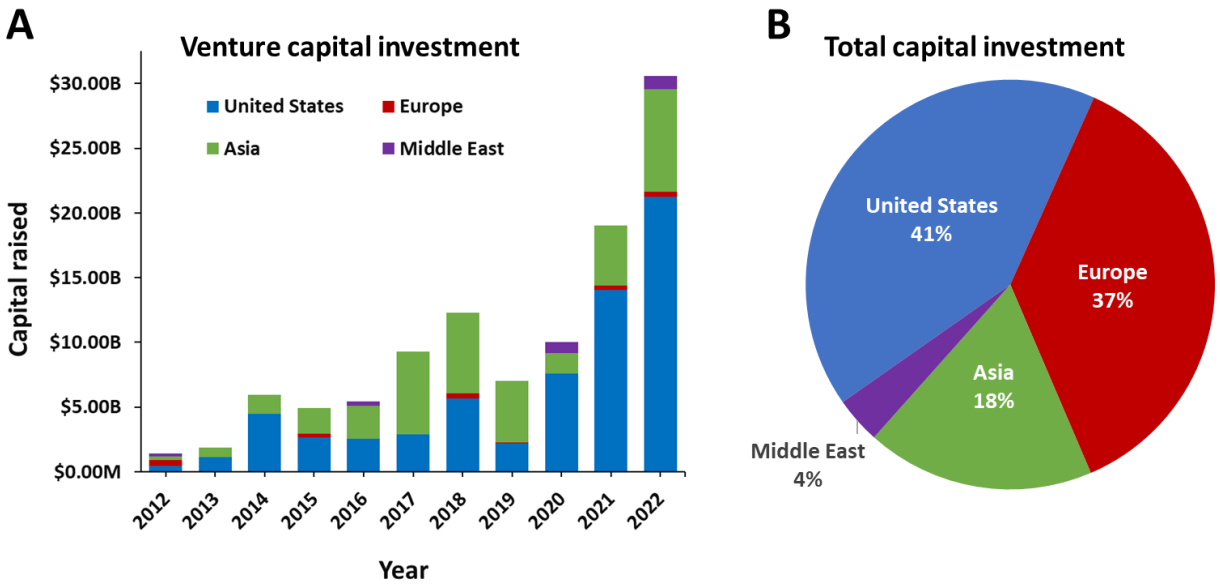


Figure 22. Overall capital raised of (A) Venture capital investment and (B) Total capital investments for the period 2012-2022 in the anti-aging field [$] distributed by regions. Source: PitchBook.com

In addition to the venture capital investments, in 2020 there was an investment of over $97B from the European Union, for the Horizon 2020 SME Instrument. [663] The SME Instrument supports high-risk, and high-potential small and medium-sized initiatives to develop and provide new products, services and business models able to drive economic growth. This large investment enhanced significantly the Europe contribution in the total capital investments distribution (Figure 22B).

7. Clinical trials

A representative selection of therapeutic anti-aging clinical trials is examined within this section to gain an overall view of the past, present, and future state of clinical development. A selection of the top 10,000 anti-aging clinical trials [664] from https://clinicaltrials.org is examined against time, clinical trial phase, status, disease indication, and anti-aging strategy. Anti-aging therapeutics are well established in clinical development, with Figure 23 showing a steady growth starting in the early 1990s and continuing through 2022.

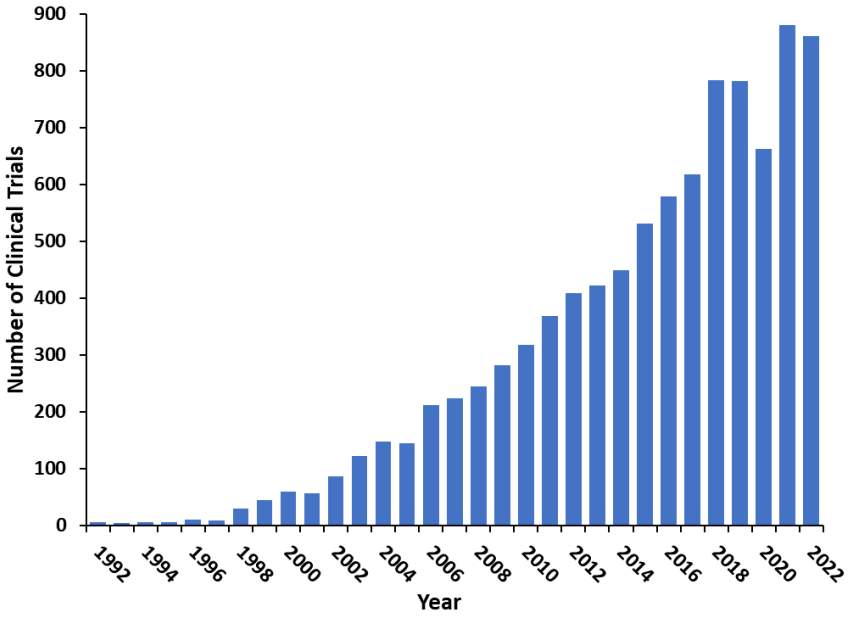


Figure 23. Number of therapeutic anti-aging clinical trials by year.

Analysis of therapeutic anti-aging clinical trial phases reveal that close to half of all trials are in Phase III and IV with the other half filtering into earlier phases. Phase II trials contain the highest percentage of all categories encompassing 33% of all trials (Supporting Information, Figure S2). Examining clinical trials, a step further, by disease indication, shows that bone, cardiovascular, and skin diseases along with sleep disorders and obesity are well established in the development pipeline having the highest percentage of clinical trials further along in phase III and IV clinical trials (Figure 24). Balance disorders, cancer, frailty, along with eye and neurological disease are the indications less established in the pipeline with the largest percentage of trials in earlier phases (Figure 24).

Indication	Early Phase I	Phase I	Phase I/II	Phase II	Phase II/III	Phase III	Phase IV
Bone disease	3%	8%	4%	14%	3%	25%	42%
Cardiovascular disease	3%	9%	6%	22%	3%	33%	23%
Skin disease	6%	14%	8%	15%	5%	22%	31%
Sleep disorders	4%	11%	4%	25%	7%	21%	29%
Obesity	6%	4%	10%	27%	4%	25%	24%
Musculoskeletal disease	1%	15%	6%	25%	4%	23%	26%
Eye diseases	6%	22%	6%	28%	0%	17%	22%
Neurological disease	4%	11%	4%	36%	6%	19%	20%
Frailty	9%	9%	10%	38%	0%	16%	18%
Cancer	0%	8%	7%	57%	3%	20%	5%
Balance/Falls	14%	14%	10%	34%	0%	14%	14%

Figure 24. Percentage of therapeutic anti-aging clinical trials in various phases for the treatment of age-related disease indications.

Next, we review therapeutic anti-aging clinical trial statuses, characterized by age-related disease indications. Current therapeutic anti-aging clinical trials in early phase trials such as neurological disease, frailty, sleep disorders, and cancer also have the highest percentage in active, recruiting, and not yet recruiting statuses (Figure 25). These early phase trials are active or getting ready to be active in the pipeline. On the other hand, it is no surprise that disease indications more well established in the development pipeline such as skin, bone, and eye diseases along with obesity also contain the highest percentage of completed trials. There is however still current advancement in these areas reflected by greater than 20% of their trials in active, recruiting or not net recruiting status (Figure 25).

Indication	Not yet recruiting	Recruiting	Active	Completed
Neurological disease	6%	30%	9%	55%
Frailty	8%	28%	8%	56%
Sleep disorders	7%	29%	6%	58%
Cancer	5%	23%	12%	60%
Cardiovascular disease	7%	21%	7%	64%
Balance/Falls	6%	20%	6%	68%
Musculoskeletal disease	5%	22%	4%	69%
Eye diseases	9%	22%	4%	64%
Obesity	5%	19%	4%	71%
Bone disease	5%	17%	6%	72%
Skin disease	8%	10%	5%	78%

Figure 25. Percentage of therapeutic anti-aging clinical trials in various statuses for the treatment of age-related disease indications.

Finally, representative clinical trials examining anti-aging therapeutics are highlighted in Table 8-16 categorized by anti-aging strategy. These are examined in further detail below to showcase a variety of anti-aging strategies, interventions, and targeted conditions in clinical development along with their status and any published results.

mTOR inhibition is widely explored in clinical trials for anti-aging therapeutics. The National eye institute researched the use of rapamycin for the treatment of geographic atrophy (GA), as part of late-stage age-related macular degeneration (AMD) in phase I/II clinical trial NCT01445548. Six participants with bilateral GA were enrolled and received intravitreal sirolimus but no benefit was detected. [665] A following phase II study NCT01675947 enrolled 52 participants with GA for monthly intravitreal sirolimus treatment. The study was terminated early due to lack of efficacy and the adverse event of sterile endophthalmitis in three participants. [666] It was determined that while immunosuppression may play some role in AMD, it might not be the main pathway for GA development. The Mayo Clinic also researched at the use of rapamycin in phase I clinical trial NCT01649960. They focused on rapamycin treatment effects on senescence markers and frailty in elderly subjects undergoing cardiac rehabilitation. 13 participants received low doses (0.5mg-2mg) of rapamycin daily for 12 weeks. While correlation between some senescence markers and physical performance were observed, the primary endpoint measurement of frailty saw no improvement. [667]

mTOR inhibition research continues however with the University of Texas Health Science Center at San Antonio currently investigating the use of rapamycin for inflammation reduction and epigenetic reversal in an active clinical trial (NCT04608448). Subjects aged 65 to 95 will topically apply rapamycin 8% ointment to a forearm daily for 6 months. Both epigenetic and inflammatory markers will be measured. The University of Nottingham is recruiting for another study (NCT05414292) looking at the use of rapamycin and resistance exercise on age related muscle loss. The study aims to recruit 16 healthy male participants over 50 years old who will take a 1mg rapamycin oral tablet daily for 16 weeks along with performing a 14-week unilateral resistance exercise program. Measurements such as changes in muscle mass, strength, power, and function will be recorded.

Table 8. Highlighted mTOR inhibition anti-aging clinical trials

Anti-aging strategy	Indication	Intervention	Status	Sponsor	NCT Number
mTOR inhibition	Geographic Atrophy	Rapamycin	Complete	National Eye Institute, USA	NCT01445548
mTOR inhibition	Aging frailty	Rapamycin	Complete	Mayo Clinic, USA	NCT01649960
mTOR inhibition	Aging|Epigenetics| Inflammatory Mediators	Rapamycin	Active, not recruiting	The University of Texas Health Science Center, USA	NCT04608448
mTOR inhibition	Age-Related Sarcopenia	Rapamycin | Resistance exercise	Recruiting	University of Nottingham, UK	NCT05414292

Targeting aging senescent cells to combat aging with **senotherapy** is currently undergoing research in clinical trials. One such study researched the use of Carlson fish oil to improve immune senescence biomarkers CD28, CD57, on the surface of CD4+ and CD8+ T lymphocytes in aging participants 40-70 years old with HIV infection. The intervention groups received 1.6 grams of omega-3

fatty acids daily for 12 weeks. [668] Published results were less than encouraging however with no significant difference in immune senescence measurements in participants. [669] Another phase I/II clinical trial NCT04063124 researching targeting aging senescent cells was recently completed by the University of Texas Health Science Center at San Antonio. Five early-stage AD patients were enrolled and received 100 mg of dasatinib and 1000 mg of quercetin every two weeks for twelve weeks. The outcome measurements of brain penetration for both compounds and AD and senescence biomarkers were collected. [670] Interpretative results have yet to be published but the results of this pilot study will guide researchers in developing a larger phase II trial researching senolytic agents for the modulation of Alzheimer's disease progression. [671] Lastly, we examine phase II clinical trial NCT04733534 currently recruiting 60 participants. St. Jude Children's Research Hospital will evaluate two senolytic regimens for their cellular senescence biomarker reduction and frailty improvement in adult survivors of childhood cancer. Treatment groups will receive either oral Dasatinib (100 mg/day) and Quercetin (500 mg twice daily) on days 1, 2, 3, 30, 31, 32 or oral Fisetin (20mg/kg/day) on days 1, 2, 30 and 31. Outcome measurement of walking speed and blood senescent cells (p16INK4A) levels will be recorded. If this pilot study is successful, it too will provide evidence needed for a continued phase II trial to determine further efficacy. [672]

Table 9. Highlighted senotherapy anti-aging clinical trials

Anti-aging strategy	Indication	Intervention	Status	Sponsor	NCT Number
Senotherapy	Immune Senescence | Inflammation	Fish oil	Complete	Rush University Medical Center, USA	NCT02102724
Senotherapy	Alzheimer's Disease	Dasatinib & Quercetin	Complete	The University of Texas Health Science Center, USA	NCT04063124
Senotherapy	Frailty|Childhood Cancer	Dasatinib & Quercetin| Fisetin	Recruiting	St. Jude Children's Research Hospital, USA	NCT04733534

Hormonal replacement trials in the clinical pipeline have shown promising results. A large phase III clinical study (NCT00799617) sponsored by the University of Pennsylvania called the testosterone trials is a coordinated set of seven clinical trials including 788 male participants with a mean age of 72. The treatment group applied 5-15g of AndroGel once daily for 12 months to determine the efficacy of increasing the testosterone levels of older men with low testosterone. [673] Results from these trials are highlighted below. [674]

- Testosterone Level: increased the median testosterone level from low to normal
- Sexual Function Trial: increased sexual activity, sexual desire, and erectile function
- Physical Function Trial: increased distance walked
- Vitality Trial: slight increase in mood and depression; did not increase energy
- Anemia Trial: increased hemoglobin

- Bone Trial: increased volumetric bone mineral density and strength of the spine and hip bones
- Cardiovascular Trial: increased coronary artery noncalcified plaque

The testosterone trials have shown that increasing testosterone levels in older men with low testosterone has documented benefits. This trial paves the way for larger trials to explore the risks and efficacy deeper and has helped influence physician decisions regarding testosterone treatment in older men.

The University of California, San Diego researched the use of estrogen with two separate drug delivery systems for the prevention of recurrent urinary tract infections (UTIs) in post-menopausal women. Their phase IV clinical trial NCT01958073 had an intervention of 0.5g of estrogen vaginal cream 2 times weekly or an estradiol ring every 3 months for 6 months. [675] More encouraging results were revealed in this trial with vaginal estrogen preventing UTIs in post-menopausal women who have been diagnosed with recurrent UTIs. [676] The University of Southern California is recruiting for its phase II trial NCT04103476 which will look at the effects of a tissue selective estrogen complex therapy on the progression of atherosclerosis and cognitive decline in 360 postmenopausal women aged 45-59 years. The treatment group will receive Bazedoxifene 20 mg/Conjugated Equine Estrogen 0.45 mg for up to a total of 3 years. Measurement outcomes will include carotid artery intima-media thickness, arterial stiffness, and three composite cognitive measures to determine cognitive decline. [677]

Table 10. Highlighted hormonal replacement anti-aging clinical trials

Anti-aging strategy	Condition	Intervention	Status	Sponsor	NCT Number
Hormonal replacement	Andropause	Testosterone	Completed	University of Pennsylvania, USA	NCT00799617
Hormonal replacement	Recurrent Urinary Tract Infection	Estrogen	Completed	University of California, San Diego, USA	NCT01958073
Hormonal replacement	Atherosclerosis	Bazedoxifene /Conjugated Estrogen	Recruiting	University of Southern California, USA	NCT04103476

Aging induces various gut microbiota changes especially the reduction in health promoting species. Researchers in the clinic are looking at **gut microbiota modulation** for disease treatment of various age-related indications. A few of these studies examined below research the use of pre and probiotics along with fecal microbiota transplant (FMT) to aid a variety of conditions such as immune function, skin health, infection, and neurological disease. One such study sponsored by Clasado discovered that their Bimuno Galacto Oligosaccharide (GOS) mixture product has the potential to increase *Bacteroides* and *Bifidobacterium* fecal bacteria during an early phase I clinical trial (NCT01303484). Forty participants (65-80 yrs old) with a dose group of 5 g/day of GOS mixture for 10 weeks revealed that supplementation with a GOS prebiotic positively affects the gut microbiota and biomarkers for immune function amongst the elderly. [678]

On the other hand, the University of Antwerp, Belgium saw no immune improvement when looking at the use of *Lactobacillus casei Shirota* (LcS) probiotic for the prevention of respiratory infection

and immune boost in elderly nursing home residents (NCT00849277). 737 volunteers aged 65 and older were enrolled and the treatment group received a daily fermented milk drink that contained greater than 6.5 B live LcS cultures for 176 days with a flu vaccine given on day 21. The results showed no significant effect on the protection against respiratory infections or regarding flu vaccine immune response. [679]

Currently in the pipeline, Chr Hansen is recruiting for their research (NCT05529693) on the use of *Bifidobacterium adolescentis* Bif-038 on low grade inflammation biomarkers. Subjects aged 65-85 years old with low and high treatment groups of 1 and 10 billion CFU for 12 weeks will be tested and various biomarkers such as C-reactive protein and TNFα will be measured. [680] Another method of gut microbiota modulation, FMT, is currently only approved for the treatment of recurrent *Clostridioides difficile* infection but researchers are branching out and researching its use for other indications with a gut-brain access connection. The University of Ghent has recently completed a clinical trial researching the effect of nasojejunal FMT (NCT03808389) on subjects with Parkinson's disease. 49 subjects aged 50 to 65 were enrolled and the treatment group received donor fecal microbiota with published results forthcoming. [681]

Table 11. Highlighted gut microbiota modulation anti-aging clinical trials

Anti-aging strategy	Indication	Intervention	Status	Sponsor	NCT Number
Prebiotics	Immunosenescence	Bimuno galacto-oligosaccharide	Complete	Clasado, UK	NCT01303484
Probiotics	Respiratory Tract Infections	*Lactobacillus casei Shirota*	Complete	University Antwerp, Belgium	NCT00849277
FMT	Parkinson's Disease	FMT	Complete	University Ghent, Belgium	NCT03808389
Probiotics	Inflammation	*Bifidobacterium adolescentis* Bif-038	Recruiting	Chr Hansen, Denmark	NCT05529693

Caloric restriction is well researched as an anti-aging strategy with its clinical development widely established. The 2-year clinical trial NCT00427193 by Duke University enrolled over 200 people to research the effect of a 25% calorie restricted diet on aging and age-related disease processes. Outcome measures included change in core body temperature, resting metabolic rate, inflammatory marker TNFα, along with fat mass. [682] The effect of calorie restriction included significant decreases in both inflammatory markers and cardiometabolic risk factors which suggests potential benefits for aging and age-related disease processes. [683] The University of Alabama also researched the use of caloric restriction along with exercise for the reduction of cardiometabolic risk. Phase III clinical trial NCT00955903 enrolled 167 participants with outcome measurements of change in abdominal fat mass, cardiometabolic risk factors, and weight change. Study results show significant improvement to relative

fat mass, biomarker adiponectin and leptin, and cardiometabolic risk measurements for the intervention arm. [684]

Researchers are also currently investigating how caloric restriction can affect biological aging and neurodegenerative diseases such as cognitive impairment and Alzheimer's disease. TruDiagnositic is conducting an active phase II trial with 50 subjects enrolled to investigate the use of Peak Human Labs calorie mimetic supplement along with a fasting mimicking diet to see their effect on biological aging. The intervention group will take the supplement for 90 days mixing in a 5-day fasting diet three times. Outcome measurements include the epigenetic age biomarkers which will test methylation at 850,000 locations on the DNA and body max index. [685] Another active study performed by the University of Kansas hopes to learn how the Mediterranean diet compared to a low-fat diet for 12 months affects cardiometabolic biomarkers, brain antioxidant status, brain volume, and memory in cognitive normal adults aged 65 or greater. Researchers plan to examine brain processes to understand health and how the Mediterranean diet may help treat Alzheimer's disease in the future. [686] The University of Genova is also currently recruiting for its phase I/II study which will investigate the use of a specific 5-day low protein fasting diet called Prolon ADTM. 40 participants will be enrolled, and the treatment group will consume the diet once a month for a 12 months. Metabolic, inflammatory, and regenerative pathways will be monitored to see the diet's effect on mild cognitive impairment and early Alzheimer's disease. [687]

Table 12. Highlighted caloric restriction anti-aging clinical trials

Anti-aging strategy	Indication	Intervention	Status	Sponsor	NCT Number
Caloric restriction	Aging	Caloric Restriction	Complete	Duke University, USA	NCT00427193
Caloric restriction	Obesity|Diabetes| Hypertension| Hyperlipidemia	Exercise| Reduced Calorie Diet	Complete	University of Alabama at Birmingham, USA	NCT00955903
Caloric restriction	Aging	Calorie mimetic supplement| Fasting Mimicking Diet	Active, not recruiting	TruDiagnostic, USA	NCT04962464
Caloric restriction	Alzheimer's Disease	Mediterranean Diet| Study Supplement| Low-fat Diet	Active, not recruiting	University of Kansas Medical Center, USA	NCT03841539
Caloric restriction	Cognitive Impairment|Early Alzheimer's Disease	Fasting-Mimicking Diet ProlonADTM	Recruiting	University of Genova, Italy	NCT05480358

Research on **physical exercise** as an intervention for aging indications are well established and clinical trials are seeing promising results. The Central Arkansas Veterans Healthcare System has

completed a clinical trial on the use of Wii-Fit exercises to improve unsteady gait and postural balance in 30 veterans aged 65 and over. The intervention was performed for 45 minutes, 3 days a week for 8 weeks. [688] Outcome measurements of gait and balance improved significantly in the intervention group, showing that the Wii-Fit exercise program was effective. [689] Another study from the University of Kansas Medical Center recently published results for its clinical trial (NCT04009629) researching moderately intensive aerobic exercise and its effects on brain blood flow and biological factors after exercise (15 mins) in participants with a genetic risk factor for developing Alzheimer's disease. 61 participants aged 65-80 years old with the apolipoprotein e4 (APOE4) gene were enrolled. [690] The results revealed increases in cerebral blood flow and neurotrophic response to acute aerobic exercise for all participants regardless of APOE4 status. [691] The long-term goal of the study team with this acquired knowledge is to create a personalized exercise prescription for the treatment of Alzheimer's disease.

Upcoming clinical trials are also continuing to examine physical exercise and its effects on the cognitive function in the aging population with heart failure. The Montreal Heart Institute is recruiting 218 participants aged 60 years and older to research physical exercise and cognitive training interventions on cognition and brain health in patients with heart failure for clinical trial NCT04970888. Cognitive training sessions will be 30 minutes and physical exercise sessions will be 60 minutes, three times a week for 6 months. [692] This combined intervention method has not been widely studied so results should be of particular interest. Another indication of post-traumatic stress disorder (PTSD) amongst elderly veterans is also currently in the clinical development pipeline (NCT04199182). The VA Office of Research and Development is recruiting to investigate this quickly emerging field of study. A three times a week supervised exercise program will continue for 6 months to see its effects on PTSD symptoms and related conditions such as sleep disorders amongst 188 older veterans. [693]

Table 13. Highlighted physical exercise anti-aging clinical trials

Anti-aging strategy	Indication	Intervention	Status	Sponsor	NCT Number
Physical Exercise	Alzheimer's Disease	Moderate Intensity Aerobic Exercise	Complete	University of Kansas Medical Center, USA	NCT04009629
Physical Exercise	Unsteady Gait|Postural Balance	Wii-Fit Exercises	Complete	Central Arkansas Veterans Healthcare System, USA	NCT02190045
Physical Exercise	Cognitive Function	Cognitive Training| Exercise Training	Recruiting	Montreal Heart Institute, Canada	NCT04970888
Physical Exercise	Post-Traumatic Stress Disorder	Exercise Training	Recruiting	VA Office of Research and Development, USA	NCT04199182

Stem cell transplantation has shown promising results in clinical trials for aging-related conditions. Longeveron studied the use of allogeneic mesenchymal stem cells (allo-MSCs) for the condition of frailty in a successful clinical trial (NCT02065245). 30 patients with a mean age of 75.5 years

received either a 100-million or 200-million cell dose infusion. Significant reduction of inflammatory marker TNF-α and early and late-stage T-cells activation occurred. B cell intracellular TNF-α and physical performance amongst participants was also improved in both treatment groups. [694] Longeveron also explored the use of MSCs through its biotherapeutic candidate Lomecel-B for the treatment of Alzheimer's disease (AD) in phase I clinical trial NCT02600130. 30 participants were enrolled with low and high dose infusion groups of 30 and 100 million cells. Significant improvement was seen for inflammatory and AD biomarkers along with neurocognitive assessments. [695] Due to these encouraging results, Alzheimer's disease treatment with Lomecel-B is further researched in phase II trial NCT05233774 currently recruiting participants.

The Vinmec Research Institute of stem cell and gene technology are exploring the use of MSC for male sexual dysfunction in a phase I/II clinical trial (NCT05345418). They are currently recruiting male subjects aged 50-70 years old with sexual functional deficiency. Treatment groups will receive two IV doses of 1.5 million cells/kg body weight spaced out by 3 months. Various biomarkers, testosterone levels, and sexual life quality information will be measured. The First Affiliated Hospital with Nanjing Medical University has an upcoming phase I clinical trial (NCT04706312) researching the use of amniotic mesenchymal stem cells (AMSCs) for the treatment of infertility is people with diminished ovarian response. Subjects will receive an IV injection of AMSCs and measurements recorded for ovarian function and *in vitro* fertilization such as stimulated follicles, number of oocyte retrieval, fertilization rate, etc. [696]

Table 14. Highlighted stem cell therapy anti-aging clinical trials

Anti-aging strategy	Indication	Intervention	Status	Sponsor	NCT Number
Stem Cell Therapy	Frailty	Allogeneic Mesenchymal Stem Cells	Complete	Longeveron, USA	NCT02065245
Stem Cell Therapy	Alzheimer's Disease	Lomecel-B (Allogeneic Mesenchymal Stem Cells)	Complete	Longeveron , USA	NCT02600130
Stem Cell Therapy	Male Sexual Dysfunction	Umbilical Cord Mesenchymal Stem Cell	Recruiting	Vinmec Research Institute of Stem Cell and Gene Technology, Vietnam	NCT05345418
Stem Cell Therapy	Diminished Ovarian Response	Amniotic Mesenchymal Stem Cells	Not yet recruiting	The First Affiliated Hospital with Nanjing Medical University, China	NCT04706312

Dietary supplementation is an anti-aging strategy that targets a wide range of indications. The University of Sherbrooke, Canada researched the use of medium chain triglycerides (MCT) combined with aerobic (AE) exercise on ketone production in a group of 20 women (prediabetic and healthy) over the age of 60 years through clinical trial NCT02678390. They discovered that MCT (30 g/day for 5 days)

combined with AE (30 mins) was more ketogenic in older women than MCT or AE alone. [697] A clinical trial (NCT02446314) researching the use of two different blueberry formulations for the treatment of cognitive decline in 125 participants 65-80 years old. Treatment groups consisted of a 6-month daily regime of 450-900 mg of blueberry powder or 100 mg of blueberry extract. The results reveal that the blueberry extract intervention can improve episodic memory and cardiovascular biomarkers over 6 months. The same effects were not observed for the blueberry powder intervention or with the measurements of executive function, working memory, or mood. [698]

Several current studies are exploring the use of collagen for age-related indications. NovoBliss Research, India is researching the effect of vegetable, bovine, fish, and chicken collagen peptide for indications such as skin elasticity, wrinkles, and hydration, hair thickness and density, along with joint pain and osteoarthritis. 125 participants are currently enrolled with treatment groups of 0.5 – 10 g/day. [699] Shenzhen Precision Health Food, China also has a registered trial (NCT05682092) researching the use of collagen peptide to increase skin moisture and elasticity. The trial is not yet recruiting but plans to enroll 70 middle aged (30-50 yrs old) women who will consume 25ml twice a day for two months if in the treatment group. Lastly, we discuss Medical College of Wisconsin's clinical trial NCT05598359 that is not yet recruiting but will be researching the use of TA-65, a purified small molecule extracted from Astragalus root. 180 participants will be recruited to investigate the use of TA-64 (250 U) taken once per day on microvascular function and blood pressure. [700]

Table 15. Highlighted dietary supplementation anti-aging clinical trials

Anti-aging strategy	Indication	Intervention	Status	Sponsor	NCT Number
Dietary supplement	Prediabetes	MCT| aerobic exercise	Complete	University de Sherbrooke, Canada	NCT02678390
Dietary supplement	Cognitive Decline	Wild Blueberry Powder or extract	Complete	University of Reading, UK	NCT02446314
Dietary supplement	Osteoarthritis| Knee	Veg, Bovine, Fish, or Chicken Collagen Peptide	Active, not recruiting	NovoBliss Research, India	NCT05613660
Dietary supplement	Skin Laxity	WonderLab Collagen Tripeptide Drink	Not yet recruiting	Shenzhen Precision Health Food, China	NCT05682092
Dietary supplement	Telomere Shortening| Vascular Diseases	TA-65	Not yet recruiting	Medical College of Wisconsin, USA	NCT05598359

8. Outline and perspectives

Aging is generally defined as the accumulation of detrimental changes taking place in cells and tissues with advancing age, which bring about the increased risk of disease and death. The emerging standpoint defines aging as a particularly complex, multifactorial process. Anti-aging research aims to identify strategies to promote healthy aging and extend lifespan. The major perspectives in the anti-aging exploration generally fall into two groups: (i) lifestyle modifications and (ii) pharmacological / genetic manipulations. More specifically, the foremost approaches in anti-aging research include the following:

- Extensive current research explore the genetic basis of aging and age-related diseases, and investigate the potential of **genetic interventions**, such as gene therapy, to prevent or reverse age-related damage.
- **Lifestyle interventions**, such as caloric restriction, exercise, and stress reduction, have been believed to promote healthy aging and extend lifespan. Widespread research is currently exploring the mechanisms underlying these effects and developing strategies to promote healthy behaviors.
- **Pharmaceutical interventions** explore the potential of drugs that target age-related pathways or senescent cells, to prevent or delay age-related diseases.
- **Regenerative medicine** aims to restore or replace damaged tissues and organs and has the potential to promote healthy aging and extend lifespan.
- **Social and environmental factors**, such as social support, access to healthcare, and exposure to toxins, can influence the aging process. The effects of these factors are being explored and interventions to promote healthy aging are being currently developed.
- **Artificial intelligence** (AI) is being used to analyze large amounts of data and identify patterns that could be used to predict or prevent age-related diseases. AI is also being used to develop personalized anti-aging interventions based on an individual's genetic and lifestyle factors.

More specifically, with particular attention to **brain health** maintenance, the following anti-aging lifestyle strategies can help prevent or slow down age-associated brain function decline:

- **Physical exercise** has been shown to improve brain function, increase brain volume, and reduce the risk of cognitive decline.
- **Mental stimulation**: engaging in mentally stimulating activities can help maintain cognitive function and reduce the risk of age-related cognitive decline.
- Eating a **healthy diet** rich in fruits, vegetables, whole grains, and lean protein can help reduce inflammation and oxidative stress in the brain.
- **Stress reduction**: chronic stress has been linked to accelerated brain aging, so finding ways to manage stress like practicing mindfulness and meditation can be beneficial.
- Staying **socially active** and connected can help maintain cognitive function and reduce the risk of cognitive decline.
- Getting adequate **sleep** is essential for brain health and has been linked to improved cognitive function and a reduced risk of cognitive decline.

All these strategies are not mutually exclusive, and anti-aging research often involves a multidisciplinary approach that combines different approaches to promote healthy aging and extend

lifespan. Yet, regardless of the extensive research for anti-aging therapeutics, based on the general understanding that aging is malleable in diverse species, to date no convincing evidence has been provided indicating that the administration of existing anti-aging remedies can markedly slow aging or increase longevity in humans. The major roadblocks that the anti-aging research and development is currently facing are summarized in Table 16.

Table 16. Major roadblocks in the anti-aging research and development

Roadblocks	Details
Complexity of aging	Aging is a complex process that involves multiple mechanisms and pathways, and it is difficult to identify specific targets for intervention.
Insufficiency of knowledge	Despite advances in anti-aging research, there is still much to be learned about the underlying mechanisms of aging and how they contribute to age-related diseases.
Heterogeneity of aging	Aging is a heterogeneous process, and there is significant individual variability in how people age. This makes it challenging to develop personalized anti-aging interventions that are effective for everyone.
Regulatory challenges	Developing and testing anti-aging interventions can be challenging due to regulatory barriers and the need for long-term clinical trials to demonstrate safety and efficacy.
Cost	Developing anti-aging interventions can be expensive, and there may be limited financial incentives for companies to invest in this area.
Ethical considerations	There are ethical considerations associated with anti-aging interventions, such as concerns about equity and access, and the potential for unintended consequences.
Perception and stigma	There is still a stigma associated with aging and a perception that aging is an inevitable and irreversible process. This can make it challenging to attract funding and support for anti-aging research and development.

Despite these multiple difficulties and complexities, anti-aging research is a rapidly growing field, and researchers are working to overcome these challenges to develop effective interventions to promote healthy aging and extend lifespan. Certain important steps forward towards the understanding of the aging process have been made, so that it is no more an incomprehensible issue. Thus, the extensive efforts and research activities in the anti-aging strategies field has led to several important outcomes:

- Identification of **biomarkers of aging**. Researchers have identified biomarkers that can predict biological age and the risk of age-related diseases. These biomarkers can be used to develop personalized anti-aging interventions and monitor the effectiveness of these interventions.
- Development of **interventions to promote healthy aging**. Anti-aging research has led to the development of interventions, such as caloric restriction, exercise, and stress reduction, that can promote healthy aging and extend lifespan.
- Identification of potential **drug targets**. Researchers have identified several pathways and targets that could be targeted by drugs to prevent or delay age-related diseases.

- Development of **regenerative medicine therapies**. Anti-aging research has led to the development of regenerative medicine therapies that can restore or replace damaged tissues and organs, which could have important implications for treating age-related diseases.
- **Extension of lifespan in animal models**. Anti-aging interventions have been shown to extend lifespan in animal models, which provides proof-of-concept for the potential of these interventions to promote healthy aging in humans.
- Improved **understanding of the biology of aging**. Anti-aging research has led to a better understanding of the biological mechanisms underlying aging and age-related diseases, which could lead to the development of new interventions and therapies.

The progress in anti-aging research has shown the potential to improve health and quality of life for older adults by promoting healthy aging and delaying the onset of age-related diseases. In pursuing a solution to the aging issues, it is necessary to keep clear in mind that the goal of research on aging hallmarks and anti-aging strategies is not to enhance human longevity, but to enhance healthy, active longevity, free from disability and functional incapacity. [657, 701] Such understanding of aging has resulted in a shift in the approach for aging interventions from anti-aging to healthy aging. In order to achieve healthy aging, it would be appropriate to abandon disease-oriented research approach and adopt health-oriented prevention strategies. [702]

Supporting Information

Table S1. Anti-aging drugs included in the CAS Content Collection

Figure S1. Yearly NIH funding for projects related to anti-aging research.

Figure S2. Overall anti-aging clinical trial phase development

Notes

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TOC figure: