# How to die young at a very old age? An overview of currently existing theories and mechanisms related to aging

Submitted by

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*Declaration*

*I hereby declare that this thesis is my own original work and that I have fully acknowledged by name all of those individuals and organizations that have contributed to the research for this thesis. Due acknowledgement has been made in the text to all other material used. Throughout this thesis and in all related publications I followed the "Standards of Good Scientific Practice and Ombuds Committee at the Medical University of Graz".*

*Date 28.07.2023".*

**“The process of scientific discovery is, in effect, a continual flight from wonder”**

**--Albert Einstein**

**"It's possible that we could change a human gene and double our lifespan"**

**--Cynthia Kenyon**

**"Unfortunately, because aging is so common and natural, we tend to think of it as destiny or something we should accept."**

**--David Andrew Sinclair**

**To my family and friends**

## Abstract

The fact that the human lifespan is perpetually increasing is undeniable. However, extending our years on earth does not fully address our aspirations as individuals. The desire for most of us extends far beyond longevity, we yearn for these additional years to be characterized by fulfillment and most crucially good health. Consequently, there has been a growing interest in identifying interventions that promote healthy aging and extend a period free from chronic diseases and disabilities, what we label as the "health-span". This thesis aims to provide a summary of current knowledge on aging processes while delving into possible interventions that hold promise in allowing individuals to „die young at an old age".

Furthermore, this literature research is driven by an increasing understanding that it may be possible to decelerate or even reverse the process of aging. The first section of the thesis provides an overview of aging while offering a precise definition for better comprehension. This section also underlines not only the significance of aging research, but also stresses on finding interventions that promote healthy aging. Following this introduction are multiple theories that shed light on how our bodies age such as the free radical theory, the hormonal stress theory, the telomere theory, the inflammation and the mitochondrial theory which explore underlying mechanisms of aging. The next section shifts towards exploring different pathways and processes associated with aging, thereby investigating specific mechanisms such as: the target of rapamycin (TOR), sirtuins and NAD+ involved in the longevity process, circadian clocks, senescence, insulin-like signaling pathway which plays pivotal role in aging process, proteostasis, autophagy, as well as AMP-activated protein kinase (AMPK) pathway. The next section of this thesis focuses on drugs currently undergoing clinical trials due to their potential of anti-aging effects. The examination specifically considers each drugs possible benefits and drawbacks while highlighting their efficacy as interventions that can promote healthy aging. These drugs encompass: rapamycin, senolytics, metformin, sirtuin activators (resveratrol) and NAD+ precursors. Additionally, this section delves deeply into promising approaches for promoting healthy aging. These approaches include the reactivation of telomerase, elimination of damaged cells, activation of chaperones and proteolytic systems, stem cell-based therapies, mitochormetics and mitophagy as well as the use of anti-inflammatory drugs and blood-borne rejuvenating factors. Furthermore, this thesis investigates the impact of nutrition on aging biology. The focus centers on

understanding how caloric restriction, macronutrients (protein, fats and carbohydrates), micronutrients (vitamins and minerals), along with specific dietary patterns within the aging process are being explored. Additionally, this thesis also explores how gut microbiota affects aging processes consciously while also discussing potential future research avenues worth considering. Lastly but just as importantly, the role of exercise in relation to the biological aspects of aging is examined. This exploration analyzes how exercise relates to hormonal balance while noting possibilities for future investigation into how exercise specifically contributes towards healthy aging. Ultimately, the thesis concludes current understandings pertaining to mechanisms underlying aging along with promising interventions with potential to foster healthy aging. The review effectively stresses on retaining a mindful approach towards maintaining a healthy diet, engaging in regular physical activity whilst making a conscious effort not to engage in detrimental habits.

# Zusammenfassung

Es ist unbestreitbar, dass die menschliche Lebenserwartung ständig zunimmt. Doch die bloße Verlängerung unserer Lebenszeit wird unseren individuellen Wünschen nicht gerecht. Der Wunsch der meisten von uns geht weit über die Langlebigkeit hinaus. Wir sehnen uns danach, dass diese zusätzlichen Jahre von Erfüllung und vor allem von guter Gesundheit geprägt sind. Infolgedessen besteht ein wachsendes Interesse an der Ermittlung von Maßnahmen, die ein gesundes Altern fördern und einen Zeitraum ohne chronische Krankheiten und Behinderungen verlängern, was wir als "Gesundheitsspanne" bezeichnen. Ziel dieser Arbeit ist es, einen Überblick über den aktuellen Wissensstand zu Alterungsprozessen zu geben und gleichzeitig mögliche Interventionen zu erforschen, die es dem Einzelnen ermöglichen, "im Alter jung zu sterben".

Darüber hinaus wird diese Literaturrecherche von der zunehmenden Erkenntnis getragen, dass es möglich sein könnte, den Alterungsprozess zu verlangsamen oder sogar umzukehren. Zu Beginn der Arbeit wird ein Überblick über das Altern gegeben und zum besseren Verständnis eine genaue Definition angeboten. In diesem Abschnitt wird nicht nur die Bedeutung der Alternsforschung hervorgehoben, sondern auch die Suche nach Interventionen, die ein gesundes Altern fördern. Im Anschluss an diese Einführung werden mehrere Theorien vorgestellt, die Aufschluss darüber geben, wie unser Körper altert, z. B. die Theorie der freien Radikale, die Hormontheorie, die Telomertheorie und die Mitochondrientheorie, die die zugrunde liegenden Mechanismen des Alterns untersuchen. Der nächste Abschnitt befasst sich mit verschiedenen Signalwegen und Prozessen, die mit dem Altern verbunden sind, und untersucht dabei spezifische Mechanismen wie Rapamycin, Sirtuine und NAD+, die am Langlebigkeitsprozess beteiligt sind, zirkadiane Uhren, Seneszenz, den insulinähnlichen Signalweg, der eine zentrale Rolle im Alterungsprozess spielt, Proteostase, Autophagie sowie den AMP-aktivierten Proteinkinase (AMPK)-Signalweg. Der nächste Abschnitt dieser Arbeit befasst sich mit Medikamenten, die derzeit aufgrund ihrer potenziellen Anti- Aging- Wirkung klinisch erprobt werden. Dabei werden die möglichen Vor- und Nachteile der einzelnen Medikamente untersucht und ihre Wirksamkeit als Mittel zur Förderung des gesunden Alterns hervorgehoben. Zu diesen Medikamenten gehören: Rapamycin, Senolytika, Metformin, Sirtuin- Aktivatoren (Resveratrol) und NAD+- Vorstufen. Darüber hinaus

befasst sich dieser Abschnitt eingehend mit vielversprechenden Ansätzen zur Förderung des gesunden Alterns.

Zu diesen Ansätzen gehören die Reaktivierung der Telomerase, die Beseitigung geschädigter Zellen, die Aktivierung von Chaperonen und proteolytischen Systemen, stammzellbasierte Therapien, mitochondriale Hormetik, Mitophagie sowie die Verwendung entzündungshemmender Medikamente und blutbasierter Verjüngungsfaktoren. Darüber hinaus werden in dieser Arbeit die Auswirkungen der Ernährung auf die Biologie des Alterns untersucht. Der Schwerpunkt liegt auf dem Verständnis, wie Kalorienrestriktion, Makronährstoffe (Proteine, Fette und Kohlenhydrate), Mikronährstoffe (Vitamine und Mineralien) und spezifische Ernährungsmuster den Alterungsprozess beeinflussen. Darüber hinaus wird in dieser Arbeit auch untersucht, wie die Darmmikrobiota den Alterungsprozess bewusst beeinflusst, und es werden potenzielle zukünftige Forschungsmöglichkeiten erörtert, die in Betracht gezogen werden sollten. Schließlich, aber ebenso wichtig, wird die Rolle der Bewegung in Bezug auf die biologischen Aspekte des Alterns untersucht. Dabei wird analysiert, wie Bewegung mit dem Hormonhaushalt zusammenhängt, und es werden Möglichkeiten für künftige Untersuchungen darüber aufgezeigt, wie Bewegung speziell zum gesunden Altern beiträgt. Schließlich werden in dieser Arbeit die aktuellen Erkenntnisse über die dem Altern zugrunde liegenden Mechanismen sowie vielversprechende Interventionen zur Förderung des gesunden Alterns zusammengefasst. Der Bericht betont, dass es wichtig ist, einen achtsamen Ansatz für eine gesunde Ernährung zu verfolgen, sich regelmäßig körperlich zu betätigen und gleichzeitig bewusst darauf zu achten, keine schädlichen Gewohnheiten zu pflegen.

## Introduction

The extension of human life has fascinated scientists and researchers for centuries. Advances in molecular biology and genetics have intensified efforts to understand the complex mechanisms underlying aging. Decoding the human genome provides further insight into this process (Collins et al., 2003) and opens new avenues for exploration like anti-aging therapies, genetic modifications and caloric restriction diets (Lopez-Otin et al., 2013). Numerous discoveries in many aging processes and mechanisms in recent years have not only advanced our understanding of the complex biology of aging but have significantly contributed towards providing information for the future assessment and treatment that can be used to help individuals maintain good quality of life as they age (Kaeberlein et al., 2016).

# Aging

## What is aging?

Aging is a complex process that can result in declining function over time leading also to fertility reduction and increased mortality as individuals age (Kirkwood et al., 2000). The survival and reproductive abilities of individuals raise several questions regarding its evolutionary origins which researchers have been investigating for better understanding (Kirkwood et al., 2000). Although aging is common among different species, it is not universal which suggests that it is not just pure result of biological wear-and-tear (Kirkwood et al., 2000).

Lopez-Otin et al. (2013) along with his colleagues proposed a framework termed “hallmarks of aging” aimed at enhancing comprehension behind the aging process. The motive behind creating such a framework was to enable better understanding about this natural event. This framework comprises nine features including alterations in intercellular communication, genomic instability, telomere attrition, epigenetic pattern alterations, disruption of protein homeostasis, impaired nutrient

sensing, mitochondrial dysfunction, cellular senescence along with exhaustion of stem cells (see Figure 1). These hallmarks provide insights into the physiology behind aging and offer clues to interventions that can slow down or even reverse aging (Lopez-Otin et al., 2013).

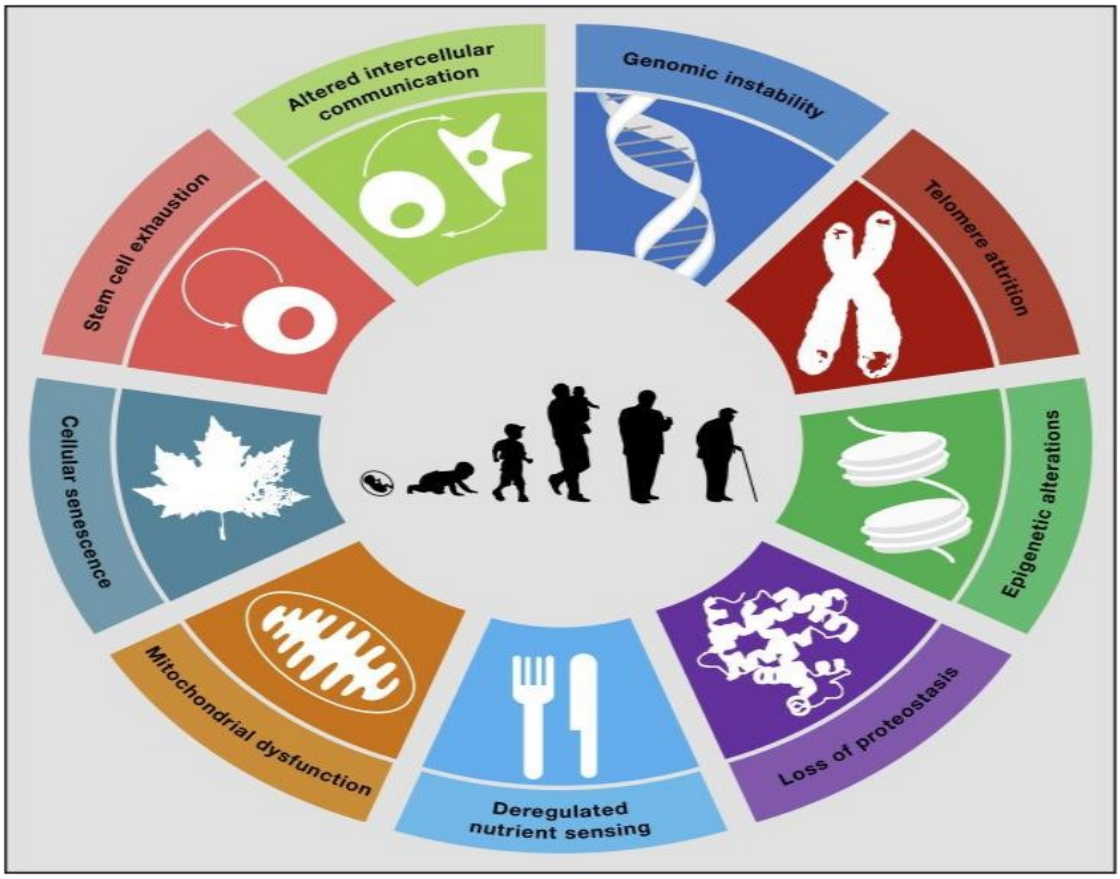


Figure 1. The Hallmarks of Aging.According to Lopez-Otin et al. (2013), these mechanisms outlined serve as the fundamental factors that primarily contribute to the process of cellular aging. (López-Otín, C., Blasco, M.A., Partridge, L., Serrano, M., & Kroemer, G., "The Hallmarks of Aging," Cell, 2013, Volume 153, Issue 6, pp. 1194–1217. Reprinted with permission from Elsevier.)

## The importance of aging research

A greater number of people worldwide are living longer than ever before. However, despite this good news, they are still likely to face physical and cognitive decline which can result in increased numbers of illnesses such as Alzheimer's disease, heart disease, cancer or stroke (Jin et al., 2014). Governments across the world

along with NGOs (non-governmental organizations) and international organizations are attempting new measures to mitigate socioeconomic and health challenges imposed by increasingly aged demographics, including increasing healthcare costs, a reduced workforce and sustain the quality of life for the elderly (Jin et al., 2014). Recent advances in gerontology examining the relationship between aging and disease have shown that longevity interventions in model organisms can also increase health span, which refers to life lived in good health (Kennedy et al., 2014). Managing an aging society involves understanding its root causes and designing interventions that promote better health outcomes while reducing economic burdens associated with complex chronic diseases (Kennedy et al., 2014)(see Figure 2). The field of aging research has immense potential to enhance the health and well-being of individuals as they age. By delving deeper into this subject, we can revolutionize the treatment of chronic diseases and improve overall health in older populations. What was once merely a subject of fascination has evolved into a legitimate area of scientific inquiry that holds the power to transform healthcare and greatly improve quality of life for the elderly (Kennedy et al., 2014).

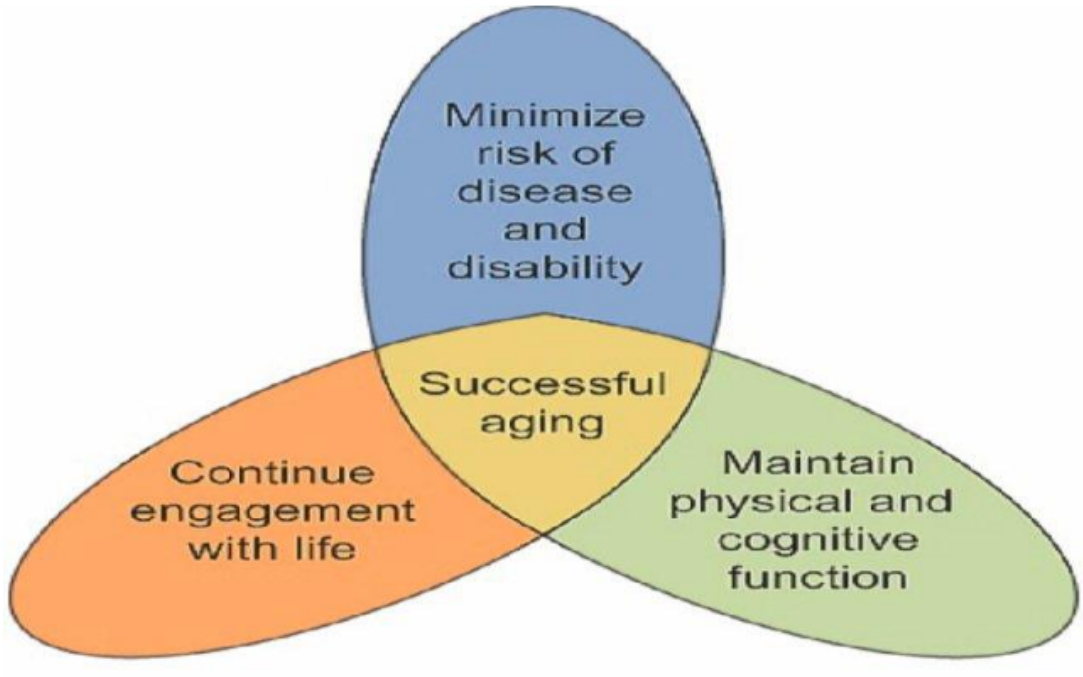


Figure 2. The Rowe and Kahn's successful aging model in 1997. (Rowe, J.W. & Kahn, R.L., "Successful Aging[1]," The Gerontologist, 1997, Volume 37, Issue 4, pp. 433–440. Reprinted with permission from Oxford University Press.)

## Theories of Aging

In an attempt to comprehend how our bodies age over time, scientists have proposed several theories including the free radical theory, the telomere theory, the mitochondrial theory, the inflammation theory and the hormonal theory.

## Free radical theory of aging

Dr. Harman proposed his Free Radical Theory of Aging in 1956 suggesting that the accumulation of free radicals overextended period causes damage of biomolecules and the development to pathological disorders resulting into cellular senescence and finally ending at organismal aging (Harman et al., 1956).

Though Free Radical Theory may encounter skepticism in some scientific communities, many researchers worldwide still accept it as one relevant explanation for why our bodies age. In brief, Free Radical Theory explains that accumulation of free radical- induced damage to cellular components and is associated in aging-related diseases and getting older. Mitochondria are considered the primary producers of ROS thus they play a central role in this theory (Finkel et al., 2000). Advancing our knowledge about how mitochondria are involved might reveal more solutions aimed at minimizing or evading age-associated issues (Finkel et al., 2000).

## The telomere theory of aging

The repetitive nucleotide-based (TTAGGG)n sequences located at the ends of chromosomes are known as telomeres (Blackburn et al., 2015). After DNA replication, cell machinery creates identical copies of DNA; however, replication problems cause telomeres to shorten rather than losing genetic information,

eventually reaching a critical length triggering cellular senescence referred to as Hayflick's limit (Olovnikov et al., 1996) (see Figure 3). Although germline and many cancer cells possess an active telomerase enzyme for maintaining their length, most somatic cells lack this ability consequently causing a decline in Telomere Length (TL) reflecting to cellular aging (Blackburn et al., 2015). Based on the telomere theory of aging, telomeres which are the protective coverings on chromosomes, experience a gradual decrease resulting in age-related diseases such as cancer and heart disease (Blackburn et al., 2006). Moreover, Harley et al.'s research from 1990 demonstrated that human fibroblasts' amount and length of telomeric DNA decrease as they age through serial passaging in vitro and possibly in vivo causing cellular senescence. Moreover, von Zglinicki et al. in 2002 examined the relationship between oxidative stress and shortened telomeres discovering that oxidative stress increases the rate of telomere length loss while antioxidants have an opposite effect. This is due to the inefficient repair of oxidative damage in telomeric DNA compared to other chromosome regions (von Zglinicki et al., 2002). The research suggests that replicative senescence driven by telomeres may primarily serve as a stress response evolved to prevent cell growth exposed to high risks of mutation. In 2004 a study by Epel et al. (2004) revealed that chronic stress led to accelerated cellular aging in healthy premenopausal women. This was shown by increased oxidative stress levels, lower telomerase activity and shorter telomeres. Such findings suggest that this type of psychological distress could cause early onsets for illnesses during one's lifetime at a cellular level. Additionally, telomere dysfunction can activate p53, which represses master regulators of mitochondrial physiology and metabolism known as PGC-1α and PGC-1β. This repression leads to impaired mitochondrial biogenesis and function, decreased gluconeogenesis, cardiomyopathy, and increased reactive oxygen species (ROS) (Sahin et al., 2011). Altogether, the telomere theory of aging provides pivotal understanding regarding biological processes governing the aging process while also giving us valuable insights into age-related diseases.

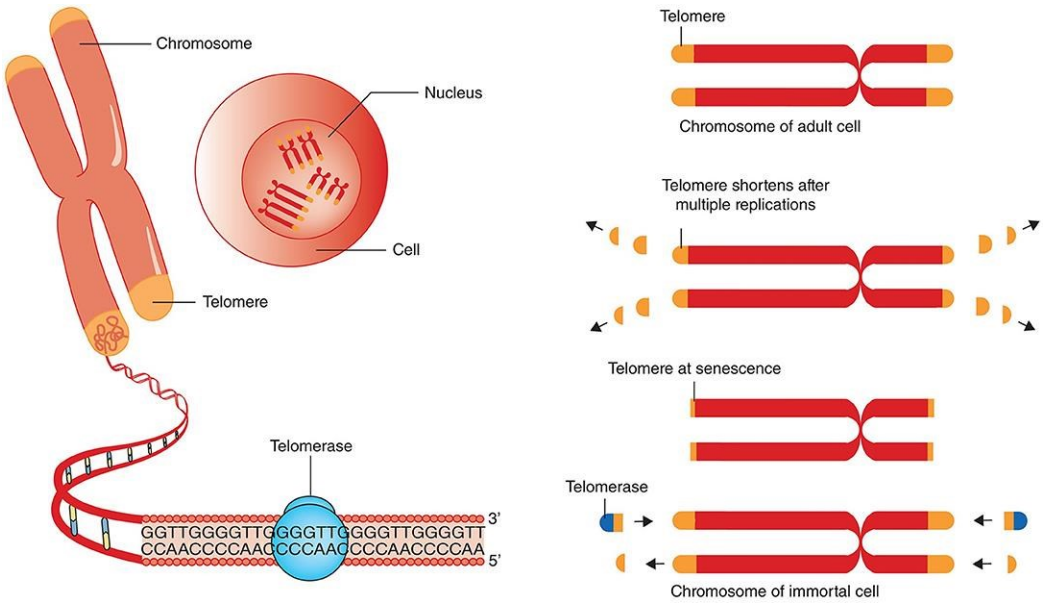


Figure 3. Aunan et al. (2016) illustrates the relationship between telomere attrition, telomere length, and telomerase. Chromosomes contain repetitive segments called telomeres that become shorter with each cell division. Telomerase, an enzyme, can extend these telomere ends, prolonging cellular lifespan and potentially leading to cell immortality, a characteristic of cancer cells. (Aunan, J.R., Watson, M.M., Hagland, H.R., & Søreide, K.S., "Molecular and biological hallmarks of ageing," British Journal of Surgery, 2016, Volume 103, Issue 2, pp. e29-e46. Reprinted with permission from Oxford University Press.)

## The hormonal stress theory of aging

Hormonal Stress Theory, also termed as Neuroendocrine Theory of Aging, implies that aging is a consequence of hormonal changes along with increasing stress responses over time (Dilman et al., 1992). The authors have suggested several instances where hormonal imbalances come with aging. They explain how declining hypothalamic sensitivity may lead towards negative cortisol reactions in both hypothalamus and pituitary gland resulting to a more prolonged reaction to stress within aging adults. Also, levels of the hormone aldosterone, secreted by adrenal glands, are seen to reduce as one ages predisposing them towards adverse effects including hypoaldosteronism. This suggests that serum concentrations of adrenal gland hormones such asdehydroepiandrosterone (DHEA) and dehydroepiandrosterone sulfate (DHEAS), serving as active precursors for estrogens and active androgens respectively decrease at old age but whether this can be linked with pathophysiology remains uncertain (Dilman et al., 1992). In his 2005 proposal on "multiple hormone deficiency", Hertoghe suggests that human

senescence occurs mainly due to a decline in most hormones in production, levels and cell receptors as individuals grow older. This hormonal decrease causes an imbalance within the endocrine system leading to an alteration in nycthemeral hormone cycles which contribute to several signs, symptoms, and diseases linked with the aging process including cardiovascular disorders, cancer, obesity, diabetes, development of osteoporosis, occurrence or susceptibility towards mental condition like dementia etc. Furthermore, the research suggests that hormonal deficiencies might also lead towards other presumed factors related to aging including formation of free radicals, glycation, protein cross-linking, waste product accumulation and immunodeficiency (Hertoghe et al., 2005).

Vance (2003) examines whether or not administering growth hormones (GH) can combat aging, referring to Rudman et al.'s research from 1990 where twelve older men were given human GH for six months. The positive results of this study gave rise to several anti-aging clinics and publications proclaiming the benefits of GH in halting or reversing the effects of old age. Recent findings have brought significant advancements in our understanding of how GH affects aging processes, shedding important new light on this crucial topic. One study conducted by Bartke et al. in 2021 demonstrated that there are considerable differences in genome maintenance (DNA damage and repair) between normal and cancerous cells exposed to GH treatment, which reveals significant health implications like progression of cancer development and age acceleration associated with this hormone use. Hence, we need comprehensive knowledge regarding how hormones like these impact overall aging and disease pathogenesis as the hormonal stress theory suggests.

## The inflammation theory of aging

As people age, they experience an increase in pro-inflammatory factors coupled with a decrease in their bodies' ability to cope with stressors, a phenomenon commonly referred to as "inflamm-aging" (Franceschi et al., 2000). Franceschi and Campisis' (2014) research on this subject finds that the chronic, low-level inflammation is a defining feature of the aging process in humans and represents a substantial contributor to the development of age-related diseases. Despite acknowledging the importance of understanding this phenomenon better, it remains

unclear precisely what causes inflamm-aging or its role affecting adverse health outcomes. Consequently, investigations and identifications into various pathways that control age-related inflammation across different systems are essential to determine if treating inflamm-aging leads to better results for older adults (Franceschi et al., 2014). Researchers have identified several potential factors that contribute to this aging-related phenomenon including damaged macromolecules and cells, detrimental microbial products, cellular senescence, coagulation system activation, immune impairment with age and defective regulation of the complement pathway. While this inflammatory response could be beneficial reinforcing existing physiological states such as normal tissue remodeling, it could also become disruptive or unhealthy when excessively activated over time due to reduced anti-inflammatory responses (Franceschi et al., 2014).

Although we know that circulating pro-inflammatory molecules are predictive of age-related diseases, there is ongoing debate regarding whether the contribution of systemic or local sources of chronic inflammation to chronic disease processes play a more significant role in driving these outcomes. Further research is required to determine the relative importance of systematic factors compared to the importance of local factors in causing age-related diseases (Franceschi et al., 2014).

In brief, according to the Inflammation Theory of Aging, persistent or constant inflammations contribute towards cellular damages leading to aging which has been backed by scientific research demonstrating the linkage between chronic inflammation and age-related diseases. A comprehensive analysis is required to fully grasp the relationship between chronic inflammation and aging and also find interventions which foster healthy aging (Franceshi et al. 2014).

## The mitochondrial theory of aging

The Mitochondrial Theory of Aging posits that damaged mitochondria accumulate throughout one's life leading to the resultant aging phenotype due to compromised energy budgets (Kowald et al., 2001). Mitochondria are the key source of energy for most eukaryotic cells, but also produce harmful substances called free radicals which make these structures unique players. For instance, these reactive molecules can harm cell components such as proteins, membranes and even DNA itself,

which overall play roles in the biological aging processes. Moreover, they enclose their own genetic material known as mtDNA but lack robust DNA repair methods, therefore making them prime targets for reactive oxygen species (Kowald et al., 2001). Chistiakov et al.s' 2014 research indicates that biological aging can be attributed significantly to age-related changes in mitochondria and mitochondrial dysfunction. As individuals grow old, there is an accumulation of mutations in mitochondrial DNA (mtDNA), which results in decline in both functionality as well as integrity of mitochondria due to reactive oxygen species (ROS). Reduced oxidative capacity, lowered oxidative phosphorylation capability, decreased ATP production values, increased ROS generation levels and diminished antioxidant defense rates are some consequences related to impaired mitochondrial functions in older individuals (Chistiakov et al., 2014).

As people grow older, mitochondrial biogenesis declines and the maintenance of proper mitochondrial quality control becomes difficult. Consequently, mitochondrial function is also compromised. Moreover, aged tissues experience heightened levels of mitochondria-mediated apoptosis, which leads to more apoptotic cells (Chistiakov et al., 2014). Nevertheless, there are still approaches like practicing caloric restriction or daily physical training that benefit and slow down these mitochondrial aging phenotypic effects in humans (Chistiakov et al., 2014). More research is necessary to attain a better understanding regarding how these mitochondrial processes relate towards various diseases associated with getting old while exploring methods for preventing or treating them successfully. Targeting mitochondrial function could be helpful towards increasing health span along with lifespan (Chistiakov et al., 2014).

In summary, these diverse theories of aging give us different perspectives regarding aging and provide us with insights into how we can unravel this intricate process of aging further. Although none of them alone is enough to explain every aspect related to ageing completely, together they highlight many different biological factors implicated in both aging itself as well as its associated age-related disorders. Researchers are continually exploring ways that might possibly be utilized to slow down or even reverse some effects caused by aging. Understanding the mechanisms of aging is vital in order to develop effective interventions targeted at improving people's general wellbeing and quality of life during their golden years.

## Aging Pathways and Processes

Aging Pathways and Processes refer to the different biological processes that occur in the body as we age. These pathways and processes are critical to understanding the biology of aging and how to potentially slow down the aging process. The main pathways and processes involved in ageing include the Insulin-Like Signaling Pathway, Target of Rapamycin (mTOR), Sirtuins and NAD+, Circadian Clocks, Autophagy, Senescence, Proteostasis and AMPK pathway.

## Insulin-Like Signaling Pathway

Insulin is a peptide hormone that is produced by beta cells within the pancreas in the islets Langerhans and plays a crucial role in managing essential biological processes such as regulating liver metabolism by binding to insulin receptors on hepatocyte plasma membranes resulting in the activation of insulin receptor substrates (IRS) (Escribano et al., 2017). The IGF (insulin-like growth factor) and insulin (INS) system IIS constitutes a complex network of proteins that modulate diverse cellular processes based on cell type and context (Massoner et al., 2010).The proteins IGF and INS act as growth hormones in autocrine, paracrine and endocrine ways, being expressed throughout the human body and having a significant impact on tissue and cell growth, differentiation and homeostasis. Moreover, these proteins are linked to a wide range of processes including apoptosis, metabolism, survival, angiogenesis, migration, wound healing and - interestingly enough - also play a role in brain functions like memory and behavior (Massoner et al., 2010). Thanks to initial research conducted in Caenorhabditis elegans (C. elegans), this pathway is evolutionary conserved and has been related to longevity across various species including humans (Kenyon et al., 2010) (see Figure 4). The identification of this aging pathway advances our knowledge on the aging process and opens doors toward potential methods for managing age-related changes (Kenyon et al., 2010). A study conducted by Longo et al. (2002) illustrates that inactivating glucose or insulin/insulin-like growth factor-1 (IGF-1) signaling pathways can elongate lifespans through transitioning organisms from reproduction

stage into non-reproductive maintenance stage. This process is supported by stress resistance pathways which are essential in enhancing longevity when food availability becomes limited. The fact that these regulatory pathways exhibit similarities across different species, suggests that insulin/IGF-1 signaling is a critical factor in controlling cellular damage and lifespan for humans as well (Longo et al., 2002). Furthermore, dysregulation of the ILS pathway can result in multiple illnesses like metabolic syndrome, diabetes, cancer and obesity (Longo et al., 2002). In summary, the ILS pathway plays an important role in preserving metabolic homeostasis, cellular maintenance and repair and prevents age-related diseases. Consequently, unraveling the regulation of this pathway and finding ways to modulate it could truly make a difference in promoting healthy aging.

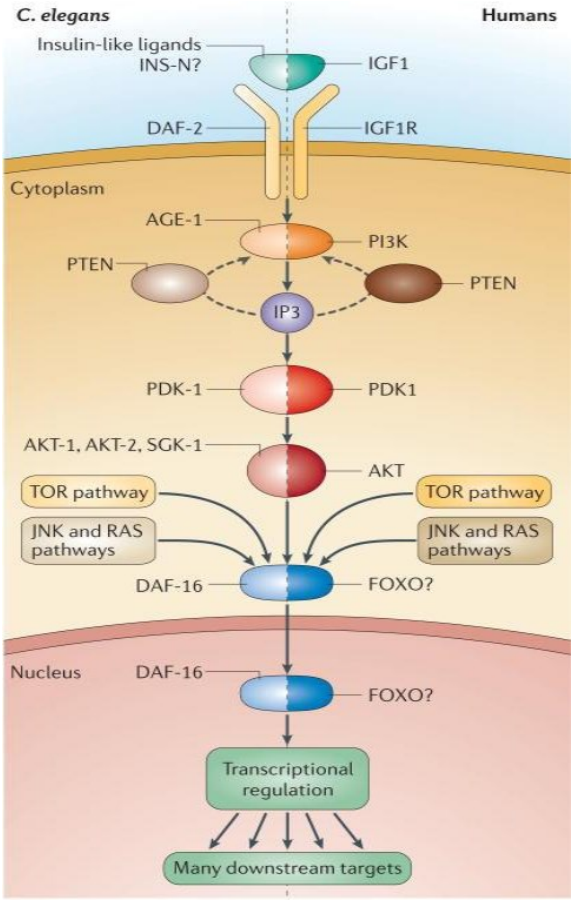


Figure 4. Illustrates the insulin/insulin-like signaling pathway in both C. elegans and humans. In C. elegans, a mutation in the DAF-2 gene results in an extended lifespan, while a mutation in the DAF-16 gene leads to a shortened lifespan. (Christensen, K., Johnson, T.E., & Vaupel, J.W., "The quest for genetic determinants of human longevity: challenges and insights," Nature Reviews Genetics, 2006, Volume 7, pp. 436-448. Reprinted with permission from Springer Nature.)

## Target of rapamycin (TOR)

The crucial role played by the TOR signaling pathway towards aging and its potential to better health span in human beings has been identified (Kapahi et al., 2010). The TOR proteins are notably involved in various processes such as initiating and extending translation phases, producing ribosomes, importing essential amino acids, transcribing multiple enzymes involved in a plethora of metabolic pathways and regulating autophagy (Raught et al., 2001) (see Figure 5). An array of studies investigating diverse model organisms ranging from yeast to mammals have consistently found evidence supporting the concept that TOR signaling network significantly impacts the aging processes (Kapahi et al., 2010). Furthermore, it is widely believed that this pathway is a central mediator of the protective effects observed with dietary restriction (DR) in terms of delaying the onset or reducing the severity of age-related diseases while prolonging life expectancy in numerous species (Kapahi et al., 2010).

In 2010, Kapahi et al.'s study focused on exploring how adjustments made to the TOR signaling network could help slow down aging. Their findings suggested that targeting downstream mechanisms such as mRNA-translation, autophagy, endoplasmic reticulum, stress signaling, stress response systems and metabolic processes can serve as potential targets for drugs aimed at enhancing lifespan while also slowing down age-related diseases. Furthermore, components like S6K, TOR and AMPK offer an underlying foundation for more extensive research. However, the researchers emphasized that further investigation must be done on the influence of various nutrients regarding DR in different species to fully comprehend TOR's role in lifespan extension (Kapahi et al., 2010).

Identifying the critical downstream-effectors of TOR and understanding the communication between different tissues in determining organismal lifespan remain challenges in the field. Other pathways modulating aging, seemingly independent of the TORC1-complex, are also being discovered. The main challenge of aging research is to create a unified understanding of the mechanisms that determine aging. Continued examination of TOR and other conserved pathways will help to shed light on the connection between diet and age-related diseases, such as cancer, neurodegeneration and diabetes in humans (Kapahi et al., 2010). TOR has been implicated in the regulation of both innate and adaptive immune responses (Soliman, 2013). Some inhibitors of the TOR pathway have already received clinical

approval, while others are under development. Although side effects prevent their use in healthy individuals, drugs targeting the TOR pathway may eventually be widely adopted to slow aging and reduce age-related pathologies in humans (Johnson et al., 2013).

The TOR pathway is a highly promising target for interventions towards supporting healthy aging and reducing age-related diseases that could potentially impact the quality of life among elderly populations.

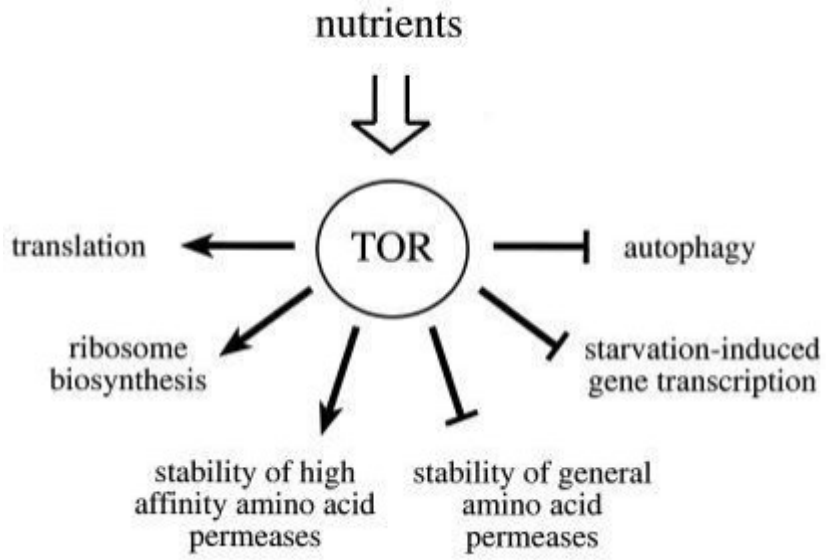


Figure 5. Illustrates that TOR signaling is situated downstream of nutrient signaling and plays a crucial role in maintaining the equilibrium between protein synthesis and degradation. (Raught B., Gingras A.C., Sonenberg N., "The target of rapamycin (TOR) proteins," Proceedings of the National Academy of Sciences of the United States of America, 2001, Volume 98, Issue 13, pp. 7037-7044. Reprinted with permission from Oxford University Press.)

## The AMP-activated protein kinase (AMPK) pathway

As an integral component of the protein kinase cascade, the AMPK is a vital element that can phosphorylate and inactivate fundamental regulatory enzymes across multiple biosynthetic pathways (Corton et al., 1994) (see Figure 6). The AMP (Adenosine Monophosphate)-activated protein kinase (AMPK) acts as a vital function in maintaining the balance of cellular energy in eukaryotic cells through the monitoring of intracellular adenine nucleotide levels. Specifically, it senses to an

increase in AMP/ADP(Adenosine Diphosphate) compared to ATP(Adenosine Triphosphate) (Carling et al., 2017).AMPK activation is believed to play a crucial role in enhancing catabolic pathways responsible for producing ATP and reducing anabolic (ATP-utilizing) pathways. Consequently, this delicately regulates the overall energy metabolism of one's body (Carling et al., 2017). Holistic improvement of an individual's health span and lifespan can be achieved through effective management of energy metabolism, enhancing resistance against stress and maintaining cellular cleanup mechanisms (Salminen et al., 2012). Emerging evidence indicates that boosting the activity level of AMP-activated protein kinase (AMPK) has enormous potential in extending lifespan among lower organisms. Further research conducted on mammals supports this claim indicating that this protein plays key roles in regulating autophagy through the signaling pathways of mTOR and ULK1 (Salminen et al., 2012). Risks arise with advancing age, whereby researchers have noticed reduced efficiency within this signal pathway leading to impaired metabolic regulation while also increasing oxidative stress levels as well as reduced autophagic clearance leading to low-grade inflammation and several metabolic disorders (Salminen et al., 2012). Regular exercise, caloric reduction and the administration of metformin, along with various natural products offer positive health benefits typically observed from increased AMPK activity (Steinberg et al., 2019). Despite these known effects and benefits, developing direct AMPK activators remains challenging for many researchers currently working in the field (Steinberg et al., 2019).

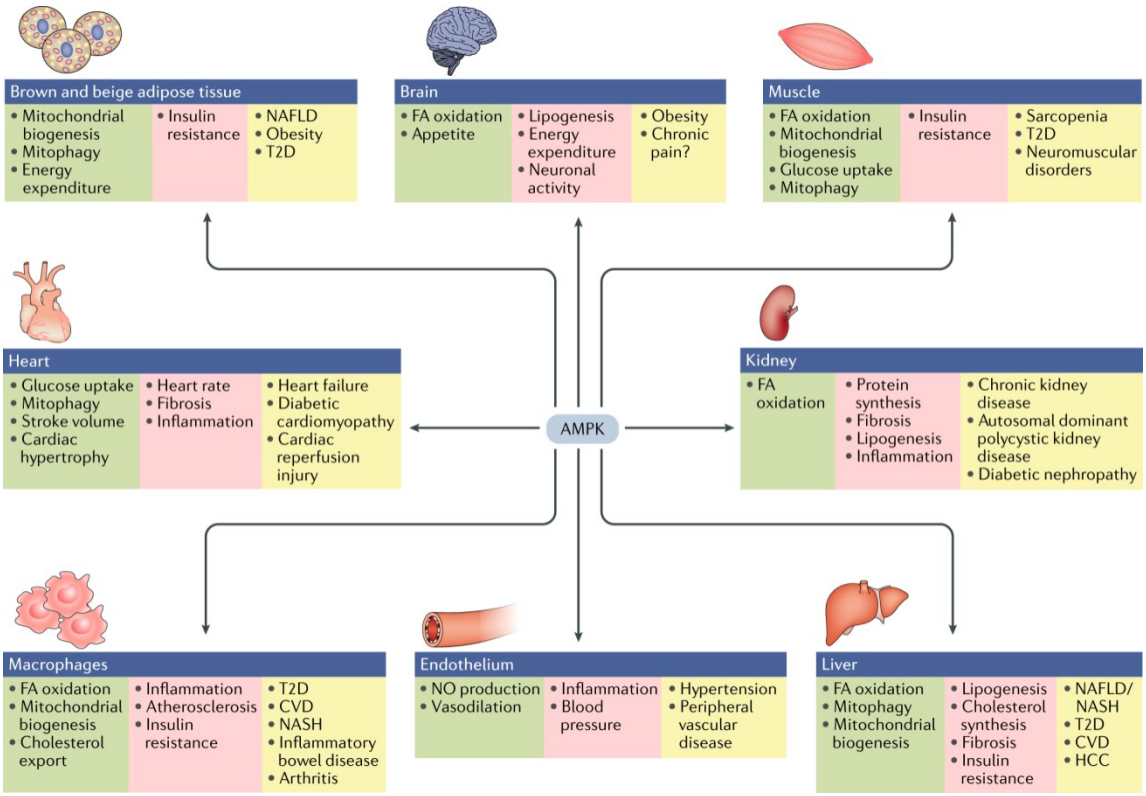


Figure 6. AMP-activated protein kinase (AMPK) plays a crucial role in the regulation of various cellular processes that have associations with human diseases. (Steinberg, G.R. & Carling, D., "AMP-activated protein kinase: the current landscape for drug development," Nature Reviews Drug Discovery, 2019, Volume 18, pp. 527-551. Reprinted with permission from Springer Nature)

## Sirtuins and NAD+

According to Amjad et al. (2021), nicotinamide adenine dinucleotide (NAD+) is a coenzyme that can be found within every living cell and bears considerable significance for numerous metabolic processes related to cellular bioenergetics. Given its relevance, this compound has garnered substantial attention in investigations concerning age-linked conditions along with oncological diseases, neurodegenerative ailments, as well as metabolic disorders (see Figure 7). Furthermore, it has been suggested that NAD+ serves an active role when it comes in regulating energy metabolism, DNA damage repair, gene expression, while also responding adequately when cellular stress occurs. Intriguingly enough though stands out the fact that there is an observable decrease of NAD+ levels throughout aging in multiple organisms, including humans themselves - which appears strongly

linked to many aging related diseases such as cognitive decline, cancer, frailty and sarcopenia (Covarrubias et al., 2021). Replenishing the low NAD+ levels has potential benefits for slowing down age-related diseases and even reverses many of these diseases. However, the safety and effectiveness of the molecular mechanisms regulating and restoring NAD+ levels and whether replenishing NAD+ levels will have beneficial effects to humans must also be examined (Covarrubias et al.,2021). Mouchiroud et al. (2013) study has shown the functional significance of the NAD+/Sirtuin pathway in regulating longevity. Critically, their study also observed that aging causes a depletion of cellular NAD+ levels on C.elegans and mice, lowering its level leads to a decline in overall lifespan, especially in C.elegans. The researchers however discovered that they could maintain metabolic rates and lengthen the lifespan of worms by reintroducing NAD+ levels. This was effectively achieved and sustained through either genetic or pharmacological means. NAD-dependent protein deacetylase sir-2.1was observed to be integral to this longevity effect.

The whole process starts with an imbalance in mitonuclear proteins, triggering stress signaling via UPRmt (mitochondrial unfolded protein response) and the activation and movement of the DAF-16 FOXO transcription factor in the nucleus. Consequently, increasing mitochondrial stress signaling elicited through modulation of NAD+ may slow down age and significantly enhance mitochondrial function. Researchers also suggest that sirtuin activity may be a key intervening factor for delayed aging. Overall, manipulating NAD+ availability through PARP activity is revealed as a crucial regulatory factor not only for mitochondrial biogenesis but also general health, contributing to organismal longevity across species. It was observed that when NAD+ levels were reduced through chemical methods or genetic manipulation it led to a decrease in the lifespan of worms. Conversely, they found that implementing strategies to preserve NAD+ levels effectively inhibited the aging process and extended the lifespan of C. elegans. To increase NAD+ levels they adopted two distinct approaches. The initial method involved employing mutation/RNAi (pme-1) or inhibiting the PARP enzymes (AZD2281 or ABT-888) responsible for NAD+ consumption. The second approach was to supplement with NAD+ precursors (NR or NAM) (Mouchiroud et al., 2013). The researchers concluded that NAD+/sirtuin activity leads to UPRmt, a SIRT1 signaling pathway, that alongside the established daf-16/sod-3 antioxidant defense, improves metabolic health and extends lifespan. This NAD+/SIRT1/UPRmt/SOD signaling pathway could potentially be used preventively or therapeutically in the context of

aging and aging-related disorders (Mouchiroud et al., 2013). However, given the varied distribution of NAD+/NADH within cells, the direct impact of impaired NAD+ dependent processes in humans is yet to be conclusively established. Future research is required to measure the fluctuations through pathways associated with NAD+ synthesis and degradation. Current human clinical trials aim to determine the benefits of NAD+ restoration using NAD precursors (Amjad et al., 2021).

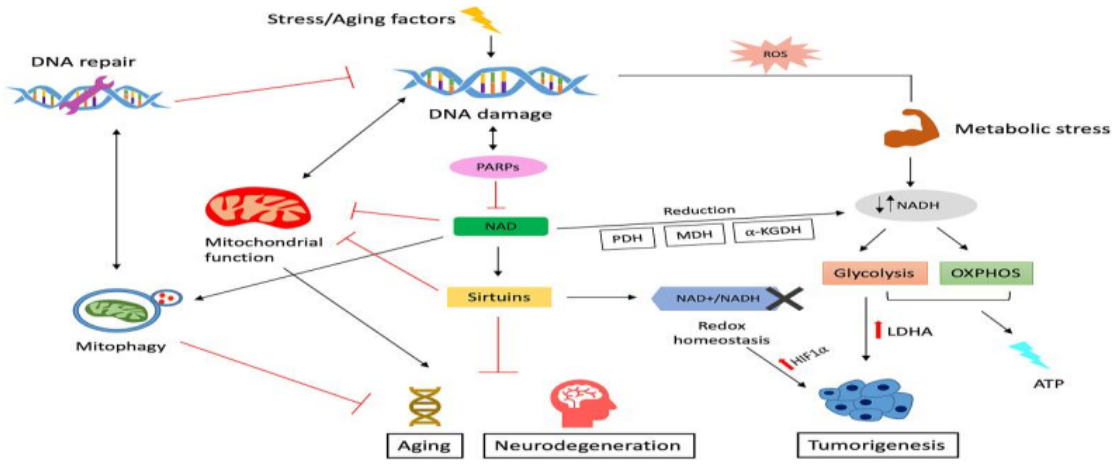


Figure 7. The involvement of NAD in aging, neurodegeneration, and cancer has been extensively studied (Amjad, S., Nisar, S., Bhat, A.A., Shah, A.R., Frenneaux, M.P., Fakhro, K., Haris, M., Reddy, R., Patay, Z., Baur, J., & Bagga, P., "Role of NAD+ in regulating cellular and metabolic signaling pathways," Molecular Metabolism, 2021, Volume 49, Article 101195. Reprinted with permission from Elsevier.)

## Circadian clocks

The circadian clock in our bodies is the one that regulates twenty-four hours of our physiological and behavioral rhythms, unfortunately aging impacts this internal system due to its decline in function over time (Kondratova et al., 2012). Kondratova et al. (2012) explains that disrupted circadian functions are correlated with severe sleep or neurodegenerative disorders. Research findings suggest that circadian clock dysfunction may contribute to aging and age-related pathologies, which indicates potential correlations between the deterioration of brain function as we grow older (Kondratova et al., 2012). Further, possible molecular mechanisms

behind this correlation involve the circadian regulation of various physiological processes, such as hormone secretion, stem cell proliferation, autophagy and reactive oxygen species homeostasis, which all follow a strict cycle under the regulation of these clocks (Kondratova et al., 2012). In organisms such as humans this internal system helps regulate metabolic and endocrine rhythms which are vital for maintaining homeostasis (Asher et al., 2015).

## Autophagy

Autophagy has come a long way since its initial identification as the bulk recycling process that breaks down intracellular material within lysosomes (see Figure 8). It is now recognized as an important player concerning metabolic and proteostatic signaling. Its role can be seen in influencing cell fate and organismal lifespan (Wong et al., 2020). But during aging processes, autophagy becomes progressively compromised due to related changes that occur within cells, which can contribute to decline in performance and is believed to cause aging (Cheon et al., 2019). Genetic mutations in autophagy genes lead to various developmental, metabolic, and pathological abnormalities, while age-induced decline in autophagy is contributing for losing homeostatic control in the cell (Tabibzadeh et al., 2022). Even though autophagy has been linked as a beneficial mechanism, several diseases and conditions like senescence and tumor progression strategically exploit its use as well, possibly due to high metabolic demands (Tabibzadeh et al., 2022).

Consequently, restoring impaired levels of autophagy to normal may have potential benefits in preventing age-related diseases while promoting longevity (Cheon et al., 2019). Rubinsztein et al. (2011) have thoroughly covered the molecular mechanisms of cellular autophagy along with its role relating to aging. Autophagy is regulated by a complex of signaling pathways such as mTOR pathway, AMPK pathway and Beclin 1 pathway, which sense stress signals and nutrient availability and regulate autophagy (Rubinsztein et al., 2011). The author also emphasized the importance of autophagy by helping maintain mitochondrial function, which is critical for cellular energy metabolism and has been linked to aging.

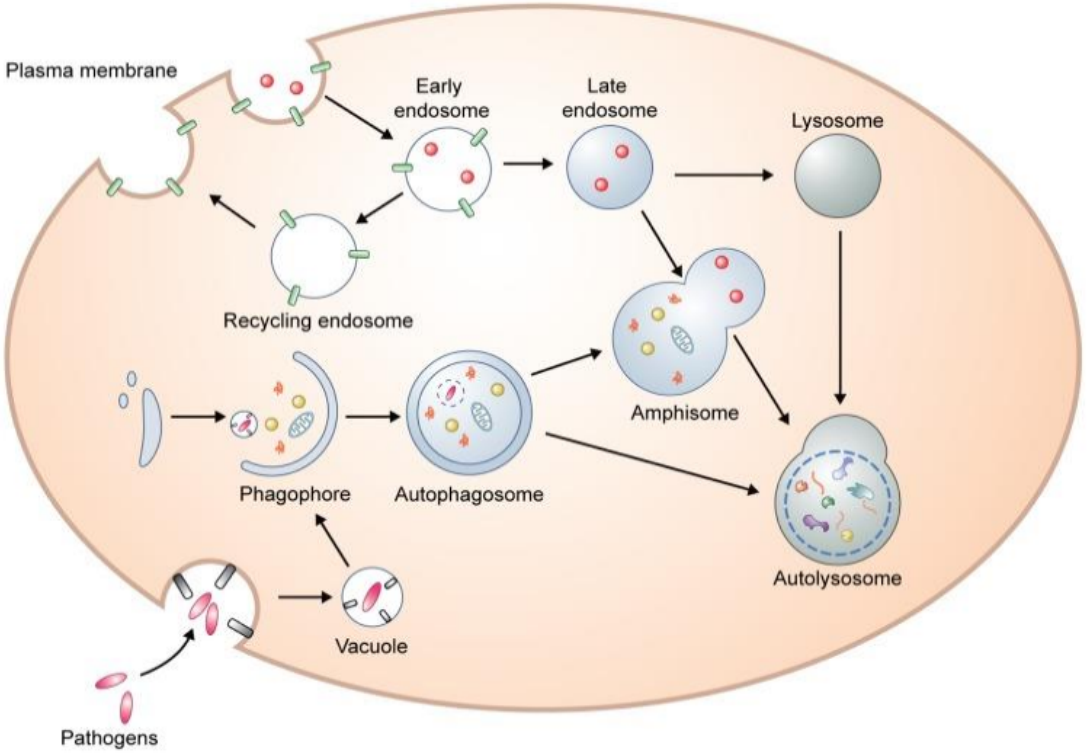


Figure 8. Graphic representation of the autophagy process. (Cheon, S.Y., Kim, H., Rubinsztein, D.C., & Lee, J.E., "Autophagy, Cellular Aging and Age-related Human Diseases," Experimental Neurobiology, 2019, Volume 28, Issue 6, pp. 643-657. Reprinted with permission from The Korean Society for Brain and Neural Sciences.)

## Senescence

Cells come into contact with several kinds of stress and damage from various sources that entail varied outcomes, ranging from total healing to cellular death (Campisi et al., 2013). In proliferating cells another response, called cellular senescence, can occur, which results in permanent inhibition of the cell cycle circuit (Campisi et al., 2013). Focusing on the reasons why this response occurs and the consequences helps understand how cells respond to genotoxic stress and how this cellular response influence complex process in organisms like ageing and cancer development (Campisi et al., 2013). Research findings revealed that cellular senescence, despite historically being viewed as irreversible cell cycle arrest mechanism against tumorigenesis, also contributes significantly to complex

biological processes like tissue repair, development, aging and age-related disabilities (van Deursen et al., 2014)(see Figure 9). By examining the sheer diversity involved in the molecular processes at play during senescence and focusing on the distinctions between acute and chronic senescent cells, newer therapies can be developed that help enhance age-linked conditions leading to a more extended lifespan (van Deursen et al., 2014).For research purposes scientists designed a novel transgene called INK-ATTAC, which facilitates inducible elimination of p16Ink4a (a protein that plays a role in regulating the cell cycle and cellular senescence) -positive senescent cells upon drug administration to investigate whether removing these cells could ameliorate age-related impairments (Baker et al., 2011).Their experiments revealed that continuous removal of cells expressing p16Ink4a throughout life results in postponed ageing related problems concerning in tissues such as fat, skeletal muscles and eyes (Baker et al., 2011). According to Baker et al. (2011) it is likely that age-related characteristics are influenced by cellular senescence. Taking steps to eliminate senescent cells could have significant implications for curbing or postponing tissue malfunction and promoting longevity (Baker et al., 2011).

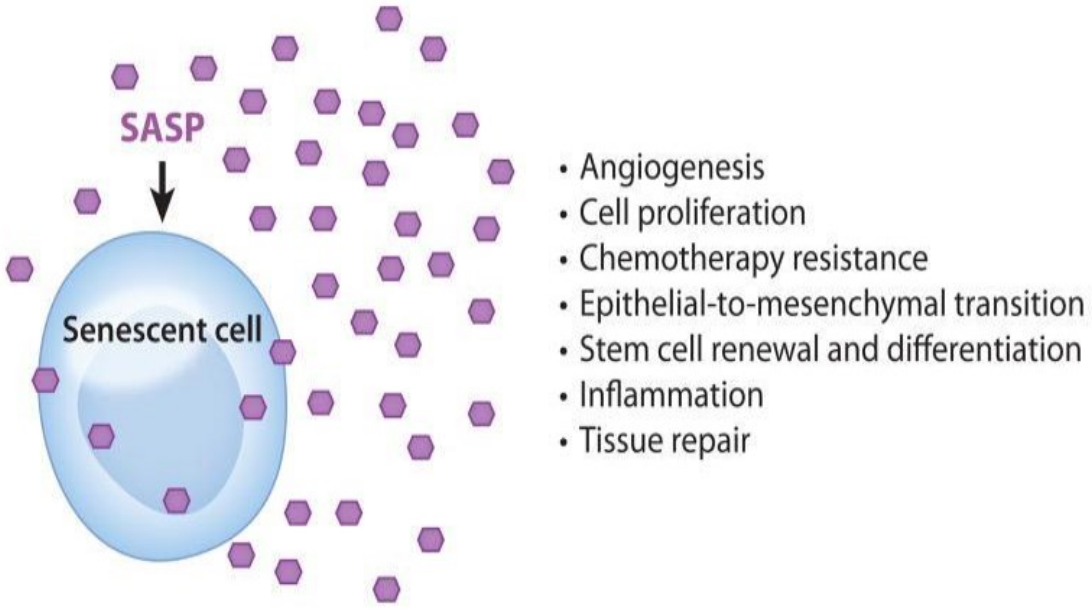


Figure 9. The diverse functions of the senescence-associated secretory phenotype (SASP) encompass a wide range of activities. (Campisi, J., "Aging, cellular senescence, and cancer," Annual Review of Physiology, 2013, Volume 75, pp. 685-705. Reprinted with permission from Annual Reviews.)

## Proteostasis

Properly maintaining protein homeostasis or proteostasis is an integral component for both maintaining cellular functionality and responding to stress (Koga et al., 2011). However, it becomes increasingly challenging for organisms as they age to preserve the stability of their proteome, leading into functional loss observed among older individuals (Koga et al., 2011). Luckily, molecular chaperones and proteolytic systems work synergistically to maintain cellular quality control by supporting ongoing protein renewal within cells (Koga et al.,2011).However, when these quality-control structures malfunction, this leads to the accumulation of abnormal proteins in aged tissues, potentially leading to unfavorable protein-inclusions or aggregation (Koga et al.,2011) (see Figure 10). Santra, Dill and de Graff (2019) present an investigation on molecular processes with regard to proteostasis involving protein folding, chaperoning, and maintenance of protein function. They developed an explanatory model that delves into how proteostasis collapses due to slowed translation with increasing age along with oxidative damage build-up over time (Santra et al., 2019).As individuals advance in age, they start acquiring irreversibly damaged proteins, causing chaperones to devote more time to handling them than folding healthy proteins that are essential to the function of a cell (Santra et al., 2019). Misfolding, accumulation of damages and aggregation build-up over time occurs when cells no longer generate functional proteins fast enough, which ultimately leads to cell death (Santra et al., 2019).C. elegans experiments show that nonlinearly lifespan decreases follow either added oxidant concentration or temperature increase and that increases in lifespan correlate with above-average quantities of chaperones or proteasomes (Santra et al., 2019). This research model proposes that oxidative damage will increase in organisms as they age, offering an explanation for the observed pattern of mortality that follows a Gompertz-like rise across different species including humans. This study emphasizes that protein instability sets the rate at which damage accumulates in our bodies with age, disrupting normal proteostasis balance within cells (Santra et al., 2019). Significantly improved proteostasis has been shown in long-lived animals and growing evidence emphasizes the strong correlation between elevated quantities and activity of diverse proteostasis components and longevity (Magalhaes et al., 2018). Further research will focus on utilizing genetic models with impaired quality control mechanisms later in life aiming to enhance protein homeostasis for healthy

aging in the future (Koga et al., 2011). Designing more effective chemical modulators for key parts of the proteostasis network will play a crucial role in advancing future anti-aging interventions as well (Koga et al., 2011).

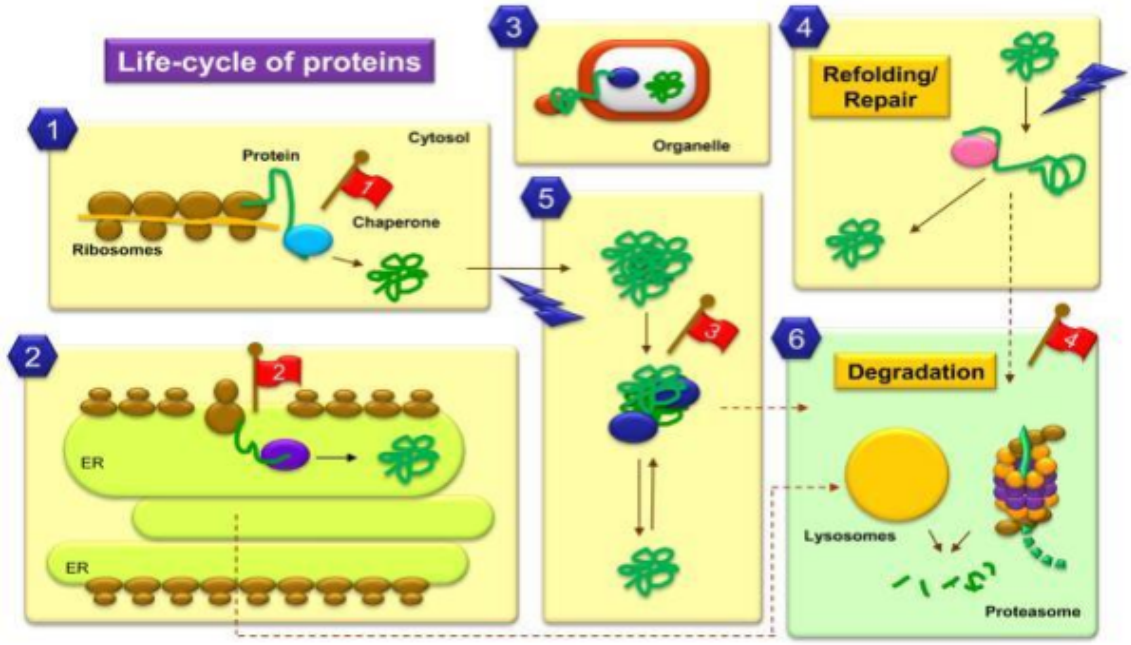


Figure 10. Cellular processes that encompass protein folding, reconfiguration, and breakdown. Most cytosolic proteins naturally fold correctly after synthesis, but sometimes they need assistance from chaperones and chaperonins to achieve their proper conformation (1). Chaperones also help fold proteins produced in the endoplasmic reticulum (ER) (2). If folding fails, both cytosolic and ER luminal proteins are marked for degradation (6). Proteins often need to unfold before crossing membranes or assembling into complexes, and chaperones aid in their refolding afterward. When proteins are damaged by external agents (4 and 5), chaperones help in refolding (4) or disaggregation (5). However, if the damage is irreversible, chaperones facilitate the degradation of the altered proteins through proteolytic systems (6). (Koga, H., Kaushik, S., & Cuervo, A.M., "Protein homeostasis and aging: The importance of exquisite quality control," Ageing Research Reviews, 2011, Volume 10, Issue 2, pp. 205-215. Reprinted with permission from Elsevier.)

## Drugs undergoing clinical trials

An expanding fascination surrounding the ability of certain drugs to extend human lifespan and delay the onset of age-related diseases has come to light recently.

Amongst them are metformin, rapamycin, senolytics, sirtuin activators (resveratrol) and NAD+ precursors showcasing encouraging anti-aging effects through animal experimentation. They are now undergoing clinical trials to assess their safety and efficacy in humans (Barzilai & Crandall, 2016; Kennedy & Lamming, 2016; Zhu et al., 2015; Howitzet al., 2003; Zhang et al., 2016). All of these drugs have major focus on different aspects of aging mechanisms like cellular metabolism, senescence and DNA damage response, simultaneously improve health span and increase lifespan. Though clinical trials are ongoing to determine their safety and effectiveness for human usage, the potential benefits in healthy aging look promising. (Barzilai & Crandall, 2016; Kennedy & Lamming, 2016; Zhu et al., 2015; Howitz et al., 2003; Zhang et al., 2016).

## Metformin

Metformin serves as one of the most widely used antidiabetic medications derived from the French lilac (Galega officinalis) (Bailey & Day, 2004). Its primary purpose lies within decreasing hepatic glucose production while at the same time increasing peripheral tissue insulin sensitivity (Foretz et al., 2014). Approximately over sixty years of use has proven effective in the management of type 2 diabetes mellitus (T2DM) (Barzilai et al., 2016). Attention has currently turned towards its role in the biology of aging and metformin's potential use as an anti-aging intervention due to metformin's multi-faceted impact on different aging pathways, such as oxidative stress, inflammation and cellular senescence (Barzilai et al., 2016)(see Figure 11).

A study conducted by Musi et al. (2002) aimed to explore the potential of metformin in enhancing AMPK activity in individuals with type 2 diabetes. The results demonstrated that administering metformin for ten weeks led to an increase in skeletal muscle by AMPK α2 activity. This rise in activity came along with greater phosphorylation of AMPK and decreased acetyl-CoA carboxylase-2 activity levels, indicating a shift in muscle energy status as the phosphocreatine- and ATP-concentrations decreased post treatment. Further analysis revealed that the rise in AMPK activity following metformin treatment was accompanied by enhanced glucose disposal rates and muscle glycogen concentrations. These effects suggest that metformin’s metabolic effects may be mainly mediated via AMPK activation.

Moreover, the study found full suppression of endogenous glucose production and lower serum insulin concentrations and improved peripheral glycose disposal as a result of metformin treatment. Although minor weight loss was noted after this ten-week treatment, it was not considered the primary factor in enhancing AMPK α2 activity. Hence, it was concluded that metformin as a reliable option when it comes to managing type 2 diabetes through AMPK activation (Musi et al., 2002). Ahmadi et al. conducted a study in 2020 which examined metformin's effect on older obese mice fed a high fat diet. The study observed beneficial effects on gut health through reduction of inflammation as well as leaky gut caused by beneficial modulation of gut microbiota. Metformin led to an enhanced mass of goblet cells forming in the gut while also promoting mucin production, which helped to mitigate gut inflammation and permeability. This was accomplished by inhibiting the Wnt signaling pathway, where the intestinal stem cells (iSCs) transformed into goblet cells.

Also, it was observed that metformin reduced glucose intolerance, inflammation within fatty tissue and liver fat accumulation in older mice. The study also revealed metformin's impact in gut microbiome and the production of beneficial metabolites like butyrate and taurine in the gut of older mice, even when the subject's diet varied. Furthermore, this study detected an improvement in cognitive function via this intervention, extending its benefits beyond metabolic improvements. In conclusion, Ahmadi et al. (2020) suggested that metformin stands as a potential therapeutic regimen to help cure age-related leaky gut syndrome, inflammation, cognitive decline and metabolic dysfunctions among elderly obese people through beneficial modulation within both gut microbiome and metabolome, while promoting goblet cell mass coupled with mucin formation. Finally, it is worth noting that Metformin also inhibits mTOR pathway which is implicated both in aging and various age-related diseases (Laplante & Sabatini 2012). Several observational studies and clinical trials provide encouraging evidence supporting both extended lifespan and health span from metformin use (Bannister et al., 2014). Nevertheless, continual usage might lead to depletion of vitamin B12 which may cause an increasing risk in peripheral neuropathy development while also affecting cognitive function negatively (Aroda et al., 2016). The current Targeting Aging with Metformin (TAME) trial aims at assessing whether metformin proves useful in prolonging health span for those without diabetes while also preventing the onset of age-related diseases (Barzilai et al., 2016). Ongoing clinical trials such as MILES (Metformin in Longevity Study) and TAME could offer definitive answers about the

anti-aging properties associated using this drug. Despite these promising findings of metformin's ability to support healthy aging, moving ahead with lifestyle modifications such as regular exercise or improved diet, is very important and should not be overlooked in favor of pharmacological interventions. It is necessary for further studies on different age groups free of chronic diseases, before making assumptions regarding the positive effects obtained from using metformin (Mohammed et al., 2021).

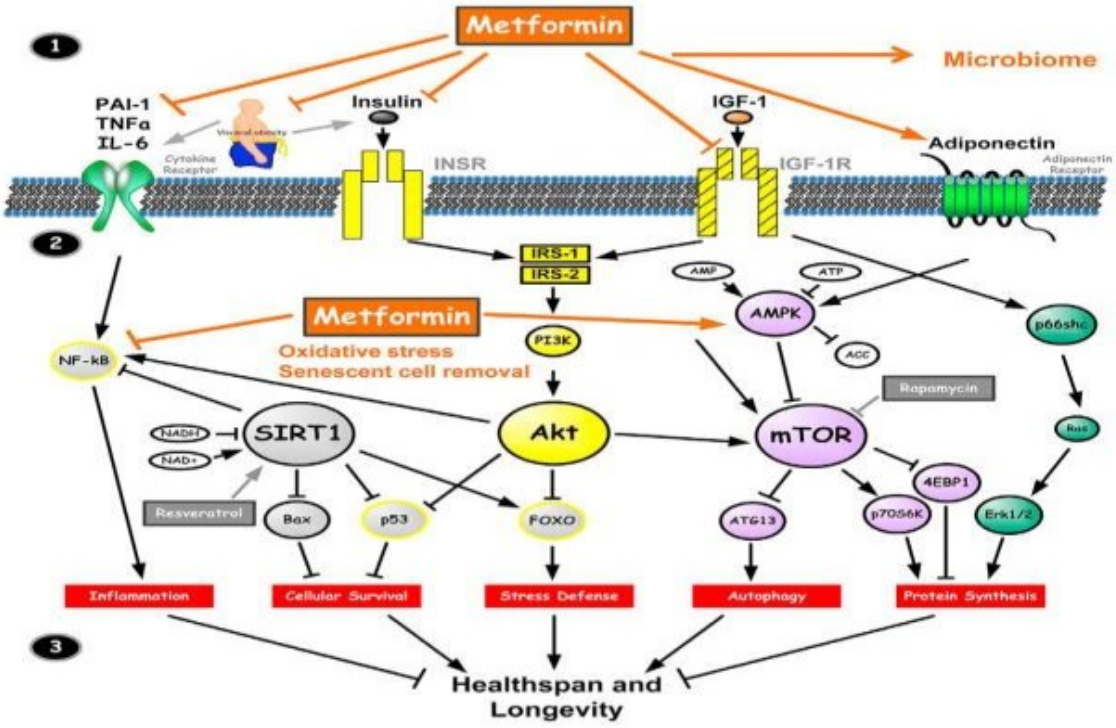


Figure 11. Demonstrates how metformin interacts with several aging-related pathways. (Barzilai, N., Crandall, J.P., Kritchevsky, S.B., & Espeland, M.A., "Metformin as a Tool to Target Aging," Cell Metabolism, 2016, Volume 23, Issue 6, pp. 1060-1065. Reprinted with permission from Elsevier.)

## Rapamycin

Rapamycin or sirolimus is a macrolide substance that has gained significant attention for its role in addressing aging. Having been discovered in soil samples collected on Easter Island in the early 70s (Vézina et al., 1975), it has been shown to prolong the lifespan of different organisms, including mice (Harrison et al., 2009),

through inhibiting the mechanistic target of rapamycin (mTOR) pathway, which regulates cell growth, proliferation and survival (Laplante & Sabatini, 2012).Studies demonstrate that inhibiting mTOR signaling through rapamycin can extend lifespan, mimicking calorie restriction in various organisms (Frontana et al., 2010, Lamming et al., 2012). Another benefit Rapamycin possesses as an anti-aging drug is improving health span (Bitto et al., 2016). Also, rapamycin studies in mice indicate that it has potential use in improving cardiac function (Flynn et al., 2013). Urfer and colleagues (2017) conducted a study on 24 middle-aged dogs over ten weeks, aiming to observe the effects of a non-immunosuppressive dose of rapamycin. The study found striking improvements in certain age-related measures of heart function in the dogs treated with rapamycin.

Despite promising outcomes for metabolism and aging-signs observed through using rapamycin, there is uncertainty regarding whether this solution is safe for healthy humans due to the drug's potential clinical risks such as hyperglycemia, insulin resistance, increased incidence of type 2 diabetes and hyperglycemia (Salmon, 2015) (see Figure 12). Nonetheless, there are believed to be alternative treatment regimens where intermittent rapamycin dosing or pairing rapamycin with specialized treatments for metabolic dysfunction could lessen these side effects without impacting the drug's positive outcomes (Salmon, 2015). Chung and his colleagues (2019) carried out a clinical investigation into how rapamycin could decrease aging signs by reducing cellular senescence in human skin. In the study were included participants over 40 years old with signs of age-related damaged skin. The study was encouraging as they observed lowered protein levels of p16INK4A, which is linked to cellular senescence along with improved protein production levels of collagen VII, which improves skin integrity (Chung et al., 2019). Even though the study only used a limited set of senescence markers, it provided preliminary indications that skin-function could improve along with decreased ageing-signs in human tissue through rapamycin treatment usage. Moreover, it was showed that low dose rapamycin is a therapy suitable for age-related illnesses with minimal side effects, as a potential solution, however extra investigation is necessary (Chung et al., 2019). To sum up, rapamycin presents enormous potential to counteract the ageing process by extending longevity and improving healthy living amongst various organisms. However, its usage in humans must be monitored for probable undesired side-effects. Due to these concerns, future research should focus on developing safer alternatives or derivatives, optimization of dosages and exploring synergistic effects with other anti-ageing strategies for

maximal utilization of rapamycin while minimizing adverse reactions.

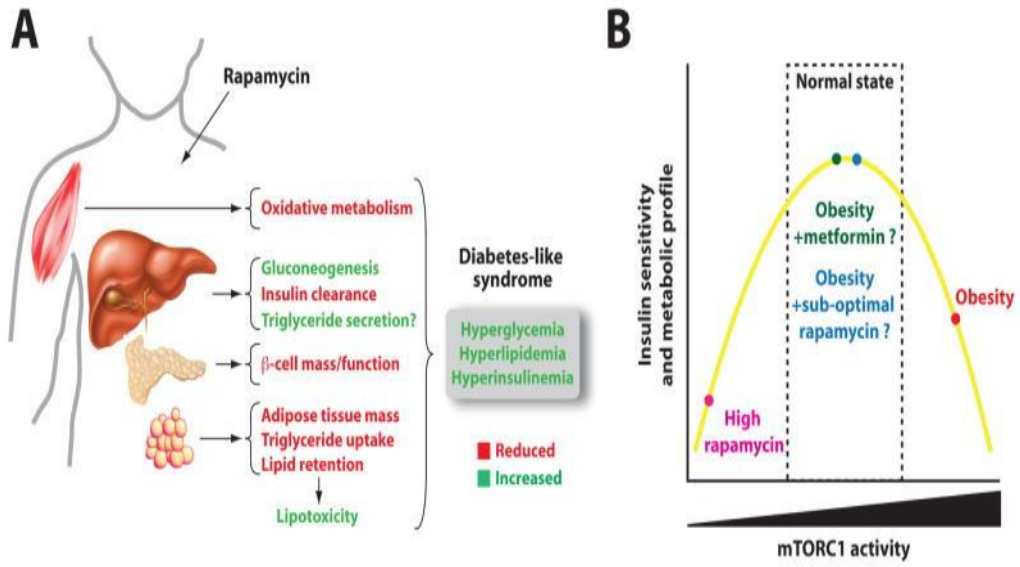


Figure 12. Depicts the influence of rapamycin on organ and systemic metabolism, illustrating its potential to trigger a diabetes-like syndrome by negatively affecting muscle, liver, adipose tissue, and pancreatic β-cell functions. Conversely, some processes are enhanced by rapamycin (Figure's part A). The figure also hypothesizes a U-shaped curve relationship between mTORC1 activation and insulin sensitivity/metabolic profile in vivo. It suggests that both excessively low and high mTORC1 activity can negatively affect systemic metabolism (Figure's part B). (Laplante, M., & Sabatini, D.M., "mTOR Signaling in Growth Control and Disease," Cell, 2012, Volume 149, Issue 2, pages 274-293. Reprinted with permission from Elsevier.)

## Senolytics

Cellular senescence refers to a state that involves irreversible arrest of cell replication, resistance against apoptosis while also triggering a harmful pro-inflammatory, tissue-destructive secretory phenotype called SASP (Kirkland et al., 2017). Currently, accumulation of senescent cells in tissues during aging has been linked to various chronic diseases (Kirkland et al., 2017). Senescent Cell Anti-apoptotic Pathways (SCAPs) have been uncovered and senolytic agents could be identified, that are able to specifically target these pathways. This makes senescent

cells more susceptible to their pro-apoptotic environment (Kirkland et al., 2017). Encouraging data from pre-clinical models reveal some promising outcomes provided by these agents in reducing numerous age-related symptoms and prompting the initiation of clinical trials. Nevertheless, the successful translation of this technology comes with multiple challenges that lie ahead, but the fact that aging mechanisms are conserved across species should somewhat increase hopes of an eventual successful translation to humans (Kirkland et al., 2017). Intermittent treatment with senolytic agents may be a viable option for humans, potentially reducing the risk of side-effects and providing a new approach to improving quality of life and managing chronic illnesses (Kirkland et al., 2017). Senolytics, such as the combination of Dasatinib and Quercetin (D + Q), specifically target these cells by temporarily disrupting their pro-survival networks (Hickson et al., 2019). A Phase 1 pilot study following an open label design was conducted on patients with diabetic kidney disease who received D + Q treatment allowing researchers to evaluate its impact on senescent cells load (Hickson et al., 2019). According to Hickson et al. (2019) the study found that 11 days after undergoing therapy, there was a noteworthy decrease in the number of senescent cells in adipose tissue. Additionally, there was a decline in specific markers of senescent cells and limited growth potential in adipocyte progenitors. The researchers also observed a significant reduction in adipose tissue macrophages and crown-like structures, both of which are attracted to, anchored by and activated by senescent cells. Furthermore, a decrease in skin epidermal senescent cells and circulating factors related to SASP, such as IL-1α, IL-6 and MMPs-9 and -12 was observed. These findings suggest that treatment with senolytics like D + Q may effectively reduce the burden of senescent cells in humans (Hickson et al., 2019). However, it is important to acknowledge that there are still many unknown factors and potential challenges. For example, our current knowledge about the rates and patterns of senescent cell accumulation in both humans and animals during normal aging and age-related diseases is limited (van Deursen, 2014).It is crucial to better comprehend senescent cell heterogeneity and the effects of senescent cells clearance on normal health and lifespan (van Deursen, 2014). Additionally, it raises concerns about using mice as models for human senescence, concerning the potential issues triggered by removal of these cells in terms of tissue dysfunction and atrophy. The reliance on different pathways (p53 and p16Ink4a) for inducing senescence in human versus mouse cells necessitates cautious validation before applying mouse study results to humans (van Deursen, 2014). Further exploration is needed

regarding immune system interaction with senescent cells for future therapeutic approaches that may aim at activating or reinforcing immune responses against these cells (van Deursen, 2014).

## Sirtuin activators (resveratrol)

Researchers have made discoveries that have identified certain molecules which may be able to delay age-related diseases and increase lifespan in mouse models. This suggests that it could theoretically lead to a longer and healthier life for humans as well (Hubbard et al., 2014). Sirtuins, which are NAD+-dependent deacylases, play a critical role in how the body responds to factors such as diet and exercise (Hubbard et al., 2014). It has been found that both natural and synthetic Sirtuin Activating Compounds (STACs) employ a shared allosteric mechanism to enhance sirtuin activity. This could potentially offer numerous health benefits not only to rodents and primates but also to humans (Hubbard et al., 2014).However, there are still several important questions that need to be addressed. One of which is identifying the most effective methods for testing STACs in humans to maximize their effectiveness (Hubbard et al., 2014). Resveratrol, a plant polyphenol, has shown potential in extending the lifespan of various organisms like yeast, worms, flies and fish. Additionally, it has been found to mitigate metabolic dysfunction in mice fed on high-fat diets (Knutson & Leeuwenburgh, 2008). One of the reasons behind the supposed benefits of resveratrol is its ability to activate SIRT1 (Knutson & Leeuwenburgh, 2008). The impacts associated with resveratrol include aspects linked to a longer lifespan, such as enhanced insulin sensitivity, decreased levels of insulin-like growth factor-1 (IGF-1), increased activity of AMP-activated protein kinase (AMPK) and peroxisome proliferator-activated receptor-gamma coactivator 1alpha (PGC-1alpha), an increase in the number of mitochondria, and enhanced mobility function (Baur et al., 2006). However, the application of resveratrol as a human dietary supplement is limited due to its low systemic bioavailability and the scarce presence of this compound in food (Knutson & Leeuwenburgh, 2008).

Also, resveratrol has been found to enhance brain health through multiple signaling pathways, with the activation of SIRT1 being a critical step in its neuroprotective properties (Cao et al., 2018). Resveratrol has been suggested to delay age-related

cognitive decline through SIRT1 by regulating anti-oxidative, anti-inflammatory, anti-apoptotic processes, and autophagy, as well as enhancing cerebral blood flow and synaptic plasticity (Cao et al., 2018). MicroRNAs, such as miR134 and miR204, which interact with SIRT1, could become promising new targets. Further, manipulating SIRT1 levels and activity has been shown to influence neurogenesis and neuronal network connections, suggesting potential clinical applications for resveratrol and SIRT1-activating agents in neurological development and nerve injury restoration (Cao et al., 2018).

## NAD+ precursors

NAD+ "precursors" act as building blocks for the essential coenzyme nicotinamide adenine dinucleotide (or NAD+) which is critical in numerous cellular processes like energy metabolism, gene expression and DNA repair (Verdin, 2015) (see Figure 13).The levels of this coenzyme naturally decline as we age which leads to a decrease in cellular function and contributing to the aging process (Massudi et al., 2012).In recent years, research focusing on NAD+ precursors has seen a consistent increase in popularity due to the potential effect they offer on the biology of aging. Nicotinamide Riboside (NR) and Nicotinamide Mononucleotide (NMN) are two ubiquitous NAD+ precursors (Bogan & Brenner, 2008). These precursors are observed in studies to increase NAD+ levels within cells. This increased concentration translates into higher activity of sirtuins, a family of enzymes responsible for cellular health and aging (Imai & Guarente, 2014). In animal studies, supplementing on NAD+ precursors increased lifespan while improving mitochondrial function and protect against neurodegenerative diseases (Zhang et al., 2016; Mills et al., 2016). As a result of such findings, interest in NAD+ supplements continuously grows within nutrition markets for their suggested potential benefits in promoting better ageing and disease prevention. However, researchers emphasize caution in monitoring long-term administration of NAD precursor supplements (Zhou, 2021).

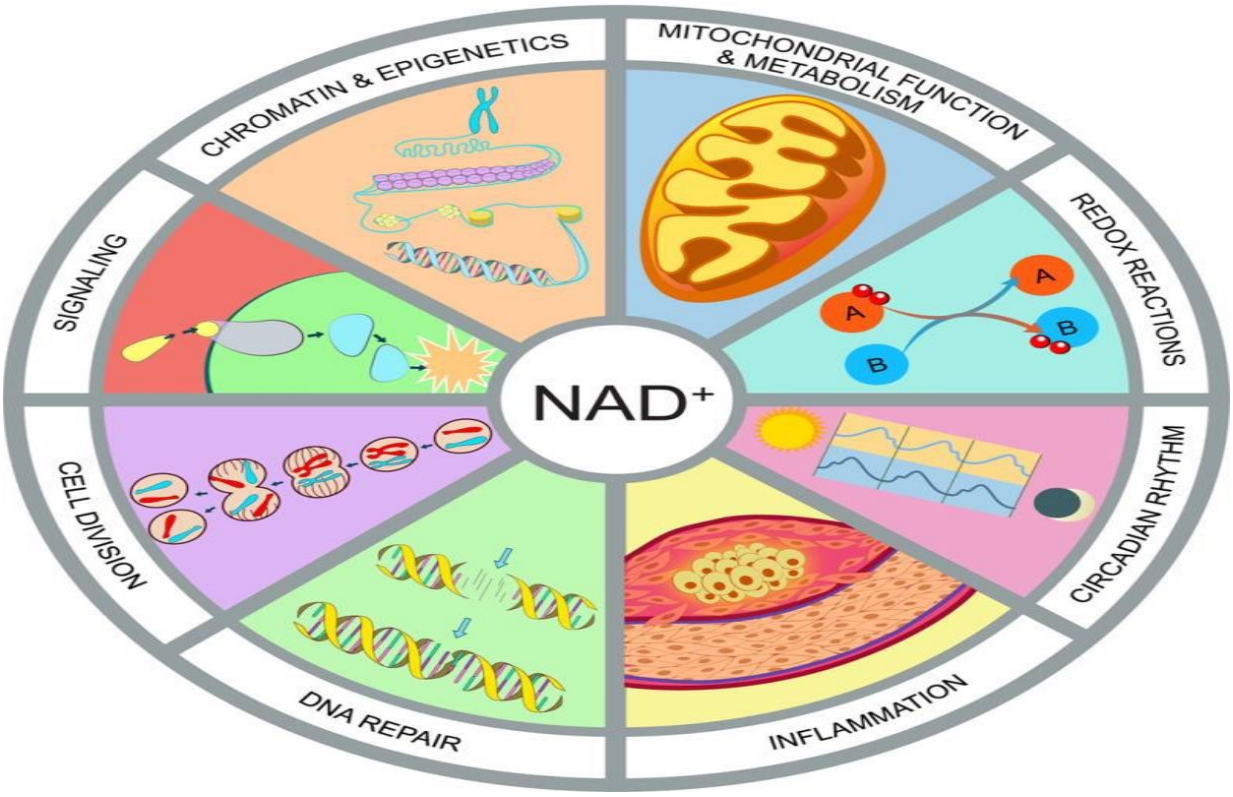


Figure 13. Hallmarks of NAD homeostasis. (Rajman, L., Chwalek, K., & Sinclair, D.A., "Therapeutic Potential of NAD-Boosting Molecules: The In Vivo Evidence," Cell Metabolism, 2018, Volume 27, Issue 3, pp. 529-547. Reprinted with permission from Elsevier.)

## Promising Approaches to Enhance Human Health and Longevity

Extensive research on the molecular and cellular mechanisms of aging has been conducted by Carlos López-Otín and colleagues. Through their work, they have proposed interventions that could potentially extend human healthspan by targeting the hallmarks of aging (López-Otín et al., 2013) (see Figure 14).

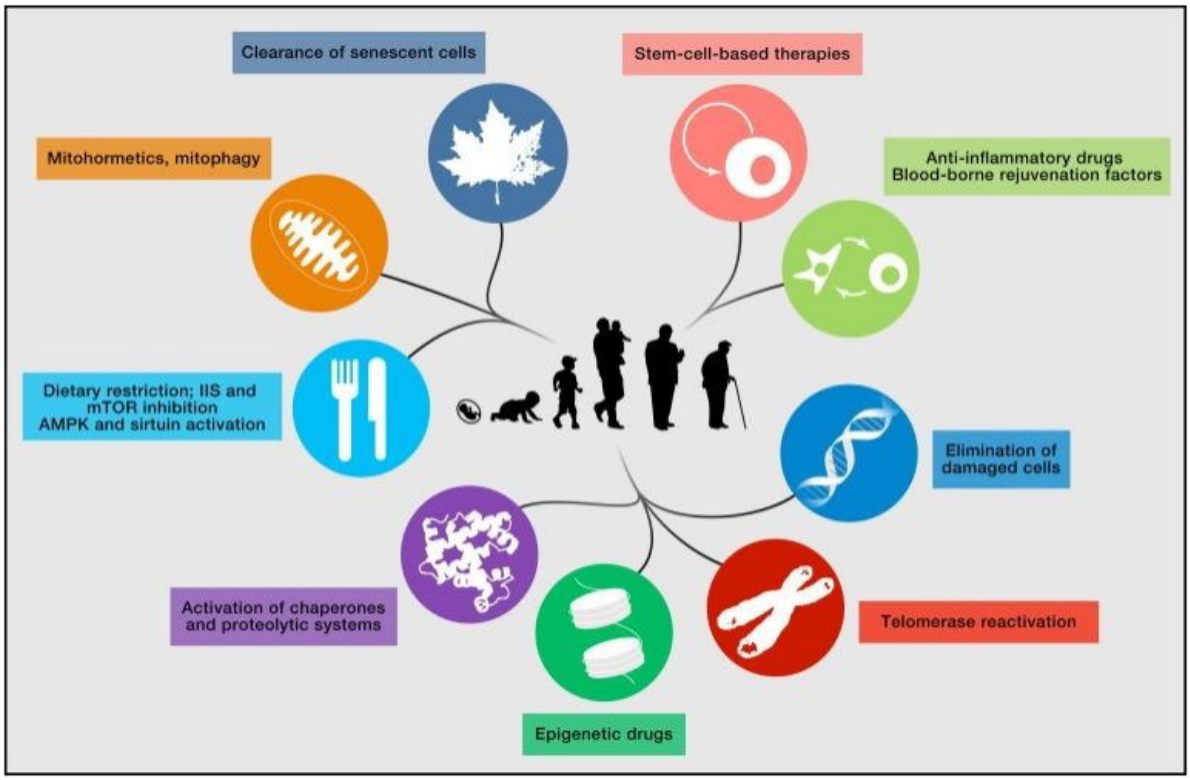


Figure 14.Targeting the hallmarks of aging. ( López-Otín, C., Blasco, M.A., Partridge, L., Serrano, M., & Kroemer, G., "The Hallmarks of Aging," Cell, 2013, Volume 153, Issue 6, pp. 1194-1217. Reprinted with permission from Elsevier.)

## Telomerase reactivation

Aging is an unavoidable part of life and often comes with various health complications and susceptibility to diseases. It is worth noting here that some age-related illnesses have been found to have an association with dysfunctional telomeres which slow down tissue-repair capacity leading to accelerated aging processes (Kuru et al., 2022). In view of this fact, researchers are exploring natural products that activate the enzyme known as telomerase - a key player in regulating good telomere health - as promising therapeutic agents against age-related degenerative diseases like neurodegenerative disorders as well as osteodegenerative or cardiovascular illnesses, where telomere dysfunction is a known factor (Kuru et al., 2022) (see Figure 15). In a study conducted by Tsoukalas et al. (2019), the researchers examined the impact of natural compounds on the activation of telomerase. Telomerase activation can potentially

reverse the shortening of telomeres. The study tested several compounds on human peripheral blood mononuclear cells (PBMCs). Results showed that some of these compounds led to a significant increase in telomerase activity compared to untreated cells (Tsoukalas et al., 2019). TA-65, a compound extracted from Astragalus membranaceus, is reported to activate telomerase both in vitro and in vivo, improving health span, particularly at the metabolic level (de Jesus et al., 2012). While telomerase activation has been associated with cancer due to its high activity in cancer cells, several studies have indicated that telomerase activation itself does not cause cancer. On the contrary, telomerase deficiency might lead to cancer as short TL has been linked with tumorigenesis (Kuru et al., 2022). Telomerase therapeutics may become a significant component of cancer chemotherapy regimens (Phatak & Burger, 2009).

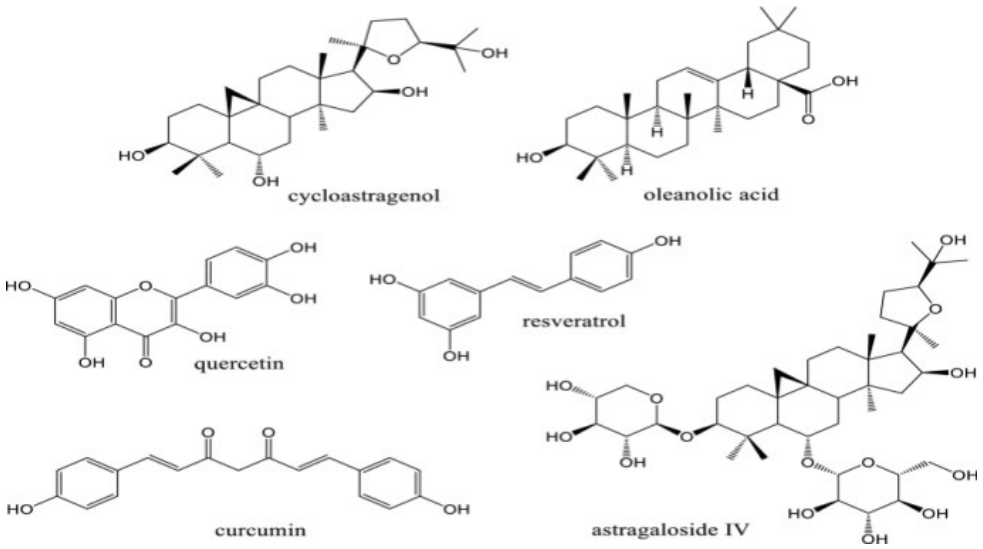


Figure 15. The molecular compositions of telomerase stimulators derived from natural origins. (Kuru, G., Üner, G., & Bedir, E., "Is telomerase a hidden player? Therapeutic potential of natural telomerase activators against age-related diseases," Phytochemistry Reviews, 2023, Volume 22, pages 35-72. Reprinted with permission from Springer Nature.)

## Elimination of damaged cells

The key to promoting healthy aging processes while avoiding age-related diseases is eliminating damaged cells and clearing out senescent ones (López-Otín et al., 2013). Senescence - a particular kind of growth-arrested cell - causes inflammation and tissue dysfunction by secreting pro-inflammatory factors (Tchkonia et al., 2013). Damaged cells which accumulate over time are leading ultimately to chronic inflammation, increasing the likelihood of cancer or other diseases including Alzheimer's and cardiovascular diseases (Franceschi & Campisi, 2014). A hopeful avenue lies in anti-aging senolytic drugs that selectively target senescent cells and induce programmed cell death through apoptosis (Zhu et al., 2015). These drugs include dasatinib, fisetin, navitoclax and quercetin, which all showed promise in preclinical models (Kirkland &Tchkonia, 2020). However, further research into their safety and effectiveness on humans is still ongoing as clinical trials advance (Hickson et al., 2019).Another novel promising method for targeting these cells and eliminating them to promote healthy aging is immunotherapy, a technique that involves using antibodies to target markers on the surface of senescent cells (Amor et al., 2020). Preclinical models have shown encouraging results with this method (Bussian et al., 2018). Other means that have been found useful in reducing the number of senescent cells and extending overall health span and lifespan include caloric restriction (Fontana & Partridge, 2015) and exercise (Safdar et al., 2016). Activating immune cells are also a mean to eliminate damaged cells. Natural-killer- and T-cells can identify senescent along with other damaged cells and eliminate them (Ovadya & Krizhanovsky, 2018). However, as the body ages, the immune system's efficiency is considerably reduced, making elimination of these cells harder (Fulop et al., 2018).To promote healthy aging while sidestepping age-related diseases caused by senescent cells, various approaches have been explored, including caloric restriction, exercise and the use of senolytic drugs and immunotherapy, which have shown the ability to target these cells when preserving the healthy ones (Kirkland &Tchkonia 2020; Fontana & Partridge 2015; Safdar et al., 2016; Amor et al., 2020). Nevertheless, Tchkonia et al. (2013) stresses the need for additional research towards discovering how cellular senescence works and the impact of damaged cells on aging and diseases, as well as to develop safe effective practices for eliminating these cells.

## Activation of chaperones and proteolytic systems

To prevent age-related diseases and promote cellular health, activation of chaperones and proteolytic systems has proven to be an effective strategy (López-Otín et al., 2013). Protein homeostasis is maintained with the critical assistance provided by chaperone proteins during protein folding (Hartl et al., 2011). Furthermore, proteolytic systems are responsible for breaking down and removing impaired proteins from cells to ensure proper functionality (López-Otín & Bond 2008). Proteostasis regulators are small molecules that enhance both chaperones and proteolytic systems that result in protein homeostasis and reducing damaged protein accumulation within cells (Powers et al., 2009).Another way to improve protein homeostasis is through caloric restrictions paired with physical activity resulting in increased expression of both chaperones as well as proteolytic systems. Studies shows that these lifestyle methods help maintain a steady balance of proteins while blocking diseases that arise from aging (Handschin & Spiegelman, 2008; Fontana & Partridge, 2015; Vainshtein et al., 2015). Alternatively, one can utilize specific compounds such as Epigallocatechin gallate present in green tea, that have demonstrated efficacy in inducing chaperone expression and proteolysis which further reduces toxicity in certain disease models as well as modulating protein misfolding (Ehrnhoefer et al., 2008). To conclude, a promising note for supporting good health is by activating chaperones along with a well-regulated proteolytic system (López-Otín et al., 2013). There are various avenues we can explore for this purpose, including proteostasis regulators or by incorporating habits like exercises and caloric restriction or particular substances like EGCG. However, further research needs to be conducted on the underlying mechanisms aiding these interventions so we may maximize their benefits towards achieving healthy aging.

## Stem cell-based therapies

Stem cell therapy has emerged as a promising way dealing with age-related complications and diseases that often accompany old age. Stem cells have a

unique position in the biology of aging field due to their capacity to regenerate and differentiate into various cell types (López-Otín et al., 2013). Significantly, advances in stem cell studies led to promising therapeutic strategies towards curing a wide range of diseases, including age-related illnesses, as well as improve life quality. One such strategy includes using induced pluripotent stem cells (iPSCs), which are adult cells reprogrammed to a pluripotent state, resembling embryonic stem cells(Takahashi & Yamanaka, 2006).These have been found to reverse specific age-related phenotypes as demonstrated in models such as progeroid mice (Liu et al. 2011). Therefore, a window of hope has opened to the possibility of rejuvenating human tissues and organs through iPSC therapies (Liu et al., 2011).

Another way for addressing age-related diseases is by using mesenchymal stem cells (MSCs) as a viable option given the significant regenerative and immunomodulatory properties of these multipotent stem cells types (Caplan, 2007). Osteoarthritis and cardiac disorders are just a few of the conditions that these multipotent stem cells have shown potential in treating (Caplan, 2007). Sources for obtaining MSCs include various tissues such as bone marrow (Friedenstein et al., 1970), adipose tissue (Zuk et al., 2002), and umbilical cord blood (Bongso & Fong, 2013). Recent studies show innovations with advanced treatment strategies involving extracellular vesicles (EVs) derived from MSCs as a cell-free therapy with incredible potential for their ability to modulate inflammation and promote tissue repair (Phinney & Pittenger, 2017).

As we age, senescent cells accumulate and contribute to age-related dysfunction (Campisi, 2013). These cells produce substances that cause inflammation known as the SASP, which negatively affects surrounding cells and promotes tissue dysfunction (Campisi, 2013). Recent studies have shown that targeting these senescent cells can improve health span and lifespan in animal models (Baker et al., 2016). At the same time, the induction of pluripotency using Yamanaka factors (Oct4, Klf4, Sox2, and c-Myc) can enhance cellular and physiological markers related to aging. Kaur et al.'s (2022) research shows how combining stem cell rejuvenation with targeted removal of senescent cells can synergistically improve health span and lifespan while preserving the intestinal stem cell pool, lowering inflammation and initiating pro stem cell signaling pathways (Kaur et al., 2022). This innovative strategy, which merges cellular reprogramming via gene therapy with the use of senolytic peptides or drugs, hold promise to promote tissue repair while reversing signs of age (Kaur et al., 2022).

While there is a great promise in utilizing stem cells as a means of resolving age associated conditions, major obstacles like optimizing the delivery and integration of stem cells into host tissues are some of the difficulties that need to be overcome. Ensuring both safety and efficacy of such treatments is crucial, as also addressing ethical concerns surrounding their utilization (Trounson & McDonald 2015).

## Mitichormetics and Mitophagy

Mitochondria are vital in eukaryotic cells for energy generation through oxidative phosphorylation. This energy production process can lead to reactive oxygen species (ROS), this may result to mitochondrial DNA damage (mtDNA), affecting mitochondrial function which results in dysfunction (Wallace, 2005).

Efficient maintenance of proper functional levels of mitochondria in cells entails two primary mechanisms, mitohormesis and mitophagy. Mitohormesis is a mechanism where minor mitochondrial dysfunction activates cellular signaling pathways. These pathways are improving overall quality-control while creating new functional mitochondria (Ristow & Zarse, 2010). On the other hand, mitophagy facilitates autophagic degradation of mitochondria which is crucial to cellular homeostasis, physiology, and disease states (Palikaras et al., 2018).A double membrane structure within a cell, called an autophagosome, absorbs damaged mitochondria which then fuses with the lysosome for degradation (Mizushima & Komatsu, 2011). The "PINK1/Parkin pathway" is one of signaling pathways in charge of regulating mitophagy (Pickrell & Youle, 2015). By ensuring the regulation of healthy mitochondrial populations, it prevents accumulation of damaged mitochondria associated with cellular aging and disease (Sun et al., 2016). In a number of different neurodegenerative diseases, including Parkinson's, Alzheimer's and Huntington's, impaired mitophagy has been identified (Fivenson et al., 2017).Conducting research into other mitochondrial quality-control pathways like the mitochondrial unfolded protein response (UPRmt) in coordination with mitophagy can enhance our understanding of neurodegenerative mechanisms significantly (Fivenson et al., 2017). Activating mitohormesis and mitophagy pathways prove to be a promising approach for promoting healthy aging while avoiding age-related diseases (Fang et al., 2019; Yun &Finkel, 2014).

## Anti-inflammatory drugs and blood-borne rejuvenating factors

As we encounter lifelong antigenic stress throughout our lives, our bodies may respond with chronic low-level inflammation - referred to as "inflamm-aging." Although this typically is an effective defense mechanism when regulated properly, it can pose risks if not accompanied by essential anti-inflammatory molecules that commonly diminish as we age (Fülöp et al., 2016). An alternative approach towards targeting inflammations caused by aging involves using blood-borne rejuvenating factors, which are proteins and peptides responsible for restoring youthful physiology while promoting tissue repair. Research suggests that when plasma obtained from younger animals is infused in older animals, their cognitive functions improved alongside with tissue regeneration, proving the presence of rejuvenating factors in young blood samples (Villeda et al., 2014). One such factor that researchers are investigating, is the alpha klotho protein which tends to decline with age and has been associated with various age-related diseases. Animal studies have indicated that raising alpha klotho levels can provide therapeutic benefits such as an improved lifespan and reduced risk of age-related diseases (Kuro-o et al., 1997).

As we progress in transitioning from tests on mice to human subjects with parabiosis treatment, there are also ethical considerations that require attention. The challenges that arise include equal access to medical resources along with the prevention of disparities (Hofmann, 2018). Further complications that need to be addressed include the use of somebody's internal substances for the purpose of rejuvenation. These elicit difficult moral dilemmas, such as creating "blood bonds" brought about by constant demand for certain blood types amongst people who have been treated through parabiosis treatment (Hofmann, 2018). Despite endless appeal of an eternal youth, we have yet to accurately assess how health, aging, medicine and society will be impacted by parabiosis or endorse its potential positive or negative outcomes (Hofmann, 2018). Sometimes, lifestyle changes such as food consumption and exercise may reduce inflammation. Eating a diet filled with whole grains, fruits, vegetables and lean proteins can decrease the scale of inflammation (Fontana & Partridge, 2015). Exercise is another useful tool that is recognized for its ability to reduce inflammation levels (Safdar et al., 2016).

## Eating for Longevity: The Role of Nutrition in Ageing Biology

Nutrition in aging biology research plays a crucial role in comprehending how diet and biological processes affect our long-term health (Fontana & Partridge, 2015; López-Otín et al., 2013). Although the phenomenon of increased life expectancy shows progress over time, it foretells unique challenges that must also be addressed. Such issues include obesity, type 2 diabetes, cardiovascular disease and fatty liver as they become more prevalent of longer lifespans. The last hundred years have demonstrated that dietary interventions can help prevent or reduce complications associated with growing older (Giacomello & Toniolo, 2022). As such, the need to review how different types of diets or their individual components actively influence the biology of aging is therefore essential for both personal well-being and public healthcare systems (Barzilai et al., 2012).

## Caloric restriction and its effects on aging and longevity

The significance of ensuring adequate nutritional status is very important when it comes on maintaining immune system functionality and preventing frailty that commonly arise among seniors (Di Giosia et al., 2022). Aging populations have shown an increase in diseases like diabetes, cancer and degenerative disorders, leading researchers towards identifying potential effective countermeasures, among them CR (caloric restriction) (Giacomello & Toniolo 2021). CR has been shown experimentally to create nutrient sensing alterations that yield beneficial outcomes such as having an overall healthier metabolic profile linked with enhanced stress resistance, improved inflammatory response levels alongside lowered oxidative stress levels (Giacomello & Toniolo, 2021).This has shown that CR as well as CR mimetics could become essential tools capable of slowing down aging processes in both experimental models and humans while extending the healthy lifespan (Giacomello & Toniolo 2021). Since researchers face ethical considerations limiting experimentation on humans with respect to testing anti-aging interventions, researchers often tend towards using experimental animal models instead (Giacomello & Toniolo, 2021). It becomes clear that despite of these models

offering significant insights into lifespan-regulating mechanisms, there is still extreme variability in aging mechanisms amongst different organisms, species and even individuals owing to the multifactorial nature of aging (Giacomello & Toniolo, 2021). Therefore, attention needs to be paid more closely when identifying which experimental model will be used when it comes to target tissues and organs that will be involved (Giacomello & Toniolo, 2021).

It is recognized that reducing caloric intake by 20-40% is an effective aging regulator and lifespan-extender in several model organisms including worms, flies, rodents, yeast and primates (Lee & Longo, 2016). However, such a severe intervention has both beneficial and detrimental effects. To avoid the risks of long-term caloric restriction while retaining health benefits, researchers have proposed alternative approaches like intermittent or periodic dietary restrictions, which increase overall health span while decreasing harmful side effects (Lee & Longo, 2016). While caloric restriction is widely studied, recent research highlights how more moderate dietary interventions could yield similar outcomes on ageing processes (Lee & Longo et al., 2016). The impact of caloric restriction on aging is not merely due to simply eating fewer calories, but also determined through the composition of one's' diet. The effects may still be attained by periodic interventions, without a consistent decrease in calorie intake or total food deprivation while fasting (Lee & Longo., 2016).

Although CR consistently improves health in both sexes across strains of mice, it does not always increase lifespan. Therefore, CR has complex and varied effects affected by factors such as genetic background, sex differences among animals under study and the degree of CR (Mitchell et al., 2016). The authors postulate that optimal benefits of CR may ultimately depend on the ways in which CR activates key physiological processes such as autophagy, maintenance of healthy and functional mitochondrial and improved lipid and carbohydrate metabolism, an area where further research is still needed (Mitchell et al., 2016).

## The role of macronutrients (protein, fats and carbohydrates) in aging

Proteins, fats and carbohydrates are macronutrients that play a significant role in aging for their importance regarding primary energy sources and building blocks for

cell structure and functionality (Solon-Biet et al., 2014). Furthermore, balanced ratio of protein-to-carbohydrate has paramount importance to foster long lived and late life healthiness (Solon-Biet et al., 2014). It seems also that different types of carbohydrates can impact aging too, Liu et al.'s study (2000) suggests that whole grains, fruits and vegetables are excellent options over refined kinds of carbohydrates which are linked to increased inflammation levels. Age related disease rates might also be linked upon the quality and type of fat consumed. Research shows a lesser chance of age-related diseases resulting from a diet with unsaturated oils such as fish and olive oil, while trans- or saturated fats are associated with a higher risk (Estruch et al., 2018). Exploring how reduced consumption of protein and amino acids influences aging and disease while focusing particularly towards enhancing lifespan across diverse organisms, Mirzaei along with co-authors conducted research in 2014 suggesting these limitations were equally powerful in comparison to calorie restriction for improving health span among different model organisms. Interventions such as decreasing intake of proteins or particular amino-acids could lead to optimization of health span (Mirzaei et al., 2014). Levine et al. (2014) conducted research aimed at determining the association between protein consumption and death rates in people over fifty years old. Their observations unveiled that taking high or moderate amounts of protein consumption resulted in more frequent deaths due to diabetes complications while having no noticeable correlation with mortalities related to cardiovascular diseases nor cancer within participants older than fifty years (Levine et al., 2014).

However, research of dietary habits amongst non-diabetic participants showed that consuming excessive amounts of protein puts individuals at a higher risk for eventual death due to diabetes-related causes (Levine et al., 2014). However, the effects of protein-intake appear to be highly variable in health outcomes depending on different age-groups. Specifically, in those aged between fifty to sixty-five years old, a substantial increase in all-cause as well as cancer mortality rates were observed in people with high protein-intake. Interestingly, when the protein derived from plant instead of animal-based ones, the relations between protein intake and health risks diminished or disappeared altogether (Levine et al., 2014). The findings show that a preference for animal-derived proteins over plant sources in food intake tends to cause elevated death risks among individuals belonging to this specific age-group. In comparison, seniors aged over sixty-six do not appear vulnerable against detrimental outcomes resulting from consuming adequate levels of protein. The results suggest that protein requirements may vary based on age and that

reducing protein consumption in middle age might be beneficial while older adults can boost their protection against some health conditions by consuming more protein (Levine et al., 2014).

## The role of micronutrients (vitamins and minerals) in aging

Micronutrients like zinc, copper andiron take center role in age-related biological changes (Mocchegiani et al., 2012).These essential nutrients act as regulatory elements responsible for maintaining and strengthening our bodies' immune and antioxidant functions while controlling genes involved for appropriate protein productions during inflammatory- or immune response (Mocchegiani et al., 2012). However, an inadequate intake in dietary minerals and vitamins may occur due to an energy-rich yet micronutrient-deficient diet, comprising mostly refined foods, which compounds the issue leading towards chronic metabolic disruption, including mitochondrial decay alongside with DNA damage (Ames et al.,2006). Micronutrient deficiencies are capable of causing late-onset diseases such as cancer and cellular aging (Ames et al.,2006). The scenario of inadequate micronutrient intake might be experienced especially by specific population groups due to complex socio-economic or environmental circumstances (Shergill-Bonner, 2017). Children and infants are majorly vulnerable with inadequate nutrition impacting growth and development at a critical stage (Shergill-Bonner, 2017). Nutritional status can further worsen in disease-states where appetite declines, reducing intake of nutrients in demanding settings. Supplementary feeding may not always yield desired results leading to nutrient deficiencies among such patients (Shergill-Bonner, 2017).

## Specific Dietary Patterns and Aging

As research has shown, there is a complex relationship between inflamm-aging (low grade chronic inflammation related to aging) and nutrition. Numerous studies have shown that adopting certain diets such as the Mediterranean diet or

implementing approaches like CR and emphasis on gut microbiota can alleviate systemic inflammation while improving clinical outcomes over time (Di Giosia et al., 2022). These dietary interventions work by specifically lowering inflammatory mediators like C-reactive protein (CRP) and Tumor Necrosis Factor α (TNF α), two typical markers of inflamm-aging (Di Giosia et al., 2022).

In her research published in 2004, Trichopoulou notes that a feature of the traditional Mediterranean eating pattern is its heavy use of fruits and vegetables as well as whole grains and healthy fats and is associated with an increased longevity especially for older people who embrace this nourishment scheme. Researches carried out across countries like Greece, Spain, Denmark and Australia revealed that senior individuals adhering closely to this particular regimen record significant decreases on death rate levels (Trichopoulou, 2004). In view of these results and lacking any significant interactions between age and dietary choices affecting overall mortality, it may be fair to assume that adhering to this traditional dietary plan constitutes a healthy nutritional strategy for older adults (Trichopoulou, 2004). A dietary shift towards plants, which entails favoring fruits, vegetables, whole grains legumes and nuts while minimizing animal products, have demonstrated some promising health advantages such as reducing risks to chronic illnesses as well increasing longevity (Orlich et al., 2013). It has been shown that such dietary changes could help lose weight more effectively whilst reducing potential hazards for heart diseases or even dying prematurely (Tuso et al., 2013).

The approach known as intermittent fasting is a popular dietary method wherein one alternates between standard feeding times with periods of reduced or non-existent food consumption. Researchers are interested in this method because it may provide benefits on aging and overall metabolism (Patterson & Sears 2017). For instance, studies suggest that an intermittent fasting regimen can boost insulin sensitivity while also reduce inflammation in the body. Additionally, this practice is linked to promote cellular repair mechanisms that may assist in warding off age-related diseases like diabetes, cancer and neurodegenerative disorders (Patterson & Sears 2017). Health gains linked to adaptive cellular responses through fasting include enhanced metabolic functioning and stress-resilience along with potential delays in aging and lowered disease risks (Longo & Mattson, 2014). While promising results have emerged, further investigation is necessary in order to understand the precise underlying mechanisms involved in order to optimize fasting practices for health purposes (Longo & Mattson, 2014).

To sum it all up, there is strong evidence that some dietary practices such as the Mediterranean diet or a plant-based style or even intermittent fasting could lead to healthier aging while also reducing susceptibility towards age associated diseases. At their core lies an emphasis on consuming whole foods that contain essential nutrients required by human bodies for optimal metabolic function alongside boosting immune systems and cellular functions engendering better health outcomes and longevity.

## Gut Microbiota and Aging

The presence of gut microbiota, a group of microorganisms present within our intestines, plays an integral role in safeguarding human health and is also linked to aging (Biagi et al., 2016). Its correlation with the aging process has been established through several studies, signifying changes concerning their diversity, composition range along with their functionality (Biagi et al., 2016). It is thought that these alterations arise due to two factors: deteriorating immune defense mechanisms (immunosenescence) and continuous low-grade inflammation known as inflamm-aging (Vaiserman et al., 2017). Dietary adjustments and probiotics are among the interventions that focus on the microbiota. They exhibit excellent results in enhancing health and lifespan by improving antioxidant activity, immune homeostasis, suppressing chronic inflammation, regulating of fat deposition and metabolism while preventing insulin resistance (Vaiserman et al., 2017). Notably, the diet affects gut microbiota significantly, therefore dietary interventions can be used to modulate this microbiota which potentially can affect age-related diseases (Claesson et al., 2012; David et al., 2014). Probiotics are live microorganisms with several health advantages when consumed sufficiently, such consumption ultimately enhances both composition and functionality of our gut microbiota (Hill et al., 2014). Regular consumption of probiotics sources such as kefir, yogurt and fermented vegetables can help ensure overall well-being by maintaining a balanced microbiota (Markowiak & Śliżewska, 2017). Prebiotics, food components that selectively foster the growth and functionality of beneficial bacteria, are not digested. Accordingly, they represent another dietary compound capable of impacting the gut microbiota positively (Roberfroid et al., 2010).Prebiotic effects are

defined as a set of processes, whereby particular dietary choices lead to selective stimulation of growth or activity for a limited number of microbial species in the gut microbiota. These ultimately result in advantageous health outcomes for the host (Roberfroid et al., 2010). Numerous rigorous studies agree with this approach, including various human interventions that prove that changing our diet significantly alters our gut microbiota composition. Currently, an increase in bifidobacteria density is considered as indicative of good intestinal health (Roberfroid et al., 2010). Additionally, prebiotic effects may affect immune system biomarkers and activities (Roberfroid et al., 2010).Infant nutrition has shown that incorporating prebiotics into a child's diet can yield significant results. One example includes changing the composition of gut microbiota leading to improvements in stool quality, decreased risk of gastrointestinal infections, enhanced general health and finally less allergic symptoms like atopic eczema (Roberfroid et al. 2010). During initial experimentation phases on patients with inflammatory bowel diseases or irritable bowel syndrome, promising results were seen when incorporating food products with prebiotic effects in the patient's diet. This indicates possible therapeutic advantages, including alterations in gut microbiota composition (Roberfroid et al., 2010). Moreover, specific food products containing prebiotics demonstrated their ability to lower tumor and cancer occurrence following experimental trials, thereby signaling key preventative potential for various types of cancer (Roberfroid et al., 2010).

A number of studies has found that innovative treatments including fecal microbiota transplantation are highly effective and safe measures that can reduce symptoms arising from age-related diseases such as Parkinson's, type 2 diabetes and atherosclerosis, among others (Vaiserman et al., 2017). These studies underline the critical involvement of gut microbiota in aging. Further highlighting the fact that targeting these microbes through anti-aging medicine can indeed achieve promising results (Vaiserman et al., 2017).Moreover, the fermentation of indigestible dietary essentials by gut microbiota produces short chain fatty acids (SCFA) having anti-inflammatory properties, which can positively impact longevity (Canfora et al., 2015, Ríos Covián et al., 2016).

## Future Research and Perspectives

Countless observations indicate that numerous nutrients and bioactive substances hold potential for impacting our natural process of aging. However, despite all efforts spent so far, it seems we have not found a conclusive understanding underlying the molecular or cellular pathways yet. Progressing methods continue exploring the role of essential nutrients in cellular key processes such as oxidative stress, DNA damage/repair, inflammation and telomere length. Similarly, on an organismal level, still remains unknown the relationship between these compounds and the relatively complex endocrine, immune and neural networks that regulate aging (Niccoli & Partridge, 2012; López-Otín et al., 2013; Barzilai et al., 2012). The way we eat not only impacts our immediate well-being but also can influence how we age through interactions with our genome and affecting gene expression (Mathers, 2006). Advances in high throughput genomics technologies, such as nutrigenomics, have opened up new opportunities for researchers seeking to explain this relationship more clearly by better understanding its molecular mechanisms (Mathers, 2006).

Mathers (2006) suggests that our diet along with lifestyle factors can manipulate gene expression, subsequently impacting cell-function and overall health throughout our lifetime. Personalized nutrition approaches must be developed, tailored to individual predispositions leading to optimal benefits concerning healthy ageing (Mathers, 2006). Human studies that are both large scale and well controlled, are vital to determining evidence-based dietary recommendations suitable for individuals across varying life stages and with different health issues (Mathers, 2006). To better understand nutrition's' impact on aging biology, future research deserve attention as they address gaps in knowledge and help researchers to develop effective dietary strategies that promote healthy aging while preventing age-related diseases (Mathers, 2006).

## The Role of Exercise in Aging Biology

As our world-population ages and age-related diseases become more prevalent,

there has been an escalating focus on understanding the impact that exercise has on aging biology through research studies. The significance of physical activity as an integral part of one's lifestyle cannot be understated when it comes to influencing how we experience aging, commonly referred to as health span, where individuals are able to live without chronic ailments or disabilities hindering them. Appreciating this link between exercise and aging holds immense value as it presents an opportunity for cost effective-interventions that are readily available for delaying age-related diseases while enhancing overall well-being among older adults (Lavie et al., 2019). Multiple studies have demonstrated the positive effects of regular physical activity on different aspects of aging biology. For instance, exercise has proven to be effective in enhancing cardiovascular health preserving muscle mass and strength, improving cognitive function as well as supporting mental well-being among older individuals (Northey et al., 2018; Pedersen & Saltin, 2015). Furthermore, engaging in physical activity has been associated with a reduced likelihood of developing age-related conditions such as heart disease, type 2 diabetes, Alzheimer's' disease, and certain forms of cancer (Booth et al., 2012).

One reason why regular exercise is beneficial to health is its ability to generate ROS within a stimulatory range (Radak et al., 2005). Moreover, this exercise-induced ROS production plays a critical role in enhancing the production of antioxidants stimulating DNA repair mechanisms and promoting protein degradation enzymes (Radak et al., 2005). These processes collectively contribute to reducing the occurrence of diseases related to oxidative stress and slowing down the aging process (Radak et al., 2005). Additionally, exercise helps maintain muscle-mass and function, which are vital for preserving physical independence and preventing age-related sarcopenia (Cruz-Jentoft et al., 2019).

The impact of exercise goes beyond its direct effects on cellular and molecular processes and can also play a role in modulating the aging process through its influence on the gut microbiota. A growing body of research points to connections between physical activity, gut microbiota composition and health outcomes among older adults. This emerging evidence suggests that exercise has potential as a mean to promote healthy aging by targeting the gut microbiota and altering its metabolic functions (Mailing et al., 2019). In spite of mounting evidence highlighting the significance of exercise in the process of aging at a biological level, a more comprehensive research approach would be beneficial to establish which types, frequencies, durations and intensities that produce ideal outcomes across different

populations with regards to promoting healthy aging. In addition, it is worth considering personalized approaches when prescribing exercises by taking into account specific genetic, epigenetic, and environmental factors, an approach that may significantly enhance effectiveness when conducting interventions aimed at improving overall health among elderly individuals (Buford et al., 2010).

## Exercise and Hormonal Balance

There are various factors that can affect hormonal secretion, such as genetic makeup, lifestyle choices, environmental conditions, diet and exercise. These hormones have an important role to play in the human body. Particularly in muscle growth, bone development, and metabolic regulation (Sellami et al., 2019). It has been found that exercise training also has an impact on hormone secretion. With the extent of the impact depending on factors like frequency, duration, intensity and mode of training (Sellami et al., 2019). However, there is still some uncertainty about how exercise training specifically affects glucoregulatory hormones in older adults (Sellami et al., 2019). Additionally, as individuals age there are various changes that occur in their endocrine system which may potentially impact human physiology as well (Sellami et al., 2019).Recent studies suggest that exercise training could have an anti-aging effect on the endocrine system, particularly affecting cortisol, growth hormone, and insulin (Sellami et al., 2019). Exercise training has been gaining interest as a therapeutic lifestyle strategy to mitigate aging and improve health. It has been observed that exercise training can attenuate many biological markers of aging by promoting a more "youthful" endocrine profile (Sellami et al., 2019). Despite this growing interest, there is a relative lack of research on the physiological effects of physical activity and exercise on glucoregulatory hormones in elderly subjects. While insulin has received considerable attention in this regard, data for other hormones are limited (Sellami et al., 2019). Given the importance of understanding the anti-aging effects of sports and physical activity the researchers emphasize the need for more attention in this field including longitudinal studies involving larger sample sizes (Sellami et al., 2019).

## Future Research and Perspectives: Understanding the Role of Exercise in Aging Biology

The concept referred to as a "metabolic clock" finds support through observations indicating alterations in metabolism and a decline in biological fitness as we age (López-Otín et al., 2016). Certain inborn defects in metabolic pathways can enhance the aging process, whereas specific genetic loci linked to extraordinary longevity can have influence on metabolism. Each of the nine hallmarks associated with the aging process is tied to unfavorable metabolic changes (López-Otín et al., 2016) (see Figure 1). Various factors commonly observed within a "westernized" lifestyle, including calorie-rich diets and a lack of physical activity can expedite the effects of aging due to their detrimental impact on metabolism. The study of metabolism is presently in a period of renewed interest, leading to an improved comprehension of how metabolites fulfill vital roles in all physiological and pathological processes related to aging (López-Otín et al., 2016).

Multiple studies have shown that lifestyle intervention strategies, which include regular exercise, can have a preventive effect on type 2 diabetes and slow down the age-related loss of skeletal muscle mass (Cartee et al., 2016). By engaging in endurance exercises, individuals can improve their insulin sensitivity and reduce body fat. Additionally, resistance exercises are effective in slowing down the decline in muscle mass and strength. These interventions play a crucial role in maintaining insulin sensitivity, body weight, and functional muscle-mass (Cartee et al., 2016). However, it is important to consider various factors such as glucose homeostasis, mitochondrial function, and muscle-mass regulation when translating this knowledge into targeted clinical care. Furthermore, it is essential to adapt exercise protocols to meet the physical limitations of older and frail individuals for them to achieve "healthy aging" (Cartee et al., 2016). Moreover, understanding how different lifestyle interventions like nutrition and physical activity in order to manage insulin resistance, obesity, and age-related muscle atrophy are a key. By early implementation of strategies to maintain insulin sensitivity and prevent muscle atrophy, the negative effects of unhealthy aging with the associated healthcare costs can be reduced. The evidence suggests that regular physical activity and exercise may hold the solution to achieving "healthy aging" by limiting secondary aging caused by modifiable lifestyle factors (Cartee et al., 2016).

Some studies indicate that regular exercise may be also beneficial for the immune systems of older adults. However, further investigation is necessary to establish the optimal forms and durations of exercise that can help preserve immune function as people age (Simpson et al., 2012). Additionally, more research is needed to explore the potential of exercise in reducing age-related cognitive declines and neurodegenerative diseases like Alzheimer's and Parkinson's. Although there is evidence to suggest that exercise can improve cognitive function and lower the risk of neurodegenerative diseases, there is limited understanding of the mechanisms underlying these effects (Bherer et al., 2013).Hence, it is crucial for future studies to identify the molecular pathways through which exercise influences brain health and investigate how exercise could potentially be used for preventing and treating neurodegenerative diseases (Bherer et al., 2013). Lastly, personalized exercise prescription has gained attention in the field of exercise and aging, given that individual responses to exercise can vary greatly due to factors such as genetics, lifestyle and health status. It is becoming increasingly important to develop tailored exercise interventions that maximize the benefits of physical activity for each individual (Pedersen & Saltin, 2015). In conclusion, there are numerous areas in exercise and aging that warrant further research. By addressing the current gaps in our knowledge, concerning the role of exercise in aging, research can contribute towards designing more effective exercise interventions and strategies for promoting healthy aging.

## Conclusion

This thesis highlights the significant findings that underscore our current understanding of aging as not an immutable process but rather one that can be influenced by a myriad of factors. This realization opens up novel avenues for generating interventions that can foster healthy aging and prolong the period of good health, ensuring that the additional years we live are satisfying, productive and free from chronic diseases or disabilities. Throughout this thesis, various theories have been explored to unravel the mechanisms underlying aging. The free radical theory, hormonal theory, telomere theory, inflammation theory and mitochondrial theory have all contributed valuable insights into the complex

interplay between genetic predispositions, cellular and environmental factors contributing to the aging process. By comprehending these mechanisms, researchers can now identify potential targets for interventions aimed at slowing down or even reversing certain aspects of aging. Furthermore, specific pathways and processes have emerged as fundamental agents in driving the process of aging. The target of rapamycin (TOR), sirtuins, NAD+ metabolism, circadian clocks, senescence, insulin-like signaling pathway, proteostasis, autophagy process and AMPK pathway have all been recognized as influential factors in the aging process. Manipulating these pathways through pharmacological interventions holds hope for extending periods of good health and mitigating age-related decline. In addition to pharmacological interventions, various approaches have been explored to promote healthy aging. Reactivation of telomerase, elimination of damaged cells, activation of chaperones and proteolytic systems, stem cell-based therapies for regenerative medicine purposes, mitochondrial hormetics, application of mitophagy as a process for removal of damaged mitochondria, as well as utilization of anti-inflammatory drugs along with rejuvenating blood-borne factors are all promising avenues for intervention. In order to enhance health span by targeting different aspects of aging while up keeping cellular and physiological function through multifaceted approaches, it must be recognized that alongside pharmaceutical interventions as well as targeted therapies holding great potential, it is important to recognize the broader context of healthy aging. Recent discoveries emphasize two key lifestyle factors- nutrition along with exercise - as important elements which largely influence one's pace of aging. Maintaining a nutritious diet inclusive of appropriate caloric restriction along with managing macronutrient balance (protein content, fats along with carbohydrates) and micronutrient intake enables profound impacts on health span. Influence of specific dietary patterns and the role of gut microbiota in aging provide immense scope for future investigation. Also, exercise undoubtedly plays an integral role in promoting healthy aging with strong links being established between regular physical activity and hormonal balance, improved cardiovascular health, enhanced cognitive functioning along with holistic well-being. Thus, for further research to fully unravel the exact mechanism through which exercise exerts its beneficial effects shall only add clarity as to how to optimize exercise regimens for healthy aging. In conclusion, it is this thesis that highlights the acknowledged fact that aging is a complex, as well as a multifaceted process, influenced by various factors. However, it is crucial to acknowledge that further research is needed to fully comprehend the intricacies of aging and to evaluate the efficacy and

safety of these interventions. Finally, adopting a comprehensive approach combining pharmacological interventions, lifestyle modifications and focusing on nutrition and exercise holds a great promise achieving the goal of "dying young at an old age", thereby ensuring that any additional years are characterized by vitality, freedom from age-related ailments along with general well-being.

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