Subcellular Biochemistry 103

J. Robin Harris
Viktor I. Korolchuk *Editors*

Biochemistry and Cell Biology of Ageing: Part IV, Clinical Science

Springer

Subcellular Biochemistry

The book series SUBCELLULAR BIOCHEMISTRY is a renowned and well recognized forum for disseminating advances of emerging topics in Cell Biology and related subjects. All volumes are edited by established scientists and the individual chapters are written by experts on the relevant topic. The individual chapters of each volume are fully citable and indexed in Medline/Pubmed to ensure maximum visibility of the work.

J. Robin Harris • Viktor I. Korolchuk
Editors

Biochemistry and Cell Biology of Ageing: Part IV, Clinical Science

Springer

Editors
J. Robin Harris
Institute for Molecular Physiology
University of Mainz
Mainz, Germany

Viktor I. Korolchuk
Biosciences Institute
Newcastle University
Newcastle upon Tyne, UK

ISSN 0306-0225 ISSN 2542-8810 (electronic)
Subcellular Biochemistry
ISBN 978-3-031-26575-4 ISBN 978-3-031-26576-1 (eBook)
https://doi.org/10.1007/978-3-031-26576-1

This Springer imprint is published by the registered company Springer Nature Switzerland AG
The registered company address is: Gewerbestrasse 11, 6330 Cham, Switzerland

Preface

J. Robin Harris and Viktor I. Korolchuk

This fourth book on Ageing in the *Biochemistry and Cell Biology of Ageing* "subseries" of *Subcellular Biochemistry* continues the approach established in our first three books, that is, to cover as many as possible relevant and interesting Ageing research topics in advanced review chapter format.

The seemingly almost endless expansion of Ageing research over recent years indicates the priority being given to the broad understanding sought by scientists, clinicians and others, with the overall aim of improving the life quality of elderly people. Thus, we anticipate incorporating new topics and updating previously included chapter topics in future books. Indeed, a fifth book, *Anti-ageing Interventions* has already been commissioned by Springer Nature.

The diverse chapters of the present book (*see* Chapter list immediately following) can be readily appreciated and should be considered alongside the content of the three earlier books at the series Website: https://www.springer.com/series/6515/books. Following a useful introductory chapter, covering the Historical Development and Progression of Clinical Research on Ageing by Carmen García-Peña and colleagues, 14 further chapters present the strong themes of the book. Indeed, there were initially due to be two other chapters, but undoubtedly owing to the increasing pressures of academic work post-COVID, these were lost. The final 16th chapter of the book, by Aurel Popa-Wagner and colleagues, presents a broad survey of Clinical Ageing, highly pertinent to the book's overall content.

Each chapter is written by authoritative clinical scientists who have published extensively in the Ageing field. We hope that this book, along with the three others, cumulatively provides an almost encyclopaedic coverage of the subject. The book will be of interest and value to undergraduate biomedical science students, medical students, postgraduate researchers, clinicians and academics involved with and interested in aspects of Ageing research. The parallel availability of the e-book and e-chapters greatly opens up the broad accessibility.

Contents

Chapter 1
Introduction: Historical Development and Progression of Clinical Research on Ageing

Carmen García-Peña, Pamela Tella-Vega, Raúl Hernán Medina-Campos, and Héctor García-Hernández

Abstract Research on ageing has developed since Greek times. It had a very slow advance during the Middle Ages and a big increase in the Renaissance. Darwin contributed somehow to the understanding of the ageing process and initiated a cumulus of ageing explications under the name of Evolutionary Theories. Subsequently, science discovered a great number of genes, molecules, and cell processes that intervened in ageing. This led to the beginning of trials in animals to retard or avoid the ageing process. Alongside this, improvements, geriatric clinical investigations (with the evidence-based medicine tools) started to consolidate as a discipline and commenced to show the challenges and deficiencies of actual clinical trials in ageing; the COVID-19 outbreak revealed some of them. The history of clinical research in ageing has already begun and is essential to affront the challenges that the world will face with the increasing ageing population.

Keywords Ageing · Clinical research · Geriatrics · Geroscience · History

Introduction

Clinical research on ageing is closely intertwined with the development of geriatrics as a medical specialty, but it is also related to and preceded by the development of the broader field of biomedical research on the ageing process itself. In this introductory chapter, we will briefly review the historical development of notions of the process of ageing. Then, we will delve into the origins and progression of clinical research on ageing. Finally, we will discuss the emergence of geroscience as a point of convergence for basic, clinical and translational research in the field of ageing.

C. García-Peña (✉) · P. Tella-Vega · R. H. Medina-Campos · H. García-Hernández
Research Division, Instituto Nacional de Geriatría, Mexico City, Mexico
e-mail: mcgarcia@inger.gob.mx; ptella@inger.gob.mx; rmedina@inger.gob.mx; hector.garcia.hernandez@facmed.unam.mx

J. R. Harris, V. I. Korolchuk (eds.), *Biochemistry and Cell Biology of Ageing: Part IV, Clinical Science*, Subcellular Biochemistry 103,
https://doi.org/10.1007/978-3-031-26576-1_1

Although ageing can occur in most animal species, it is a relatively rare phenomenon in nature. Our own species is an example of this—for the most part of our history on Earth since the mid-Pleistocene, the life expectancy of *Homo sapiens* is thought to have been less than 25 years. No prehistoric human remains have been found that can be assigned an age at death older than 50 years (Hayflick 2004). Life expectancy in ancient Greece and the Roman Empire is thought to have been between 18 and 22, while in medieval Britain, it is estimated to have ranged from 17 to 35 (Omran 1971). It was not until the twentieth century that life expectancy dramatically increased from 47 to 72 years (United Nations 2015). One can argue that old age, as a massive, everyday phenomenon, is a very recent occurrence in human history. Notwithstanding, ageing has been the source of multiple philosophical, theological, and biological questions throughout the history of human thought. It has generated remarkably diverse views and explanations on the matter, which have changed in accordance with the dominant paradigm.

From the biological point of view, there might be three main broad questions that have driven scientific research on ageing, two of them pertaining to the nature of ageing itself: why do we age? and how do we age? The first question pursues an explanation of the very existence of a biological process that seems to be, at first sight, counterintuitive from the evolutionary point of view. There are no clear survival advantages or fitness traits that come with ageing, but on the contrary, ageing entails an increase in the risk of dying from extrinsic and intrinsic causes. Older individuals are more susceptible to dying from degenerative disease, infection, environmental harm and depredation than younger—*fitter*—individuals. The second question pertains to the biological mechanisms that transform a young, fit individual into an old, frail one. A large corpus of research has focused on these mechanisms of ageing, especially through the second half of the twentieth century (Kirkwood and Austad 2000; López et al. 2013).

A third relevant broad question in the field of ageing is whether the mechanisms of ageing can be modified or intervened to prevent, slow or reverse ageing itself, and whether the diseases that are associated with ageing and old age can be prevented, delayed, treated, rehabilitated, or cured. These three questions pertain not only to a biological perspective but to the field of clinical research. Biological and clinical research on ageing have found an intersection in the emerging field of geroscience, which we will briefly touch upon later in this chapter.

From Early Civilizations to the Age of Enlightenment

In his *Republic*, Plato argued that people gained experience at every stage of life, which conferred a natural wisdom to old age (Scarre 2016). Most importantly, he also posed that ageing and disease are different concepts (Weintrob 2022).

Aristotle considered ageing as a natural process, but one with negative connotations, as can be noted in his work *On Youth, Old Age, Life and Death, and Respiration* (Woodcox 2018). He viewed old age as lack of virtue, responsibility,

moderation, and rationality (Scarre 2016) and associated it with the decay of the body and intellect (Weintrob 2022).

Cicero had a notion of old age closer to Plato's. In his work *De Senectute*, he argued that old age could be enjoyable, and that physical health could be enhanced by practicing virtue, thus suggesting the possibility of a healthy ageing (Scarre 2016).

During the Middle Ages, the dominant worldview was marked by religious determinism. As such, the course of human life was considered the reflection of the divine will. Older persons were not subject of any considerations, as far as it is known (Shahar 1993). The charity of the church was intended for orphans, widows and those disabled by war or illness, but not for older persons (Gilleard 2002). However, it is curious to find that in some medieval evidence older age was related to wealthiness and to have benefits, like non-payment of taxes. Also, the scientific and intellectual advance was too small in that period and the ideas of sickness were dominated by the miasmatic and the humors equilibrium theories. Thus, the study of ageing had no significant progress during this time (Shahar 1993).

By the late Middle Ages, several economic, cultural, political, and social changes took place in Europe. The figure of the State emerged and began to claim relevance. Population censuses started being undertaken during the fifteenth and sixteenth centuries, which would end up making older persons visible (Gilleard 2002). However, life expectancy was still not higher than 30 years (Shahar 1993).

Also, during this period, many works were developed about ageing. Their content was about how to delay ageing by having healthy lifestyles. The books were written mostly by Italian physicians, because in Italy the "scholar" medical discipline began. But they were greatly influenced by the Galenic notion of sickness (the "non-naturals" external factors that attack somehow the body and cause ageing) that dominated the paradigm of getting old. Now these are considered as precursors of modern gerontology and geriatrics (Gilleard 2013).

An interesting fact during the Renaissance was that the humoral model of old age, which prevailed in the Middle Age (the "non-natural" forces like magic or witch-craft), was not challenged or modified by the Renaissances physicians. However, none of them treated old age as a pathological status. It was not until the nineteenth century that old age was discussed as a form of pathology through scientific discoveries (Gilleard 2013).

Modern Ageing Research

The social, economic, and political changes that followed the Industrial Revolution, together with the advancement of science and technology, paved the way for an accelerated increase in life expectancy for the human species during the nineteenth and twentieth centuries. This demographic transition occurred first in European countries and later spread to the rest of the world, with some regions such as Africa still lagging but nonetheless moving in the same direction. The main features of the

demographic transition are reduced mortality, reduced fertility, and increased life expectancy. An epidemiological transition followed, with chronic, non-transmissible diseases gradually replacing transmissible diseases as the main causes of morbidity and mortality (Omran 1971). Ageing and old age gradually became a more common occurrence in human societies, thus increasing the interest in this topic.

Evolutionary Theories of Ageing

Prior to the nineteenth century, there was little scientific interest in the mechanisms of ageing. The common belief was that organisms deteriorated gradually in a similar way to inanimate objects. Charles Darwin changed the paradigm in the biological sciences with his survival of the fittest theory of evolution published in *The Origin of Species* in 1859 (Goldsmith 2017). Elaborating on Darwin's theory, August Weissman—a recognized biologist—suggested that the death of older individuals was part of the natural selection as it favored the evolution process by making space for the younger, reproductively active individuals (Fabian and Flatt 2011). However, this theory failed to explain certain ageing processes in mammals and other multiparous species (Goldsmith 2017). Moreover, in some cases the long-lived individuals may produce more offspring (Fabian and Flatt 2011).

In the middle of the 1900s, the biologists Peter B. Medawar and George C. Williams argued that ageing appeared because of a gradual deterioration of the natural selection process that resulted in a malfunction at old age (Medawar 1957; Williams 1957) which varied across species and among individuals of the same species. This spawned several theories, collectively known as non-programmed theories of ageing: the mutation accumulation theory (MA), antagonistic pleiotropy theory (AP) and disposable soma theory (DS) (Goldsmith 2017). In the MA theory, Medawar hypothesized that mutations producing the deleterious effects of ageing were not suppressed because of a weak effect of natural selection in late life, thus allowing these mutations to be carried on generation after generation. The AP theory suggested that genetic traits with pleiotropic effects might be responsible for ageing and be allowed to subsist by natural selection because of their positive effects at the early stages of life, despite deleterious effects in late life. Kirkwood in his disposable soma theory, posed that the main purpose of individuals is to reproduce, and that their bodies (somas) are maintained and repaired for as long as their reproductive fitness is relevant to the survival of the species; once reproductive fitness begins to decline with age, maintenance, and repair systems decline as well, thus resulting in the appearance of ageing (Kirkwood and Austad 2000).

Although evolutionary theories did not originally consider ageing as a regulated process, evidence began to emerge during the twentieth century that changed this perspective. Researchers in this field continue to identify and describe key factors involved in the rate of ageing. Processes and changes at the cellular and molecular level that increase morbidity and influence the response to related interventions were

identified. The importance of these discoveries now lies in the identification of factors that lead to successful ageing and improved quality of life.

Molecular Research on Ageing

Biological and biochemical techniques for research started to develop at the beginning of the twentieth century, but it was not until the second half that they had an impact on the field of ageing research. At the same time, the idea that ageing was the cause of age-related diseases emerged. Even though formal causality has never been proven, it is now established that ageing is the most important risk factor for many of these diseases (Campisi et al. 2019).

One of the first breakthroughs in the understanding of the mechanisms of ageing was the finding that calorie restriction (CR) increased lifespan in mice in 1939. Later, it was observed that human diseases such as Hutchinson-Guilford and Werner Syndromes appeared to accelerate the ageing process, with the early appearance of features of old age, including alopecia, osteoporosis, cataracts, skin atrophy, and an elevated risk of malignant tumors. These observations led to the notion that the pace of ageing can be modified (Zainabadi 2018).

Fundamental discoveries followed that helped the understanding of the mechanisms of ageing, including apoptosis, telomere function, insulin-like growth factor signaling pathway (ILS), target of rapamycin (TOR) proteins, sirtuins (Sir), NAD+ coenzyme, reactive oxygen species and senolytics (molecules that destroys old cells) (Goldsmith 2017; Campisi et al. 2019; Zainabadi 2018). Eventually, the manipulation of some of these mechanisms would prove to prolong lifespan in several animal models, including mammals (Zainabadi 2018). Research in humans followed, with relevant findings such as the association of ILS genes and the sirtuin pathway with increased longevity in human populations across the globe (Campisi et al. 2019; Zainabadi 2018).

At least five major classes of drugs had being tested in humans for their geroprotective potential. These include metformin, rapamycin analogues that inhibit the TOR pathway, senolytics that eliminate senescent cells, sirtuin activators which enhance sirtuin activity and NAD+ precursors, which counter the decrease in intracellular NAD (Campisi et al. 2019).

Yet, the intervention which most consistently prolongs lifespan and improves health in animals and humans is the combination of exercise and a healthy diet. Exercise has shown a great efficacy in reducing the incidence of age-related disease, increasing lifespan and improving quality of life. Healthy diets, although variable in their characteristics, have been shown to favor longevity. Common features to nutritional interventions of healthy diet include a minimum of processed foods, predominantly plant-based, low alcohol level and a lack of overeating (Campisi et al. 2019).

The advances in genetical, biological, and biochemical sciences in the past century have already demonstrated that ageing is a complex process that involves

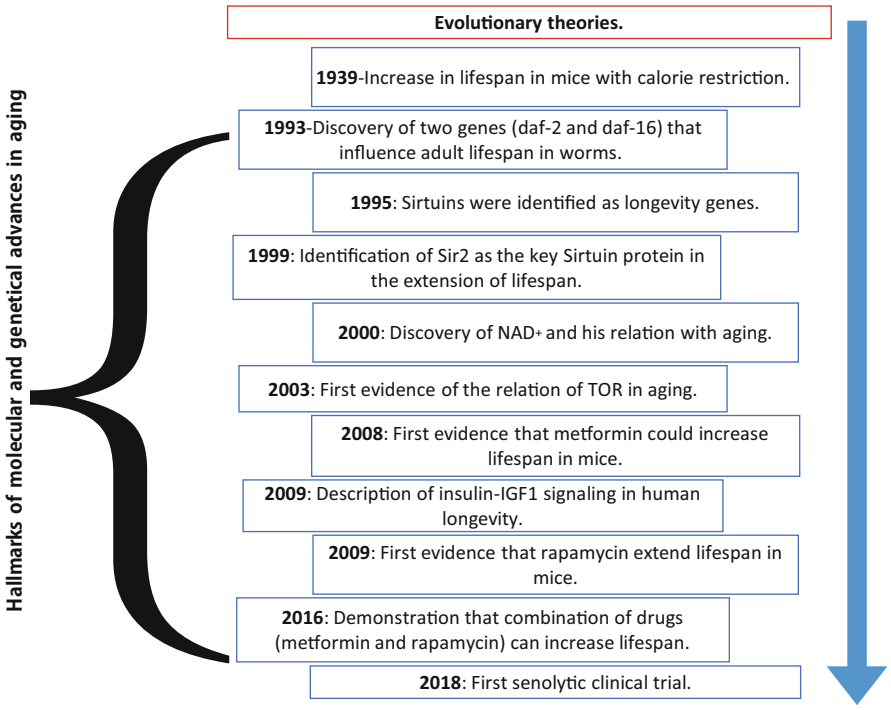


Fig. 1.1 Hallmarks of molecular and genetical advances in ageing. Self-made figure based on Campisi et al. (2019) and Zainabadi (2018)

a lot of genes, multiple molecules pathways and various mechanisms inside cells that could somehow be modified and regulated. Also, there is sufficient evidence to affirm that ageing is not part of a big evolutionary plan of nature, and obviously, it is not a disease. As well, science has brought us a bunch of pharmacological drugs that could intervene to prolong life and improve health in populations (Zainabadi 2018).

In Fig. 1.1 we summarize the hallmarks of molecular and genetical advances in ageing.

Geroscience: An Integrative Approach

With ageing research advancing at the molecular level, it soon became evident that many age-related diseases shared fundamental biological mechanisms with the process of ageing itself. This led to the development of geroscience, a new, transdisciplinary field that aims to understand the link between ageing and disease, while recognizing the importance of both genetic and environmental influences throughout the life course in the ageing process.

One of the main goals of geroscience is to achieve translation of scientific knowledge into clinical practice and public health policies. This can be achieved by five main routes: (1) Moving geroprotective therapies from animals to clinical trials; (2) Performing geroprotective trials incorporating expertise in inclusive sampling, adherence and retention; (3) Having solid measures of ageing and outcome metrics (noninvasive, inexpensive, repeatable, reliable, and sensitive to biological change); (4) Appropriately identifying fast- and slow-agers in clinical trials, and (5) Bringing the benefits of geroscience to people who need it most (Sierra et al. 2021; Sierra 2019).

Clinical Research on Ageing

The term "geriatrics" was introduced in 1909 by Ignatz Leo Nascher in a homonymous article and later in his book "Geriatrics: The Diseases of Old Age and Their Treatment" (Nascher 1914). The development of modern geriatrics is usually attributed to Marjory Warren and several other physicians in the United Kingdom (UK) during the twentieth century. In that country the development of modern geriatric treatments was taking place: innovations to enhance the environment, introduction of active rehabilitation programs, the implementation of domiciliary (home) visits for rehabilitation, environmental modification to prevent falls, the creation of the first geriatric syndromes terms (instability, immobility, intellectual impairment, and incontinence), among others (Morley 2004).

Also, geriatric scientific journals were developed principally in the United States and UK to disseminate ageing research, for example: The Journal of Gerontology in 1946, The Gerontologist in 1961 or the Journal of the American Geriatrics Society in 1953 (Morley 2004).

During the end of the second half of the twentieth century, a great advance in clinical geriatric investigation field took place around the globe. It was boosted by the concept of evidence-based medicine (EBM). EBM was defined as the conscientious, explicit, and judicious use of current best evidence in making decisions about the care of individual patients (Guyatt 1991). This practice started to have a great progress based on the multiple clinical trials that were carried out. However, the evidence generated was obtained principally from younger persons participating in such clinical trials. The validity of the conclusions of such trials for older persons was soon brought into question, because of the evident differences between these age groups (Mooijaart et al. 2015). Physiologic changes that accompany ageing, a higher degree of multimorbidity and a greater probability of multiple-system involvement with every episode of disease, make older persons a very distinct population to study in clinical trials (Fontana et al. 2014). In addition, older persons are more likely to drop out of trials, be lost to follow-up and tend to exhibit higher risks—for example, more side effects or complications—and lesser benefits than younger adults (Mooijaart et al. 2015).

Evidence from clinical trials involving younger adults cannot be readily translated into clinical practice for older persons. In randomized controlled trials (RCTs), older persons are often underrepresented because the inclusion and exclusion criteria discriminate against them. Most of the time treatment recommendations are based on younger populations, as older adults are more vulnerable to adverse effects. Seldom are older persons with multimorbidity, cognitive impairment, frailty, and polypharmacy included in randomized controlled trials (Tan et al. 2018).

Certainly, clinical research in older adults has specific complications that differ in essential aspects from research in other fields. RCT are complicated to implement due to issues related to recruitment, selection, and follow-up of participants, as well as monitoring the safety and appropriateness of interventions in this population group (Faes et al. 2007).

A higher burden of comorbidities—chronic diseases, cognitive impairment, and frailty—make it difficult to discriminate whether a specific outcome resulted from the disease being studied or from a concomitant condition (Tan et al. 2018; Faes et al. 2007). Older persons are less likely to be able to attend to the study center multiple times or may have difficulty recalling important self-reported data. Most of these trials require long-term follow-up, so attrition rates (total loss to follow-up) are higher compared to other age groups (Gardette et al. 2007). So, there is limited evidence for decision-making in prescribing treatments in this group.

The global research response to the COVID-19 pandemic highlighted the underrepresentation of older persons in research. Clinical researchers appear to have not understood the specific needs of this population. Clinical research has focused on treatment more than prevention, on younger rather than older persons and on hospital care rather than community-based care. During the pandemic, most publications in the geriatric field focused on COVID-19 vaccines, frailty, outcome prediction and managing nursing home outbreaks (Witham et al. 2021).

In order to better respond to the care needs of older persons, it is necessary to prioritize clinical research on ageing, adapt research protocols and implement an interdisciplinary approach (Witham et al. 2021). Also, it is of great importance that the knowledge can be translated into clear and useful strategies that impact clinical practice, public health policies and the health and quality of life of older populations.

Innovative tools are being developed to improve and facilitate clinical research in older persons. Artificial intelligence (AI) and machine learning techniques can be used to analyze large amounts of routinely collected data (RCD). These data are usually available in clinical records, census databases, hospital databases and other sources, and can be used to understand differentiated clinical presentations, diagnoses, health status, health service use, economic health impact and other relevant outcomes for the care of older persons. These approaches, however useful, may fail to capture what in geriatrics is called "what matters the most to older persons"—i.e., they may not be useful for understanding patient-centered outcomes (Todd et al. 2020).

RCD has the potential to reduce research costs and allow the inclusion of older persons that would not be able to readily participate in clinical trials because of disability, distance, discrimination, or other reasons. Moreover, they could improve

the accuracy of prediction tools and allow for a more thorough assessment of the performance of health systems and their ability to meet older persons' care needs (Todd et al. 2020).

For RCD to be used in clinical research in older persons, a suitable data platform is needed that contains all data and makes it comparable and shareable. Subsequently, a collaborative web of clinical (and multidisciplinary) researchers should support each other to generate protocols, clean data and develop analytic methods and tools. An example of such a data platform is the Strategic Information System on Health, Functional Dependence and Ageing (Sistema de Información Estratégica en salud, Dependencia funcional y Envejecimiento—SIESDE) that was developed by the National Institute of Geriatrics in Mexico.

Challenges for Future Trials

Several challenges lie ahead in the path to achieving clinical research that yields interventions, drugs, and technologies to increase longevity and the quality of life of older persons. The first is related with animal research. Studies that assess molecules and processes related with age are often done under conditions with little relevance to human ageing. For example, experiments where inflammation effects are induced by a certain concentration of molecules that will never be seen in humans or the studies that tried to explore the effect of obesity on ageing in mice that are fed with high trans and saturated fats diet that no human would tolerate. Thus, experimental studies in animals should resemble human conditions as much as possible (Fontana et al. 2014). Also, the use of young animal models instead of aged models is inaccurate, since most biologic processes leading to age-related diseases occur predominantly in aged individuals (Kaeberlein and Tyler 2021).

As previously stated, plenty of molecular pathways are involved in the ageing process. Most of the experimental evidence in this regard comes from animal models, including worms, flies, and rodents. This evidence needs to be corroborated and validated in humans, within the appropriate ethical framework (Fontana et al. 2014). This is the second challenge for the clinical investigation in ageing.

One more challenge is the inclusion of real-world, older persons in clinical trials, so that the results can be readily translated to the point of care (Mooijaart et al. 2015).

The fourth challenge is guaranteeing a continuous financial funding for clinical ageing research. This field receives a little budget when compared to disease-centered research, such as Alzheimer's disease. In the USA, the National Institute of Ageing receives less than 1% of the National Institutes of Health's budget even though healthy ageing is one of the most important priorities in our time due to its financial, social, and health implications. A similar scenario is observed in Japan and Europe (Fontana et al. 2014).

The fifth is about the translational integration of all the knowledge generate in the basic and clinic science (Fontana et al. 2014). Scientific discoveries should benefit all societies.

While the sixth challenge is to ensure the clinical research investigation legitimacy. At the end of the twentieth century, the growing understanding of the ageing process and the identification of molecules and interventions that could extend the lifespan in animal models provided a fertile field for pseudoscience. By posing that ageing can be arrested and avoided, or treated as a disease, the Anti-ageing movement has developed mainly as a pseudoscientific field, albeit with considerable commercial success. Several anti-ageing books and publications have been followed by an expanding market of compounds, cosmetic treatments, exercise programs, therapies, foods, beverages, and supplements that claim to prevent, treat, or reverse ageing. It is a billion-dollar business that has brought struggle to the credibility of biomedical and clinical research in ageing (Binstock 2004).

On the other hand, both (biomedical and clinical research and the anti-ageing movement) have a big ethical issue. If one of them could accomplish the increase in lifespan beyond the "natural human process," humanity would face important political, social, and economic challenges related to poverty, inequity, violence, and discrimination in all its forms (Binstock 2004).

At present, the question remains whether scientific research should continue to pursue the extension of the human lifespan, or instead focus on enhancing quality of life, improving health status and promoting well-being in old age.

Final Remarks

This chapter focuses principally on history of clinical experimental research but we should not forget that observational designs in clinical ageing research are also important. Longitudinal studies are a source of fundamental evidence of the multifactor changes over time, but also case-control and descriptive designs are crucial to study causality, prognostic, and diagnostic topics. However, the discussion of observational research is beyond the scope of the present chapter (García et al. 2018).

Research in ageing requires interdisciplinary work from different perspectives that can address the complex problems and situations associated with this process. With the emergence of COVID-19 in 2020, a further challenge has been added to this work. This global situation has led researchers from different disciplines to adapt by modifying methods and procedures in their work. In the area of ageing, the social distancing and safety of participants and researchers had a major impact on the follow-up of many cohorts of older adults. In response to this scenario, it will be necessary to work on the introduction of important changes in the design of protocols in older adults and in the implementation of interdisciplinary approaches in response to the situations inherent to ageing and those derived from COVID-19.

There is currently an imbalance between the magnitude of the health needs of older adults and research activities. Although new service models have been developed in clinical practice, there is still a lack of rigorous evaluation of them. On the other hand, much of the clinical research that is conducted in geriatrics most often

lacks the impact to directly influence practice. For this reason, not only is a holistic and multidisciplinary approach essential, but it is also of great importance that this knowledge is translated into clear and useful messages that can be applied in different areas.

References

Binstock RH (2004) Anti-ageing medicine and research: a realm of conflict and profound societal implications. J Gerontol 59(6):523–533. https://doi.org/10.1093/gerona/59.6.b523

Campisi J, Kapahi P, Lithgow GJ, Melov S, Newman JC, Verdin E (2019) From discoveries in ageing research to therapeutics for healthy ageing. Nature 571(7764):183–192. https://doi.org/10.1038/s41586-019-1365-2

Fabian D, Flatt T (2011) The evolution of ageing. https://www.nature.com/scitable/knowledge/library/the-evolution-of-ageing-23651151/. Accessed 20 May 2022

Faes M, Van Iersel M, Rikkert MO (2007) Methodological issues in geriatric research. J Nutr Health Ageing 11(3):254–259

Fontana L, Kennedy B, Longo V, Seals D, Melov S (2014) Medical research: treat ageing. Nature 511:405–407. https://doi.org/10.1038/511405a

García C, Gutiérrez LM, Pérez MA (eds) (2018) Ageing research- methodological issues, 2nd edn. Springer, Switzerland

Gardette V, Coley N, Toulza O, Andrieu S (2007) Attrition in geriatric research: how important is it and how should it be dealt with? J Nutr Health Ageing 11(3):265–271

Gilleard C (2002) Ageing and old age in medieval society and the transition of modernity. J Ageing Identity 7(1):25–41. https://doi.org/10.1023/A:1014358415896

Gilleard C (2013) Renaissance treatises on 'successful ageing'. Ageing Soc 33(2):1–27. https://doi.org/10.1017/S0144686X11001127

Goldsmith TC (2017) Evolvability, population benefit, and the evolution of programmed ageing in mammals. Biochemistry 82(12):1423–1429. https://doi.org/10.1134/S0006297917120021

Guyatt GH (1991) Evidence-based medicine. Am Coll Physicians J 114(2):A16. https://doi.org/10.7326/ACPJC-1991-114-2-A16

Hayflick L (2004) "Anti-ageing" is an oxymoron. J Gerontol Biol Sci 59(6):576–578. https://doi.org/10.1093/gerona/59.6.b573

Kaeberlein M, Tyler J (2021) A new era for research into ageing. eLife 10:e65286. https://doi.org/10.7554/eLife.65286

Kirkwood T, Austad S (2000) Why do we age? Nature 9408(6809):233–238. https://doi.org/10.1038/35041682

López C, Blasco MA, Partridge L, Serrano M, Kroemer G (2013) The hallmarks of ageing. Cell 153(6):1194–1217. https://doi.org/10.1016/j.cell.2013.05.0392013

Medawar PB (1957) The uniqueness of the individual, 1st edn. Routledge, London

Mooijaart SP, Broekhuizen K, Trompet S, de Craen AJ, Gussekloo J, Oleksik A, van Heemst D, Blauw GJ, Muller M (2015) Evidence-based medicine in older patients: how can we do better? Neth J Med 73(5):211–218

Morley JE (2004) A brief history of geriatrics. J Gerontol 59(11):1132–1152. https://doi.org/10.1093/gerona/59.11.1132

Nascher L (1914) Geriatrics; the diseases of old age and their treatment: including physiological old age, home and institutional care, and medico-legal relations, 1st edn. University of California, California

Omran AR (1971) The epidemiologic transition: theory of the epidemiology of population change. Milbank Mem Fund Q 49(4):509–538

Scarre G (2016) The Palgrave handbook of the philosophy of ageing, 1st edn. Palgrave Macmillan, London

Shahar S (1993) Who were old in the middle ages? Soc Hist Med 6(3):313–341. https://doi.org/10.1093/shm/6.3.313

Sierra F (2019) Geroscience and the challenges of ageing societies. Ageing Med 2(3):132–134. https://doi.org/10.1002/agm2.12082

Sierra F, Caspi A, Fortinsky RH, Haynes L, Gordon I, Lithgow J, Moffitt TE, Olshansky S, Perry D, Verdin E, Kuchel GA (2021) Moving geroscience from the bench to clinical care and health policy. J Am Geriatr Soc 69(9):2455–2463. https://doi.org/10.1111/jgs.17301

Tan EC, Sluggett JK, Johnell K, Onder G, Elseviers M, Morin L, Vetrano DL, Wastesson JW, Fastbom J, Taipale H, Tanskanen A, Bell JS (2018) Research priorities for optimizing geriatric pharmacotherapy: an international consensus. J Am Med Dir Assoc 19(3):193–199. https://doi.org/10.1016/j.jamda.2017.12.002

Todd O, Burton JK, Doods RM, Hollinghurst J, Lyons RA, Quinn TJ, Schneider A, Walesby KE, Wilkinson C, Conroy S, Gale CP, Hall M, Walters K, Clegg PC (2020) New horizons in the use of routine data for ageing research. Age Ageing 49:716–722. https://doi.org/10.1093/ageing/afaa018

United Nations (2015) World population prospects - 2015. Key findings and advance tables. United Nations, New York

Weintrob G (2022) History of ageing research. https://www.research.colostate.edu/healthyageingcenter/2022/02/21/history-of-ageing-research/. Accessed 20 May 2022

Williams GC (1957) Pleiotropy, natural selection, and the evolution of senescence. Evolution 11:398–411. https://doi.org/10.2307/2406060

Witham MD, Gordon A, Henderson E, Harwood H (2021) Pandemic research for older people: doing it better next time. Age Ageing 50(2):276–278. https://doi.org/10.1093/ageing/afaa273

Woodcox A (2018) Aristotle's theory of ageing. https://journals.openedition.org/etudesanciennes/1040. Accessed 21 May 2022

Zainabadi K (2018) A brief history of modern ageing research. Exp Gerontol 104:35–42. https://doi.org/10.1016/j.exger.2018.01.018

Chapter 2
Bone Cells Metabolic Changes Induced by Ageing

Anca Cardoneanu, Ciprian Rezus, Bogdan Ionel Tamba, and Elena Rezus

Abstract Bone is a living organ that exhibits active metabolic processes, presenting constant bone formation and resorption. The bone cells that maintain local homeostasis are osteoblasts, osteoclasts, osteocytes and bone marrow stem cells, their progenitor cells. Osteoblasts are the main cells that govern bone formation, osteoclasts are involved in bone resorption, and osteocytes, the most abundant bone cells, also participate in bone remodeling. All these cells have active metabolic activities, are interconnected and influence each other, having both autocrine and paracrine effects. Ageing is associated with multiple and complex bone metabolic changes, some of which are currently incompletely elucidated. Ageing causes important functional changes in bone metabolism, influencing all resident cells, including the mineralization process of the extracellular matrix. With advancing age, a decrease in bone mass, the appearance of specific changes in the local microarchitecture, a reduction in mineralized components and in load-bearing capacity, as well as the appearance of an abnormal response to different humoral molecules have been observed. The present review points out the most important data regarding the

A. Cardoneanu · E. Rezus
Department of Rheumatology, "Grigore T. Popa" University of Medicine and Pharmacy, Iasi, Romania

Clinical Rehabilitation Hospital, 1st Rheumatology Clinic, Iasi, Romania
e-mail: anca.cardoneanu@umfiasi.ro; elena.rezus@umfiasi.ro

C. Rezus
Department of Internal Medicine, "Grigore T. Popa" University of Medicine and Pharmacy, Iasi, Romania

IIIrd Medical Clinic, "Saint Spiridon" Clinic Emergency County Hospital, Iasi, Romania
e-mail: ciprian.rezus@umfiasi.ro

B. I. Tamba (✉)
Advanced Research and Development Center for Experimental Medicine (CEMEX), "Grigore T. Popa" University of Medicine and Pharmacy, Iasi, Romania
e-mail: bogdan.tamba@umfiasi.ro

J. R. Harris, V. I. Korolchuk (eds.), *Biochemistry and Cell Biology of Ageing: Part IV, Clinical Science*, Subcellular Biochemistry 103,
https://doi.org/10.1007/978-3-031-26576-1_2

formation, activation, functioning, and interconnection of these bone cells, as well as data on the metabolic changes that occur due to ageing.

Keywords Ageing · Metabolic changes · Osteoblast · Osteoclasts · Osteocytes · Bone marrow stem cells

Introduction

Bone is a living organ with complex properties, having an essential role in the body. It is in a perpetual dynamic, having both metabolic functions and an important role in protection, support, and locomotion. Moreover, the bone system participates directly in the mechanism of hematopoiesis and mineral homeostasis (Asada et al. 2015; Su et al. 2019).

According to World Health Organization (WHO) estimates, the world's population is ageing at a rapid manner, with the number of people over 60 doubling by 2051 (Veronese et al. 2021). Ageing causes an important alteration in the functionality of the bone system. The main bone changes highlighted are decrease in bone mass, modification of specific microarchitecture, reduction of mineral components, reduction of load-bearing capacity, and the appearance of an abnormal response to different humoral molecules (Corrado et al. 2020).

The most important secondary clinical manifestations of ageing are osteoporosis and the increased risk of osteoporotic fracture, advanced age being an independent risk factor for the development of fractures (Boros and Freemont 2017; Corrado et al. 2020). The results of a study that included laboratory mice confirm the fact that both trabecular and cortical bone deteriorates with ageing (Ramanadham et al. 2008). At the level of the cortical bone, thinning and an increase in bone marrow volume have been observed due to periosteal bone production and increased endocortical resorption (Tong et al. 2017; Corrado et al. 2020). At the level of the trabecular bone, a decrease in the number and trabecular thickenings is found, accompanied by a widening of the trabecular space (Corrado et al. 2020; Kim et al. 2020). Moreover, due to the increased porosity of the bone cortex associated with increased osteoclastic resorption, a non-smooth endosteal area can be observed (Zhang et al. 2019). Therefore, the mechanism of ageing bone mass loss is particularly complex, not yet fully understood and includes both local and systemic pathogenic factors (Khosla et al. 2018; Feehan et al. 2019).

Bone Cells and Ageing

At the bone tissue level, there is well-defined homeostasis between the formation and resorption processes, bone remodeling being governed by hormones such as 1,25-hydroxyvitamin D3 (1,25-D3) or parathyroid hormone (PTH), as well as by mechanical loading (Hadjidakis and Androulakis 2006).

Osteoblasts and Ageing

Bone formation is governed by *osteoblasts,* actively present in all three osteoforming phases: the production of the organic matrix rich in type I collagen, its maturation and mineralization (Hadjidakis and Androulakis 2006). Osteoblasts originate from the differentiation of multipotent mesenchymal stem cells (BMSC) from which other cells such as osteocytes, adipocytes, fibroblasts, or chondrocytes are also formed (Bianco et al. 2001). The differentiation of MBSC into pre-osteoblasts and then into osteoblasts is achieved by binding of certain growth factors such as transforming growth factor β (TGFβ), fibroblast growth factor 3 (FGF3) or bone morphogenetic proteins-2 (BMP-2) (Saedi et al. 2020). Then follows the activation of transcription molecules such as osterix and runt-related transcription factor 2 (Runx2). In addition, the TGFβ signal can also be transmitted through the mitogen-activated protein kinase (MAPK) pathway. Moreover, the tyrosine kinase domains through which the FGF receptor signals determine the phosphorylation and activation of protein kinase C (PKC) and MAPK, the latter being associated with the differentiation, mobility, and survival of osteoblasts (Chen et al. 2004; Galea et al. 2014; Pan et al. 2018).

The most important mechanism of differentiation, activation, and proliferation of osteoblasts is realized through the Wingless/Integrated (Wnt) pathway. Depending on the ligand molecules and the biological processes they carry out, there are 2 Wnt pathways: canonical and non-canonical Wnt signaling paths (Corrado et al. 2020). The canonical Wnt pathway is closely related to the intracellular concentration of β-catenin and modulates bone formation by activating and differentiating osteoblasts and participates in bone mineralization. The non-canonical Wnt pathway is independent of β-catenin. The activation of the canonical Wnt pathway is achieved by some proteins (low-density lipoprotein receptor-related protein (LRP) 5 and 6) that, by binding to the transmembrane frizzled (FZZ) receptors, prevent the phosphorylation and degradation of β-catenin and favor gene transcription (Maruotti et al. 2013; Cici et al. 2019). The inactivation of canonical pathway is due to the phosphorylation of β-catenin by glycogen synthase kinase-3β (GSK3β) and caseine kinase I (CKI), followed by degradation via the ubiquitin-proteasome pathway (Corrado et al. 2020).

Differentiation and maturation of osteoblasts can be blocked by extracellular antagonists of the Wnt pathways such as dickkopfs (Dkk-1), secreted frizzled-related proteins, and sclerostin (Westendorf et al. 2004; Pinzone et al. 2009). Naturally, after differentiation, osteoblasts settle on the bone surface in areas called bone structural units where they determine the synthesis and mineralization of the matrix (Hadjidakis and Androulakis 2006). At the end of this process, 15% transform into osteocytes, the rest following the process of apoptosis (Heino and Hentunen 2008).

In older age, osteoblasts directly participate in the loss of bone volume and quality through several mechanisms. The main pathogenic mechanisms are represented by the decrease in cellular activity and a reduction in the differentiation capacity (Corrado et al. 2020). Senescent osteoblasts lose their ability to secrete type

I collagen, osteocalcin, decorin and C1CP, a marker of collagen synthesis (Corrado et al. 2013; Zhang et al. 2018). In addition, osteoblasts respond more less to stimulation by growth factors such as insulin growth factor I (IGF-I) (Wei and Sun 2018). Also, with advancing age, the secretion of cAMP at the level of osteoblasts decreases, even under the stimulation of PTH (Wei and Sun 2018).

Another important mechanism related to ageing is represented by alterations in the Wnt activation pathway characterized by the imbalance between Wnt inhibitors and Wnt ligands. Thus, the studies showed an important decrease in Wnt inhibitors such as Dkk-1 or secreted frizzled-related protein 1 (sFRP1) and a reduction in the expression of numerous Wnt proteins (1, 4, 5, 7, 10b) and LRP-5 (Rauner et al. 2008; Stevens et al. 2010). All of these affect the activity of osteoblasts and decrease osteoblastogenesis.

Other data support the fact that ageing osteoblasts can stimulate the formation of osteoclasts by developing an IL-6 secretory phenotype (Luo et al. 2016). In addition, senescent osteoblasts respond poorly to fluid flow and flow-induced intracellular calcium oscillations (Ota et al. 2013). Other pathogenic mechanisms involved in the decrease in bone mineral density in the elderly refer to the decrease in receptor activator of NFkb ligand (RANKL), matrix metalloproteinase 9 (MMP-9), osteopontin, osteocalcin, osterix and runt-related transcription factor 2 (RUNX-2) (Becerikli et al. 2017; Corrado et al. 2020).

Last, but not least, it seems that an increased rate of osteoblast apoptosis is associated with ageing, leading to bone loss (Komori 2016). Among the pathogenic mechanisms, it seems that increased oxidative stress plays a decisive role in accelerating this programmed cell death for both osteoblasts and osteocytes (Almeida et al. 2007).

Figure 2.1 summarizes the mechanisms of activation and differentiation of osteoblasts, as well as the main metabolic changes that correlate with advanced age.

Osteoclasts and Ageing

The main bone cells involved in the resorption process are *osteoclasts*. These are large multinucleated cells that have precursors in the bone marrow, originating from monocyte/macrophage precursors (Corrado et al. 2020). The most important ways of activation and differentiation of osteoclasts are represented by RANK-RANKL-osteoprotegerin (OPG) and by macrophage-colony stimulating factor (M-CSF) (Corrado et al. 2020). Achieving the RANK- RANKL link at the level of pre-osteoclasts and osteoclasts determines the expression of specific genes for the osteoclast family (Saedi et al. 2020). OPG, secreted by many cells, including osteoblasts, is an inhibitor of osteoclastogenesis, being a trap receptor for RANKL (Boyce and Xing 2008).

Initially, tumor necrosis factor receptor (TNFR)- associated factor 6 (TRAF6) has an important role in the activation of signal transduction pathways for osteoclast formation (Tan et al. 2017; Park et al. 2017). Then, the differentiation and activation

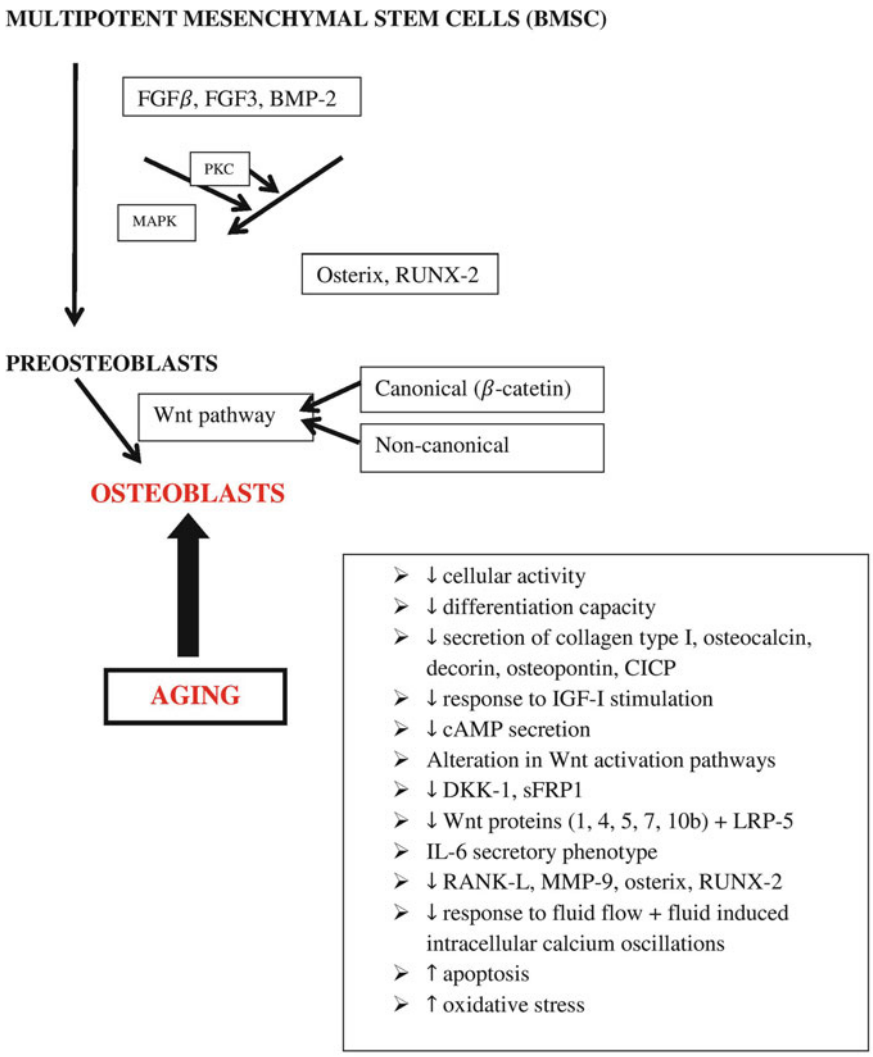


Fig. 2.1 The mechanism of activation and differentiation of osteoblasts and the main metabolic changes occurring due to old age. FGFβ transforming growth factor β, FGF3 fibroblast growth factor 3, BMP-2 bone morphogenetic proteins-2, RUNX2 runt-related transcription factor 2, MAPK mitogen-activated protein kinase, PKC protein kinase C, LRP lipoprotein receptor-related protein, DKK-1 dickkopfs-1, CICP C-terminal collagen propeptide, IFG-I insulin growth factor I, IL-6 interleukin 6, RANKL receptor activator of NFkb ligand

of osteoclasts are dependent on four major signaling pathways that include: protooncogene tyrosine-protein kinase (Src), protein kinase inhibitor of IkB kinase (IKK), extracellular signal-regulated kinase (ERK), and c-Jun N-terminal kinase (JNK) (Cappariello et al. 2014; Saedi et al. 2020). In addition, there are specific

transcription factors for osteoclasts such as Fos, p50, or nuclear factor of activated T-cells cytoplasmic 1 (NFATc1) (Saedi et al. 2020). After achieving the RANK-RANKL link, TRAF6 determines the following: (1) it will bind to JNK and activate the c-Fos transcription factor involved in osteoclast differentiation; (2) it will bind to interleukin-1 receptor-associated kinase 1 (IRAK1), then to the non-canonical NFkB signaling pathway and activate osteoclastogenesis; (3) it will induce phosphatidylinositol 3-kinase (PI3K) formation, Akt phosphorylation and mammalian target of rapamycin (mTOR) activation (Wesche et al. 1999; Park et al. 2017; Shi and Sun 2018).

Another important way of osteoclastic differentiation and activation is represented by macrophage-colony-stimulating factor (M-CSF). Binding of M-CSF to c-Fms receptors on the surface of pre-osteoclasts leads to an increase in RANK expression (Kim et al. 2016). Moreover, by this link, TRAF6 signaling is favored through motif kinase (EMK)/ERK/NFATc1 and PI3K/Akt/mTOR (Yamashita et al. 2012).

Older age is characterized by an increased bone turnover determined by a high number of osteoclasts, as well as by their increased activity. The data support the presence of an accelerated osteoclastogenesis mediated by osteoblasts, which leads to increased expression of M-CFS and RANKL at the level of bone stromal cells and osteoblasts (Cao et al. 2003, 2005). In addition, a decrease in OPG has been identified to the elderly subjects (Makhluf et al. 2000).

Other essential factors associated with ageing and favoring osteoclastogenesis, resorption and loss of bone mass are: changes in the extracellular matrix, microfractures, reduced mechanical loading, increased inflammation, sclerostin production, decreased testosterone and estrogens, secondary hyperparathyroidism, increased expression of c-Fms, RANK, and RANKL (Chung et al. 2014; Boskey and Imbert 2017). Estrogen deficiency leads to increased secretion of pro-inflammatory cytokines such as IL-1β, TNFα, IL-6, TGFβ and these modulate the RANK signaling pathway, thus stimulating the formation and activation of osteoclasts (Shulman 2009; Gibon et al. 2016). IL-1 and TNF receptors act through TRAF6 and have similar effects on RANK-mediated TRAF6 activation (Yan et al. 2001). Stimulation of osteoclast differentiation is also achieved by TGFβ which, through Smad1, activates the RANK pathway (Battaglino et al. 2002).

On the other hand, the degradation of the extracellular matrix can be associated with the activation and differentiation of osteoclasts. Thus, in the elderly, an important increase, up to 300%, of type I collagen β-isomerization of C-telopeptide has been observed (Henriksen et al. 2007). Moreover, for differentiation and good functionality, osteoclasts need very low levels of reactive oxygen species (ROS). In the elderly, a decrease in the oxidative stress-induced apoptosis of the osteoclasts has been highlighted due to the loss of caspase-2 (Sharma et al. 2014).

Figure 2.2 summarizes the mechanisms of activation and differentiation of osteoclasts, as well as the main metabolic changes that correlate with advanced age.

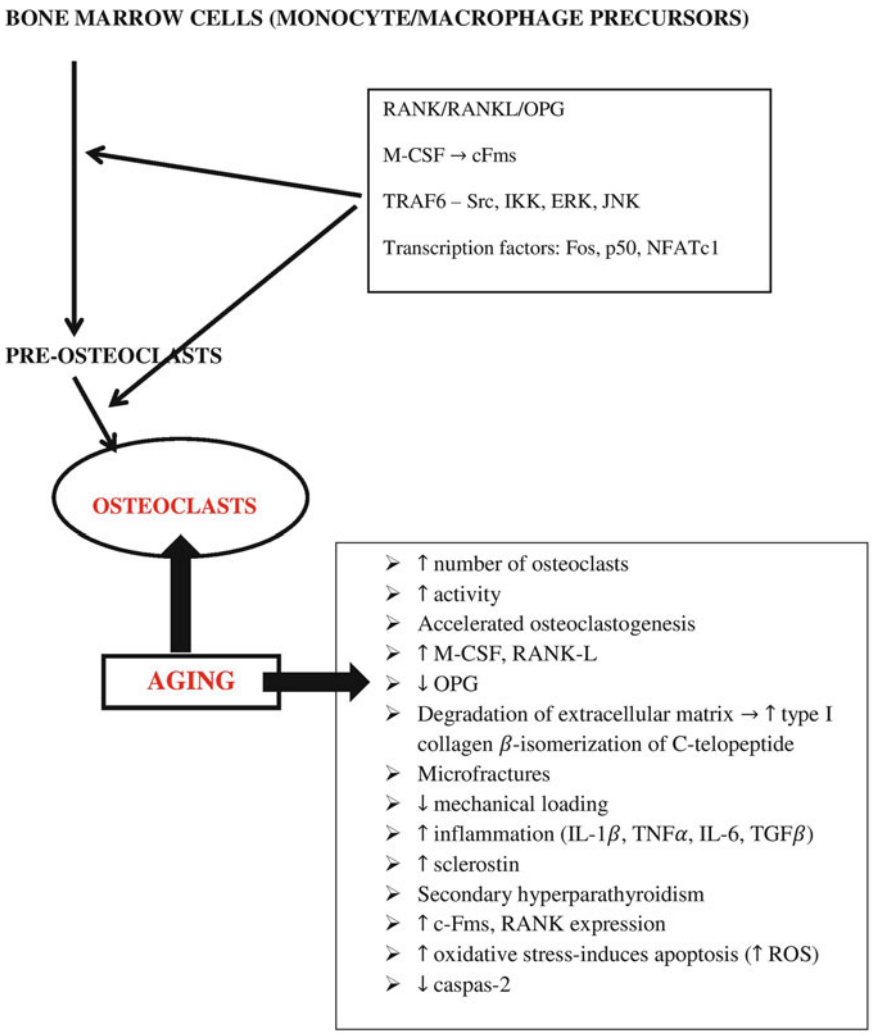


Fig. 2.2 The mechanism of activation and differentiation of osteoclasts and the main metabolic changes occurring due to old age. RANK receptor activator of NFkb, RANKL receptor activator of NFkb ligand, OPG osteoprotegerin, M-CSF macrophage-colony stimulating factor, TRAF6 tumor necrosis factor receptor-associated factor 6, Src protooncogene tyrosine-protein kinase, IKK protein kinase inhibitor of IkB kinase, ERK extracellular signal-regulated kinase, JNK c-Jun N- terminal kinase, IL-1 interleukin 1, TNF tumor necrosis factor, IL-6 interleukin 6, TGF transforming growth factor, ROS reactive oxygen species

Osteocytes and Ageing

Osteocytes, similar to osteoblasts, are formed from mesenchymal stem cells (BMSC). They can survive up to 50 years and represent 90% of bone cells (Manolagas and Parfitt 2010). The main role of osteocytes is to modulate bone remodeling by secreting RANKL and OPG (Jilka et al. 2010; Jilka and O'Brien 2016). Osteocytes are interconnected and also connected with other cells through a network made up of long cytoplasmic connections called dendrites (Bonewald 2011). Due to their increased survival, these cells accumulate molecular damage over time (Jilka and O'Brien 2016). Osteocytes control bone turnover and maintain a balance between bone formation and resorption through apoptosis (Chen et al. 2015).

Normally, osteocytes, due to the secretion of sclerostin, modulate the activity of osteoblasts through the Wnt signaling pathway, while the activation of osteoclasts is achieved through the secretion of RANKL and macrophage-colony-stimulating factor (M-CSF) (Nakashima et al. 2011). Sclerostin binds to osteoblasts through LRP5 and 6, thus inactivating the Wnt pathway and osteoblast differentiation, leading to bone loss (Gaudio et al. 2010). On the other hand, the activation of osteoclasts is achieved by the increased expression of RANKL (Ominsky et al. 2014). Other molecules produced by osteocytes and participating in bone homeostasis are: nitric oxide (NO), bone morphogenic proteins (BMPs) and prostaglandin E2 (PGE2) (Corrado et al. 2020).

Osteocytes participate in the bone mineralization process and in phosphocalcium metabolism by secreting proteins such as dentin matrix acidic phosphoprotein 1 (DMP1), bone sialoprotein (BSP), fibroblast growth factor 23 (FGF23), phosphate-regulating gene with homologies to endopeptidases on the X chromosome (PHEX) and matrix extracellular phosphoglycoprotein (MEPE) (Dallas et al. 2013). In addition, these cells have receptors for PTH and vitamin D. PTH favors bone resorption, stimulates RANKL expression and decreases OPG secretion (Ma et al. 2001; Onal et al. 2012; Saini et al. 2013).

Ageing is associated with a decrease in the number of osteocytes and lacunar density (Corrado et al. 2020). The deterioration of the canalicular structure due to old age is associated with the decrease of connections between osteocytes, thus leading to changes in mechanotransduction (Hemmatian et al. 2017). The lacuno-canalicular system is indispensable for the normal flow of canalicular fluid which, apart from its important role in bone nutrition, in moments of mechanical loading, represents the stimulus for osteocytes to mediate mechanotransduction (Fritton and Weinbaum 2009). Ageing causes a decrease in bone response to mechanical stimuli. Also, reducing physical activity with older age is associated with a decrease in mechanical load, favoring bone loss (Javaheri and Pitsillides 2019).

With age, the morphology of the lacunae changes, becoming smaller and more spherical (Hemmatian et al. 2017). This seems to be due to an increase in the activity of the pro-apoptotic system and secondary to mechanical factors (Hunter and Agnew 2016). The data have shown that there is no correlation between the number of

osteocytes and the number of lacunae, the percentage of empty lacunae increasing with older age (Piemontese et al. 2017; Heveran et al. 2018).

Further, in old age it was observed that osteocytes show increased rates of apoptosis due to the decrease of connexin 43, a protein that participates in mechanotransduction (Davis et al. 2018). The pathogenic mechanisms underlying the accelerated apoptosis of osteocytes include: increased cortisol levels, increased ROS and NOS in osteocytes, increased ATP release and damage-associated molecular patterns (DAMPs) accumulation and impaired autophagy (Jilka and O'Brien 2016; Komori 2016). Osteocyte autophagy, the mechanism by which cellular debris is presented to lysosomes for degradation, shows a lower rate with ageing, which favors apoptosis and bone loss (Jilka and O'Brien 2016).

Last but not least, it should be remembered that the network of osteocytes is essential in maintaining bone mass, the reduction of connections between these cells being associated with a poor bone quality and a decrease in mechanical properties (Tiede-Lewis et al. 2017). Studies that used electron microscopy highlighted a reduction in osteocyte dendrites that was associated with thinning of the bone cortex (Milovanovic and Busse 2019).

Figure 2.3 summarizes the mechanisms of activation and differentiation of osteocytes, as well as the main metabolic changes that correlate with advanced age.

Multipotent Mesenchymal Stem Cells and Ageing

Bone marrow also plays an important role in bone homeostasis. It consists of extracellular matrix, stromal cells that include hematopoietic precursors of osteoclasts and bone *multipotent mesenchymal stem cells* (BMSC), as well as numerous cytokines that participate in cell survival and activity (Farr and Khosla 2019). BMSCs are formed from mesoderm and ectoderm cells and have the ability to self-renew and transform into numerous cells such as bone cells (osteoblasts, osteocytes), chondrocytes or adipocytes (Farr and Khosla 2019). BMSCs are some of the most important stem cells of the bone marrow, maintaining a balance between the formation of osteoblasts, osteoclasts and hematopoiesis (Bianco and Robey 2015).

Advanced age is associated with the decrease of bone tissue and its replacement with fat cells formed in the bone marrow from BMSC (Paccou et al. 2019). This increased differentiation of BMSC into adipocytes and the decrease in the number and functionality of osteoblasts is the main factor involved in the pathogenesis of osteoporosis (Stenderup et al. 2003; Hu et al. 2018; Qadir et al. 2020). These fat cells of the bone marrow have a special metabolism, depending on the lipolysis of their own lipids stored intracellularly to release fatty acids for oxidative metabolism (Bartelt et al. 2017). Then follows an accumulation of free saturated fatty acids that exert a negative effect on the bone marrow (Gasparrini et al. 2009). One of the most toxic and intensively secreted free fatty acids is palmitate, directly involved in bone destruction processes in ageing (Elbaz et al. 2010). Studies have highlighted its

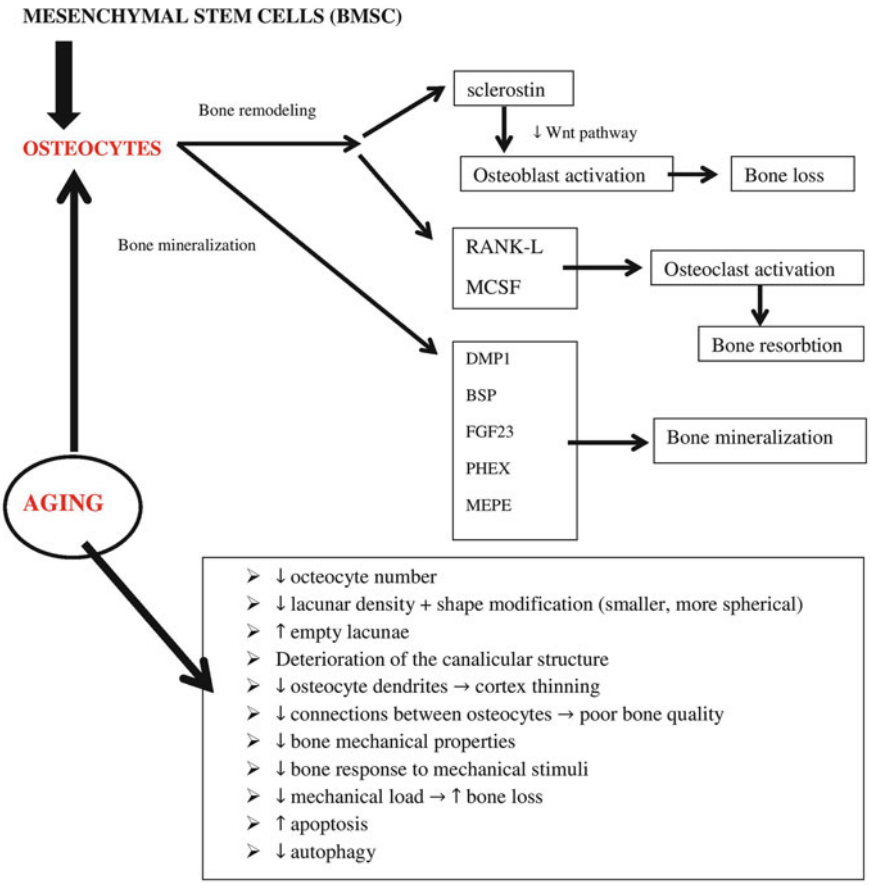


Fig. 2.3 The main actions of osteocytes in bone homostasy and the most important metabolic changes occurring due to old age. BMSC multipotent bone mesenchymal stem cells, RANKL receptor activator of NFkb ligand, M-CSF macrophage-colony stimulating factor, DMP1 dentin matrix acidic phosphoprotein 1, BSP bone sialoprotein, FGF23 fibroblast growth factor 23, PHEX phosphate-regulating gene with homologies to endopeptidases on the X chromosome, MEPE matrix extracellular phosphoglycoprotein

toxic effect on both osteoblasts and osteocytes (Gunaratnam et al. 2014; Al Saedi et al. 2019). The main pathogenic mechanisms involved in the loss of bone mass are the decrease in the formation of osteoblasts, the reduction in the deposition and mineralization capacity of the extracellular organic matrix, the increase in the process of apoptosis and dysfunctional autophagy of osteoblasts (Gunaratnam et al. 2014; Al Saedi et al. 2019).

The main factors that participate in the differentiation of BMSCs into osteoblasts are osterix, RUNX2, and forkhead transcription factor P (FOXP) (Ducy et al. 1997; Li et al. 2017). With ageing, RUNX2 expression decreases, which leads to decreased

bone matrix formation, osteoblast differentiation being blocked (Jiang et al. 2008). On the other hand, the decrease in FOXP directly influences BMSC, leading to an increase in adipogenesis, a decrease in the formation of osteoblasts and, finally, to the degradation of the bone structure (Li et al. 2017). Other metabolic changes attributed to BMSC and observed in old age are decrease in response to bone morphogenic protein (BMP), decrease in alkaline phosphatase (ALP), osteocalcin, and reduction in type I collagen secretion (Fleet et al. 1996; Abdallah et al. 2006).

The pathogenic mechanisms involved in the adipose transformation of the bone marrow are not yet fully understood (Boros and Freemont 2017). Some results support the role of the increased expression of miRNAs and peroxisome proliferator-activated receptor γ (PPARγ), as well as the decrease of nuclear factor erythroid-related factor 2 (NRF2) (Sen et al. 2014; Bionaz et al. 2015; Sun et al. 2015; Li et al. 2015). miRNA, especially miR188, acts through rapamycin-sensitive companion of mammalian target of rapamycin (RICTOR) mRNAs and histone deacetylase 9 (HDAC9), the expression of which decreases with age, leading to an increase in PPARγ and consequently to the adipose transformation of BMSC (Sen et al. 2014; Li et al. 2015).

Last but not least, it seems that the inactivation of osteoblasts and the stimulation of the formation of medullary adipose tissue is influenced by the Wnt signaling pathway whose activity is reduced in old age (especially the Wnt10b pathway) (Stevens et al. 2010).

Figure 2.4 summarizes the mechanisms of activation and differentiation of BMSC, as well as the main metabolic changes that correlate with advanced age.

Concluding Remarks

Ageing strongly influences bone dynamics characterized by increased resorption processes and decreased bone formation. Thus, the most important clinical manifestations are the occurrence of osteoporosis and the risk of fracture, advanced age being considered an independent risk factor. Senile osteoporotic bone is characterized by the decrease in the number and thickness of trabecular bone, by the increase in trabecular spacing, by the bone cortex thinning and the expansion of the bone marrow. Due to the complex metabolic processes secondary to ageing, there is an increase in bone resorption related to the increase in the number and activity of osteoclasts, as well as a decrease in new bone formation due to the decrease in osteogenic differentiation from BMSC, the increase in osteoblast apoptosis and the decrease in their metabolic activity. Moreover, there is a reduction in the bone anabolic response to mechanical loading, which has as a substrate the decrease in the number of osteocytes and dendrites, as well as the reduction in lacunar density. Finally, the increase in oxidative stress correlates with the acceleration of cellular apoptosis processes, which translates into bone mass loss.

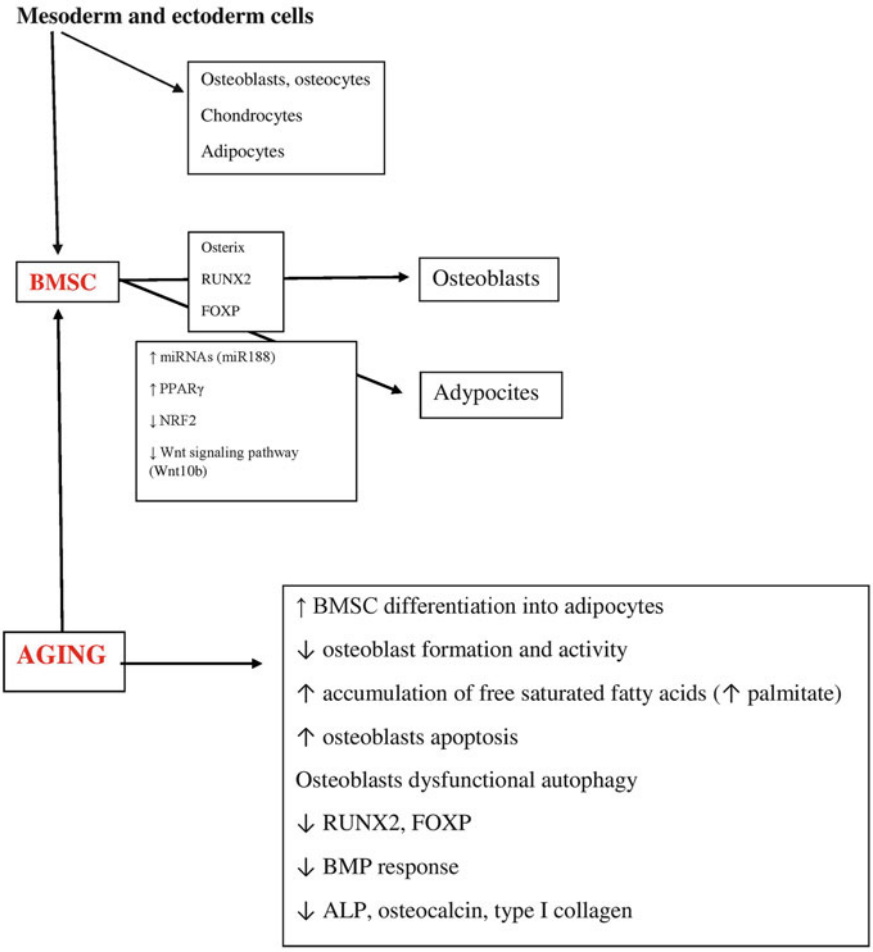


Fig. 2.4 Formation, differentiation and the main metabolic effects of ageing on BMSC. RUNX2 Runt-related transcription factor 2, FOXP forkhead transcription factor P, PPARγ peroxisome proliferator-activated receptor γ, NRF2 nuclear factor erythroid 2-related factor 2, BMSC bone marrow stem cells, ALP alkaline phosphate, BMP bone morphogenic protein

References

Abdallah BM, Haack-Sørensen M, Fink T, Kassem M (2006) Inhibition of osteoblast differentiation but not adipocyte differentiation of mesenchymal stem cells by sera obtained from aged females. Bone 39:181–188

Almeida M, Han L, Martin-Millan M, Plotkin LI, Stewart SA, Roberson PK, Kousteni S, O'Brien CA, Bellido T, Parfitt AM et al (2007) Skeletal involution by age-associated oxidative stress and its acceleration by loss of sex steroids. J Biol Chem 282:27285–27297

Al Saedi A, Bermeo S, Plotkin L, Myers DE, Duque G (2019) Mechanisms of palmitate-induced lipotoxicity in osteocytes. Bone 127:353–359

Asada N, Sato M, Katayama Y (2015) Communication of bone cells with hematopoiesis, immunity and energy metabolism. Bonekey Rep 4:748

Bartelt A, Koehne T, Todter K, Reimer R, Muller B, Behler-Janbeck F et al (2017) Quantification of bone fatty acid metabolism and its regulation by adipocyte lipoprotein lipase. Int J Mol Sci 18(6):E1264. https://doi.org/10.3390/ijms18061264

Battaglino R, Kim D, Fu J, Vaage B, Fu XY, Stashenko P (2002) C-myc is required for osteoclast differentiation. J Bone Miner Res 17(5):763–773. https://doi.org/10.1359/jbmr.2002.17.5.763

Becerikli M, Jaurich H, Schira J, Schulte M, Döbele C, Wallner C, Abraham S, Wagner JM, Dadras M, Kneser U et al (2017) Age-dependent alterations in osteoblast and osteoclast activity in human cancellous bone. J Cell Mol Med 21:2773–2781

Bianco P, Robey PG (2015) Skeletal stem cells. Development 142:1023–1027

Bianco P, Riminucci M, Gronthos S, Robey PG (2001) Bone marrow stromal stem cells: nature, biology, and potential applications. Stem Cells 19(3):180–192. https://doi.org/10.1634/stemcells.19-3-180

Bionaz M, Monaco E, Wheeler MB (2015) Transcription adaptation during in vitro adipogenesis and osteogenesis of porcine mesenchymal stem cells: dynamics of pathways, biological processes, up-stream regulators, and gene networks. PLoS ONE 10:e0137644

Bonewald LF (2011) The amazing osteocyte. J Bone Miner Res 26(2):229–238

Boros K, Freemont T (2017) Physiology of ageing of the musculoskeletal system. Best Pract Res Clin Rheumatol 31:203–217

Boskey AL, Imbert L (2017) Bone quality changes associated with ageing and disease: a review. Ann N Y Acad Sci 1410(1):93–106. https://doi.org/10.1111/nyas.13572

Boyce BF, Xing L (2008) Functions of RANKL/RANK/OPG in bone modeling and remodeling. Arch Biochem Biophys 473(2):139–146. https://doi.org/10.1016/j.abb.2008.03.018

Cao J, Venton L, Sakata T, Halloran BP (2003) Expression of RANKL and OPG correlates with age-related bone loss in male C57BL/6 mice. J Bone Miner Res 18:270–277

Cao JJ, Wronski TJ, Iwaniec U, Phleger L, Kurimoto P, Boudignon B, Halloran BP (2005) Ageing increases stromal/osteoblastic cell-induced osteoclastogenesis and alters the osteoclast precursor pool in the mouse. J Bone Miner Res 20:1659–1668

Cappariello A, Maurizi A, Veeriah V, Teti A (2014) The great beauty of the osteoclast. Arch Biochem Biophys 558:70–78. https://doi.org/10.1016/j.abb.2014.06.017

Chen D, Zhao M, Mundy GR (2004) Bone morphogenetic proteins. Growth Factors 22(4):233–241. https://doi.org/10.1080/08977190412331279890

Chen H, Senda T, Kubo KY (2015) The osteocyte plays multiple roles in bone remodeling and mineral homeostasis. Med Mol Morphol 48:61–68

Chung PL, Zhou S, Eslami B, Shen L, LeBo MS, Glowacki J (2014) Effect of age on regulation of human osteoclast differentiation. J Cell Biochem 115:1412–1419

Cici D, Corrado A, Rotondo C, Cantatore FP (2019) Wnt signaling and biological therapy in rheumatoid arthritis and spondyloarthritis. Int J Mol Sci 20:5552

Corrado A, Neve A, Macchiarola A, Gaudio A, Marucci A, Cantatore FP (2013) RANKL/OPG ratio and DKK-1 expression in primary osteoblastic cultures from osteoarthritic and osteoporotic subjects. J Rheumatol 40:684–694

Corrado A, Cici D, Rotondo C, Maruotti N, Cantatore FP (2020) Molecular basis of bone ageing. Int J Mol Sci 21(10):3679

Dallas SL, Prideaux M, Bonewald LF (2013) The osteocyte: an endocrine cell and more. Endocr Rev 34(5):658–690

Davis HM, Aref MW, Aguilar-Perez A, Pacheco-Costa R, Allen K, Valdez S, Herrera C, Atkinson EG, Mohammad A, Lopez D et al (2018) Cx43 overexpression in osteocytes prevents osteocyte apoptosis and preserves cortical bone quality in ageing mice. JBMR Plus 2:206–216

Ducy P, Zhang R, Georoy V, Ridall AL, Karsenty G (1997) Osf2/Cbfa1: a transcriptional activator of osteoblast differentiation. Cell 89:747–754

Elbaz A, Wu X, Rivas D, Gimble JM, Duque G (2010) Inhibition of fatty acid biosynthesis prevents adipocyte lipotoxicity on human osteoblasts in vitro. J Cell Mol Med 14(4):982–991. https://doi.org/10.1111/j.1582-4934.2009.00751.x

Farr JN, Khosla S (2019) Cellular senescence in bone. Bone 121:121–133

Feehan J, Al Saedi A, Duque G (2019) Targeting fundamental ageing mechanisms to treat osteoporosis. Expert Opin Ther Targets 23:1031–1039

Fleet JC, Cashman K, Cox K, Rosen V (1996) The effects of ageing on the bone inductive activity of recombinant human bone morphogenetic protein-2. Endocrinology 137:4605–4610

Fritton SP, Weinbaum S (2009) Fluid and solute transport in bone: flow-induced mechanotransduction. Annu Rev Fluid Mech 41:347–374

Galea GL, Meakin LB, Williams CM, Hulin-Curtis SL, Lanyon LE, Poole AW, Price JS (2014) Protein kinase Calpha (PKCalpha) regulates bone architecture and osteoblast activity. J Biol Chem 289(37):25509–25522. https://doi.org/10.1074/jbc.M114.580365

Gasparrini M, Rivas D, Elbaz A, Duque G (2009) Differential expression of cytokines in subcutaneous and marrow fat of ageing C57BL/6J mice. Exp Gerontol 44(9):613–618. https://doi.org/10.1016/j.exger.2009.05.009

Gaudio A, Pennisi P, Bratengeier C, Torrisi V, Lindner B, Mangiafico RA, Pulvirenti I, Hawa G, Tringali G, Fiore CE (2010) Increased sclerostin serum levels associated with bone formation and resorption markers in patients with immobilization-induced bone loss. J Clin Endocrinol Metab 95:2248–2253

Gibon E, Lu L, Goodman SB (2016) Ageing, inflammation, stem cells, and bone healing. Stem Cell Res Ther 7:44

Gunaratnam K, Vidal C, Gimble J, Duque G (2014) Mechanisms of palmitate-induced lipotoxicity in human osteoblasts. Gen Endocrinol 155(1):108–116

Hadjidakis DJ, Androulakis II (2006) Bone remodeling. Ann N Y Acad Sci 1092:385–396. https://doi.org/10.1196/annals.1365.035

Heino TJ, Hentunen TA (2008) Differentiation of osteoblasts and osteocytes from mesenchymal stem cells. Curr Stem Cell Res Ther 3(2):131–145. https://doi.org/10.2174/157488808784223032

Hemmatian H, Bakker AD, Klein-Nulend J, van Lenthe GH (2017) Ageing, osteocytes and mechanotransduction. Curr Osteoporos Rep 15:401

Henriksen K, Leeming DJ, Byrjalsen I, Nielsen RH, Sorensen MG, Dziegiel MH, Martin TJ, Christiansen C, Qvist P, Karsdal MA (2007) Osteoclasts prefer aged bone. Osteoporos Int 18:751–759

Heveran CM, Rauff A, King KB, Carpenter RD, Ferguson VL (2018) A new open-source tool for measuring 3D osteocyte lacunar geometries from confocal laser scanning microscopy reveals age-related changes to lacunar size and shape in cortical mouse bone. Bone 110:115–127

Hu L, Yin C, Zhao F, Ali A, Ma J, Qian A (2018) Mesenchymal stem cells: cell fate decision to osteoblast or adipocyte and application in osteoporosis treatment. Int J Mol Sci 19:360

Hunter RL, Agnew AM (2016) Intraskeletal variation in human cortical osteocyte lacunar density: implications for bone quality assessment. Bone Rep 5:252–261

Javaheri B, Pitsillides AA (2019) Ageing and mechanoadaptive responsiveness of bone. Curr Osteoporos Rep 17:560–569

Jiang Y, Mishima H, Sakai S, Liu YK, Ohyabu Y, Uemura T (2008) Gene expression analysis of major lineage-defining factors in human bone marrow cells: effect of ageing, gender, and age-related disorders. J Orthop Res 26:910–917

Jilka RL, O'Brien CA (2016) The role of osteocytes in age-related bone loss. Curr Osteoporos Rep 14(1):16–25. https://doi.org/10.1007/s11914-016-0297-0

Jilka RL, O'Brien CA, Bartell SM, Weinstein RS, Manolagas SC (2010) Continuous elevation of PTH increases the number of osteoblasts via both osteoclast-dependent and -independent mechanisms. J Bone Miner Res 25(11):2427–2437. https://doi.org/10.1002/jbmr.145

Khosla S, Farr JN, Kirkland JL (2018) Inhibiting cellular senescence: a new therapeutic paradigm for age-related osteoporosis. J Clin Endocrinol Metab 103:1282–1290

Kim HJ, Ohk B, Kang WY, Seong SJ, Suk K, Lim MS, Kim SY, Yoon YR (2016) Deficiency of Lipocalin-2 promotes proliferation and differentiation of osteoclast precursors via regulation of c-Fms expression and nuclear factor-kappa B activation. J Bone Metab 23:8–15

Kim HN, Xiong J, MacLeod RS, Iyer S, Fujiwara Y, Cawley KM, Han L, He Y, Thostenson JD, Ferreira E, Jilka RL, Zhou D, Almeida M, O'Brien CA (2020) Osteocyte RANKL is required for cortical bone loss with age and is induced by senescence. JCI Insight 5

Komori T (2016) Cell death in chondrocytes, osteoblasts, and osteocytes. Int J Mol Sci 17(12):2045

Li CJ, Cheng P, Liang MK, Chen YS, Lu Q, Wang JY, Xia ZY, Zhou HD, Cao X, Xie H et al (2015) MicroRNA-188 regulates age-related switch between osteoblast and adipocyte differentiation. J Clin Investig 125:1509–1522

Li H, Li P, Xu S, Li Y, Dekker JD, Li B, Fan Y, Zhang Z, Hong Y, Yang G et al (2017) FOXP1 controls mesenchymal stem cell commitment and senescence during skeletal ageing. J Clin Investig 127:1241–1253

Luo X, Fu Y, Loza AJ, Murali B, Leahy KM, Ruhland MK et al (2016) Stromal-initiated changes in the bone promote metastatic niche development. Cell Rep 14(1):82–92

Ma YL, Cain RL, Halladay DL, Yang X, Zeng Q, Miles RR et al (2001) Catabolic effects of continuous human PTH (1--38) in vivo is associated with sustained stimulation of RANKL and inhibition of osteoprotegerin and gene-associated bone formation. Endocrinology 142(9): 4047–4054. https://doi.org/10.1210/endo.142.9.8356

Makhluf HA, Mueller SM, Mizuno S, Glowacki J (2000) Age-related decline in osteoprotegerin expression by human bone marrow cells cultured in three-dimensional collagen sponges. Biochem Biophys Res Commun 268:669–672

Manolagas SC, Parfitt AM (2010) What old means to bone. Trends Endocrinol Metab 21:369–374

Maruotti N, Corrado A, Neve A, Cantatore FP (2013) Systemic effects of Wnt signaling. J Cell Physiol 228:1428–1432

Milovanovic P, Busse B (2019) Inter-site variability of the human osteocyte lacunar network: implications for bone quality. Curr Osteoporos Rep 17:105–115

Nakashima T, Hayashi M, Fukunaga T, Kurata K, Oh-Hora M, Feng JQ, Bonewald LF, Kodama T, Wutz A, Wagner EF et al (2011) Evidence for osteocyte regulation of bone homeostasis through RANKL expression. Nat Med 17:1231–1234

Ominsky MS, Niu QT, Li C, Li X, Ke HZ (2014) Tissue-level mechanisms responsible for the increase in bone formation and bone volume by sclerostin antibody. J Bone Miner Res 29(6): 1424–1430

Onal M, Galli C, Fu Q, Xiong J, Weinstein RS, Manolagas SC, O'Brien CA (2012) The RANKL distal control region is required for the increase in RANKL expression, but not the bone loss, associated with hyperparathyroidism or lactation in adult mice. Mol Endocrinol 26(2):341–348. https://doi.org/10.1210/me.2011-1149

Ota K, Quint P, Ruan M, Pederson L, Westendorf JJ, Khosla S et al (2013) Sclerostin is expressed in osteoclasts from aged mice and reduces osteoclast-mediated stimulation of mineralization. J Cell Biochem 114(8):1901–1907

Paccou J, Penel G, Chauveau C, Cortet B, Hardouin P (2019) Marrow adiposity and bone: review of clinical implications. Bone 118:8–15

Pan BL, Tong ZW, Li SD, Wu L, Liao JL, Yang YX et al (2018) Decreased microRNA-182-5p helps alendronate promote osteoblast proliferation and differentiation in osteoporosis via the Rap1/MAPK pathway. Biosci Rep 38(6):BSR20180696. https://doi.org/10.1042/bsr20180696

Park JH, Lee NK, Lee SY (2017) Current understanding of RANK signaling in osteoclast differentiation and maturation. Mol Cells 40(10):706–713. https://doi.org/10.14348/molcells.2017.0225

Piemontese M, Almeida M, Robling AG, Kim HN, Xiong J, Thostenson JD, Weinstein RS, Manolagas SC, O'Brien CA, Jilka RL (2017) Old age causes de novo intracortical bone remodeling and porosity in mice. JCI Insight 2:93771

Pinzone JJ, Hall BM, Thudi NK, Vonau M, Qiang YW, Rosol TJ, Shaughnessy JD Jr (2009) The role of Dickkopf-1 in bone development, homeostasis, and disease. Blood 113(3):517–525. https://doi.org/10.1182/blood-2008-03-145169

Qadir A, Liang S, Wu Z, Chen Z, Hu L, Qian A (2020) Senile osteoporosis: the involvement of differentiation and senescence of bone marrow stromal cells. Int J Mol Sci 21:349

Ramanadham S, Yarasheski KE, Silva MJ, Wohltmann M, Novack DV, Christiansen B, Tu X, Zhang S, Lei X, Turk J (2008) Age-related changes in bone morphology are accelerated in group VIA phospholipase A2 (iPLA2beta)-null mice. Am J Pathol 172:868–881

Rauner M, Sipos W, Pietschmann P (2008) Age-dependent Wnt gene expression in bone and during the course of osteoblast differentiation. Age (Dordr) 30:273–282

Saedi A, Stupka N, Duque G (2020) Pathogenesis of osteoporosis. Bone regulators and osteoporosis therapy. Springer, Cham, pp 353–367

Saini V, Marengi DA, Barry KJ, Fulzele KS, Heiden E, Liu X et al (2013) Parathyroid hormone (PTH)/PTH-related peptide type 1 receptor (PPR) signaling in osteocytes regulates anabolic and catabolic skeletal responses to PTH. J Biol Chem 288(28):20122–20134. https://doi.org/10.1074/jbc.M112.441360

Sen B, Xie Z, Case N, Thompson WR, Uzer G, Styner M, Rubin J (2014) mTORC2 regulates mechanically induced cytoskeletal reorganization and lineage selection in marrow-derived mesenchymal stem cells. J Bone Miner Res 29:78–89

Sharma R, Callaway D, Vanegas D et al (2014) Caspase-2 maintains bone homeostasis by inducing apoptosis of oxidatively-damaged osteoclasts. PLoS One 9(4):e93696

Shi JH, Sun SC (2018) Tumor necrosis factor receptor-associated factor regulation of nuclear factor kappaB and mitogen-activated protein kinase pathways. Front Immunol 9:1849

Shulman LP (2009) Androgens and menopause. Minerva Ginecol 61(6):491–497

Stenderup K et al (2003) Ageing is associated with decreased maximal life span and accelerated senescence of bone marrow stromal cells. Bone 33:919–926

Stevens JR, Miranda-Carboni GA, Singer MA, Brugger SM, Lyons KM, Lane TF (2010) Wnt10b deficiency results in age-dependent loss of bone mass and progressive reduction of mesenchymal progenitor cells. J Bone Miner Res 25:2138–2147

Su N, Yang J, Xie Y, Du X, Chen H, Zhou H, Chen L (2019) Bone function, dysfunction and its role in diseases including critical illness. Int J Biol Sci 15:776–787

Sun YX, Xu AH, Yang Y, Li J (2015) Role of Nrf2 in bone metabolism. J Biomed Sci 22:101

Tan EM, Li L, Indran IR, Chew N, Yong EL (2017) TRAF6 mediates suppression of osteoclastogenesis and prevention of ovariectomy-induced bone loss by a novel prenylflavonoid. J Bone Miner Res 32(4):846–860. https://doi.org/10.1002/jbmr.3031

Tiede-Lewis LM, Xie Y, Hulbert MA, Campos R, Dallas MR, Dusevich V, Bonewald LF, Dallas SL (2017) Degeneration of the osteocyte network in the C57BL/6 mouse model of ageing. Ageing (Albany NY) 9:2190–2208

Tong X, Malo MKH, Burton IS, Jurvelin JS, Isaksson H, Kroger H (2017) Histomorphometric and osteocytic characteristics of cortical bone in male subtrochanteric femoral shaft. J Anat 231:708–717

Veronese N, Kolk H, Maggi S (2021) Epidemiology of fragility fractures and social impact. In: Falaschi P, Marsh D (eds) Orthogeriatrics: the management of older patients with fragility fractures. Springer, Cham (CH), pp 19–34

Wei Y, Sun Y (2018) Ageing of the bone. Ageing Ageing-Related Dis:189–197

Wesche H, Gao X, Li X, Kirschning CJ, Stark GR, Cao Z (1999) IRAK-M is a novel member of the Pelle/interleukin-1 receptor-associated kinase (IRAK) family. J Biol Chem 274(27):19403–19410. https://doi.org/10.1074/jbc.274.27.19403

Westendorf JJ, Kahler RA, Schroeder TM (2004) Wnt signaling in osteoblasts and bone diseases. Gene 341:19–39. https://doi.org/10.1016/j.gene.2004.06.044

Yamashita T, Takahashi N, Udagawa N (2012) New roles of osteoblasts involved in osteoclast differentiation. World J Orthop 3(11):175–181. https://doi.org/10.5312/wjo.v3.i11.175

Yan T, Riggs BL, Boyle WJ, Khosla S (2001) Regulation of osteoclastogenesis and RANK expression by TGF-beta1. J Cell Biochem 83(2):320–325

Zhang X, Zhao G, Zhang Y, Wang J, Wang Y, Cheng L, Sun M, Rui Y (2018) Activation of JNK signaling in osteoblasts is inversely correlated with collagen synthesis in age-related osteoporosis. Biochem Biophys Res Commun 504:771–776

Zhang C, Xu S, Zhang S, Liu M, Du H, Sun R, Jing B, Sun Y (2019) Ageing characteristics of bone indicated by transcriptomic and exosomal proteomic analysis of cortical bone cells. J Orthop Surg Res 14:129

Chapter 3
Chronic Inflammation as an Underlying Mechanism of Ageing and Ageing-Related Diseases

Ki Wung Chung, Dae Hyun Kim, Hee Jin Jung, Radha Arulkumar, Hae Young Chung, and Byung Pal Yu

Abstract Age-related chronic inflammation is characterized as the unresolved low-grade inflammatory process underlying the ageing process and various age-related diseases. In this chapter, we review the age-related changes in the oxidative stress-sensitive pro-inflammatory NF-κB signaling pathways causally linked with chronic inflammation during ageing based on senoinflammation schema. We describe various age-related dysregulated pro- and anti-inflammatory cytokines, chemokines, and senescence-associated secretory phenotype (SASP), and alterations of inflammasome, specialized pro-resolving lipid mediators (SPM), and autophagy as major players in the chronic inflammatory intracellular signaling network. A better understanding of the molecular, cellular, and systemic mechanisms involved in chronic inflammation in the ageing process would provide further insights into the potential anti-inflammatory strategies.

Keywords Ageing · Senoinflammation · Senescence-associated secretory phenotype (SASP) · NF-κB

K. W. Chung · D. H. Kim · H. J. Jung · H. Y. Chung
Department of Pharmacy, College of Pharmacy, Pusan National University, Busan, Republic of Korea
e-mail: kieungc@pusan.ac.kr; bioimmune@hanmail.net; hjjung2046@pusan.ac.kr; hyjung@pusan.ac.kr

R. Arulkumar
Interdisciplinary Research Program of Bioinformatics and Longevity Science, Pusan National University, Busan, Republic of Korea
e-mail: radhuspn@pusan.ac.kr

B. P. Yu (✉)
Department of Physiology, The University of Texas Health Science Center at San Antonio, San Antonio, TX, USA
e-mail: bpyu@usa.com

J. R. Harris, V. I. Korolchuk (eds.), *Biochemistry and Cell Biology of Ageing: Part IV, Clinical Science*, Subcellular Biochemistry 103,
https://doi.org/10.1007/978-3-031-26576-1_3

Introduction

The chronic inflammatory response is an essential immune defense function that has evolved to promote survival under specific stressors. Acutely activated inflammation is the first line of defense against harmful agents such as pathogens, toxins, and allergens. Normal conditions enable the elimination of pathogens, infected cells, and damaged tissue for full recovery through the tightly coordinated actions of various defense components, including immune cell function and tissue remodeling processes (Freire and Van Dyke 2013).

When the acute inflammatory response does not subside, the immune system responds in a more complex, long-term manner. The chronic inflammatory response is usually low in intensity and involves many proinflammatory cellular components, including leukocytes enriched with macrophages and lymphocytes (Chen and Xu 2015). The ageing process and numerous age-related chronic diseases are characterized by chronic inflammation due to changes in the cellular redox state and cell death signaling pathways (Chung et al. 2006).

One of the well-known characteristics of age-related dysregulation of immune response is low-grade systemic inflammatory activity. Cytokines and chemokines are among the many dysregulated proinflammatory mediators that contribute to prolonged chronic inflammation and immunosenescence. According to previous studies, the expression of cytokines, such as interleukin-6 and tumor necrosis factor (TNF)-α, increases substantially in aged tissues (Chung et al. 2006; Franceschi et al. 2000). Several studies have linked high levels of chemokines, C-reactive protein (CRP), and prostanoid synthesis to age-related diseases and ageing development (Wyczalkowska-Tomasik et al. 2016). We have previously reported that several important intra- or intercellular signaling pathways are closely associated with chronic inflammation and age-related inflammatory changes in cellular status during ageing (Park et al. 2013; Kim et al. 2016).

In the literature on ageing, two major hypotheses exist regarding chronic inflammation associated with ageing: inflammageing (Franceschi et al. 2000) and senoinflammation (Chung et al. 2019). These two hypotheses are complementary, as the widely observed inflammatory phenomenon with ageing is the major point of the inflammageing hypothesis, whereas senoinflammation focuses on a broad spectrum of proinflammatory molecular, cellular, and systemic components that underlie the chronic inflammatory process. Importantly, recent research on chronic inflammation has produced more supportive experimental data for senoinflammatory hypothesis, necessitating an in-depth discussion of molecular, cellular, and systemic aspects of chronic inflammation, as described in this chapter.

Key Factors Influencing Chronic Inflammation in Ageing

Pro- and Anti-inflammatory Cytokines and SASP

Inflammation is an essential aspect of the body's immune response and defense. An acute state of inflammation over a short period promotes immunity and protects the host in various ways (Chung et al. 2019). Because of its potentially adverse effects, inflammation is generally transient and tightly regulated. The development of chronic diseases, such as cancer, dementia, and atherosclerosis, is an inevitable consequence when inflammation is prolonged and unresolved, that is when it becomes chronically dysregulated immune system (Chung et al. 2019). Therefore, the disruption of the homeostatic inflammatory response is a significant risk factor for ageing process. The influence of the major mediators of chronic inflammation and immunosenescence, cytokines, and chemokines persists throughout this scenario. Chronic inflammation has a much more complex and intricate connection to ageing. M1-like macrophages have been found to release proinflammatory molecules such as TNFα, IL-1β, and IL-12 as a result of prolonged overeating and obesity, which have been linked to numerous metabolic disorders (Li et al. 2018). Additionally, PPARα and SREBP-1c have been implicated in lipid accumulation associated with proinflammatory IL-1β activation by inflammasomes (Chung et al. 2015). Moreover, chronic adipokine-mediated systemic inflammation is widely recognized to be exacerbated by chronic inflammation in adipose tissues (Bluher 2016).

Senescence-associated secretory phenotype (SASP) has recently proved to be a major proinflammatory contributor to numerous pathophysiological conditions (Wiley and Campisi 2021). Senescent cells secrete extracellular modulators such as cytokines, chemokines, proteases, growth factors, and bioactive lipids (Lopes-Paciencia et al. 2019). As a result, macrophages are activated to eliminate senescent cells that produce a senescence-associated (SA) secretome containing inflammatory senescence-associated protein. However, aged macrophages may not properly eliminate senescent cells, leading to a chronic inflammatory state (Oishi and Manabe 2016).

The secretion of inflammatory mediators such as cytokines and chemokines is dependent on the activation of redox-sensitive nuclear factor (NF-κB) (Chung et al. 2019). NF-κB is now regarded as one of the most important proinflammatory transcription factors. NF-κB signaling during ageing has been shown to involve cytokines (IL-1β, IL-2, and IL-6), chemokines (IL-8, RANTES, and T cells), and adhesion molecules, all of which contribute to chronic diseases and symptoms associated with ageing (Chung et al. 2006). Substantial evidence suggests that NF-κB plays an important role in cancer progression and initiation (Xia et al. 2014). Many other proinflammatory mediators are also induced by NF-κB stimulation, leading to major age-related chronic diseases (Wang et al. 2022; Esparza-Lopez et al. 2019).

NF-κB also interacts with various factors such as signal transduction factors and transcriptional activators 3 (STAT3) and p53. These factors are implicated in age-related chronic inflammatory diseases such as cancer and type 2 diabetes mellitus (Fan et al. 2013; Lowe et al. 2014). Crosstalk has been reported between upstream signaling components as well as at the transcriptional level. In addition to GSK3, MAPK, and protein kinase B (PKB), kinases that regulate NF-κB transcription can also regulate the activity of other cancer-related kinases (Park and Hong 2016). Numerous proinflammatory genes, metabolic signaling pathways, and SASP have been identified to be systemically involved in inflammatory and metabolic disorders (Wiley and Campisi 2021). A more detailed discussion on NF-κB is presented below.

Anti-inflammatory cytokines also play essential roles in balancing the immune response and preventing immune homeostasis from falling into proinflammatory ageing and disease-induced states. These cytokines play a key role in alleviating inflammation. By blocking or modulating IL-1α, TNF, and other major proinflammatory cytokines, they dampen and ultimately resolve the inflammation response. In addition to soluble receptor antagonists, chemokines, microRNAs, and siRNAs, specific cytokine receptors for IL-1, TNF-α, and IL-18 also inhibit proinflammatory cytokines (Rea et al. 2018). The anti-inflammatory cytokines interleukin 10 (IL-10) and IL-37, members of the IL-1 family, are crucial factors in controlling inflammation, along with TGF-β released by monocytes and platelets. Inflammatory pathways are reduced by the soluble receptors TNFR and IL-1 receptor (IL-1R), which bind to cytokines and neutralize them (Levine 2008). Many other anti-inflammatory mediators are observed, including stress hormones—primarily corticosteroids and catecholamines—and negative regulators such as microRNAs (MiR-146 and MiR-125) (Schulze et al. 2014; Lee et al. 2016). Previously reported observations clearly demonstrate that proinflammatory and anti-inflammatory cytokines and SASP modulate the outcome of chronic inflammation underlying the ageing process and age-related disease pathogenesis.

Changes in Endogenous Anti-inflammatory Lipid Mediators During Ageing

The complete resolution of an inflammatory response is essential for maintaining cellular homeostasis, and inflammation resolution is a highly sophisticated process that involves important anti-inflammatory mediators. Endogenous anti-inflammatory mediators, including lipoxins, resolvins, and protectins, play key roles in resolving inflammation (Basil and Levy 2016). These mediators are derived from lipid precursors and produced by the enzymatic activity of lipoxygenases. Among these mediators, lipoxins are the most well-characterized. Lipoxins and their epimers are bioactive autacoid metabolites of arachidonic acid that are produced by several cell types. Initially, two lipoxins were identified: lipoxin A4

(LXA4) and lipoxin B4 (LXB4), and further studies have identified epimers of these two lipoxins. In addition, other lipid mediators including resolvins and protectins were found to be derived from omega 3 fatty acids or other families of polyunsaturated fatty acids with functions and activities similar to those of lipoxins (Kohli and Levy 2009). Because of their specific role in inflammation resolution, these anti-inflammatory lipid mediators are often called specialized pro-resolving lipid mediators (SPMs). In addition to the chemical properties of SPMs, their physiological roles and mechanisms at the site of inflammation have been extensively studied during the last decade (Basil and Levy 2016).

Accumulating evidence suggests that changes in lipid mediators are age-related. Gangemi et al. (2005) first reported that ageing was associated with reduced LXA4 levels. They evaluated urinary arachidonic acid metabolites, including anti-inflammatory LXA4 and proinflammatory cysteinyl leukotrienes in volunteers aged 26 to over 100 years. A significant inverse correlation between age and LXA4 levels was found, suggesting that reduced LXA4 levels over the course of ageing may contribute to the development of disease. Another study conducted by Dunn et al. (2015) showed a similar reduction in LXA4 levels in the ageing brain. The same researchers assessed age-related changes in proinflammatory leukotriene B4 (LTB4) and pro-resolving LXA4 levels in the brain. Age-dependent increases in LTB4 levels and decreases in LXA4 levels have been detected during brain ageing. Furthermore, these changes were exacerbated in the 3xTG Alzheimer mouse model. Pamplona et al. (2021) showed interesting results in aged brains by showing that neurons and microglia are responsible for LXA4 production in the brain, and ageing reduces the brain and systemic LXA4 levels in mice. Also, LXA4 levels in cerebrospinal fluid decrease with age and dementia in humans.

Crucial Role of Oxidative Stress in Age-Related Chronic Inflammation

Among the several well-known hypotheses of ageing, the most widely accepted theory is that ageing is caused by oxidative stress (Yu 1996). The oxidative stress hypothesis explains the characteristic changes during ageing as a net effect of redox imbalances caused by the difference between oxidative stress and reactive antioxidant forces (Kim et al. 2002). This redox imbalance is likely due to an increase in ROS and reactive lipid aldehydes associated with a weakened antioxidant defense system. The main contributors to the redox imbalance caused by age-related oxidative stress are uncontrolled production of reactive species such as reactive oxygen species (ROS), reactive nitrogen species, and reactive lipid species, in conjunction with a weakened antioxidant defense capacity. A gradual increase in oxidative stress due to impaired redox regulation during ageing may affect gene transcription and signal transduction pathways.

A seminal finding in oxidative stress and inflammatory processes is the profound activation of NF-κB, a highly sensitive and critical proinflammatory mediator (Kim et al. 2002). By activating proinflammatory cells and expressing various cytokines and chemokines, NF-κB plays a crucial role in maintaining an immune response while ageing. According to motif mapping of gene promoters, NF-κB is the transcription factor most closely associated with ageing (Adler et al. 2007). Furthermore, chronic activation of NF-κB has been demonstrated in various tissues, such as the skin, kidneys, cardiac muscle, and brain (cerebellum and hypothalamus) (Helenius et al. 1996; Korhonen et al. 1997; Zhang et al. 2013; Tilstra et al. 2012).

Molecular and Cellular Constituents in Inflammation-Related Pathophysiological Conditions

NF-κB Involvement

The NF-κB signaling pathway is implicated in ageing, along with the insulin-like growth factor-1 (IGF-1), mTOR, SIRT, and p53 pathways. Several lines of evidence have shown that NF-κB activity increases with age. NF-κB/p65 DNA binding increased in the skin, liver, kidney, muscle, and gastric mucosa of aged mice. In addition, chronic NF-κB activation has been observed in various age-related diseases, including muscular atrophy, atherosclerosis, osteoporosis, heart diseases, type 1 and type 2 diabetes, osteoarthritis, and neurodegenerative diseases, such as Alzheimer's disease and Parkinson's disease (Chung et al. 2019).

Another line of evidence further demonstrated the tissue-specific role of NF-κB in ageing phenotypes. Cai et al. (2004) identified NF-κB activation through muscle-specific transgenic expression of activated IKK beta. These mice exhibited profound muscle wasting resembling clinical cachexia. Muscle loss occurred due to increased protein breakdown through E3 ligase MuRF1 expression, and pharmacological or genetic inhibition of the NF-κB pathway reversed muscle atrophy. Similar results were also obtained in the Duchenne muscular dystrophy model. Another study, by Zhang and colleagues, showed that IKK-NF-κB signaling is involved in the hypothalamic programming of systemic ageing (Zhang et al. 2013). They found ageing-dependent hypothalamic NF-κB activation with an increase in the innate immune pathway. NF-κB inhibition in genetically engineered mice increased lifespan with a less age-related phenotype, suggesting that hypothalamus NF-κB activation has a unique role in the development of systemic ageing. They further showed that activation of NF-κB mediates gonadotropin-releasing hormone (GnRH) decline in the aged hypothalamus, and GnRH treatment adjusts ageing-impaired neurogenesis and decelerates ageing.

Inflammasome in Chronic Inflammation

Recent studies have revealed that NACHT, LRR, and PYD domain-containing protein 3 (NLRP3) inflammasome are major regulators of age-related inflammation (Gritsenko et al. 2020). The inflammasome is activated by a wide range of extra and intracellular stimuli include pathogen-associated molecular patterns and danger-associated molecular patterns (Broz and Dixit 2016). Activation of NLRP3 proteins further oligomerizes and recruits an adaptor protein known as ASC, which consists of two death-fold domains, a pyrin domain (PYD) and a caspase recruitment domain (CARD). These domains allow ASC to bridge the upstream inflammasome sensor molecule with caspase 1. Activated caspase 1 further facilitates the release of interleukin-1β (IL-1β) and IL-18 and induces inflammation and pyroptosis, which is a lytic form of cell death. Inflammasome activation plays a crucial role in host defense against pathogens; however, dysregulated inflammasomes are also linked to the development of numerous age-related diseases.

Experimental data clearly demonstrated the role of inflammasome in ageing-associated phenotypic changes. Youm et al. (2013) utilized *Nlrp3* deficient mice to observe age-related functional changes. NLRP3 inflammasome-deficient mice were protected from age-related increases in innate immune activation, alterations in CNS transcriptomes, and astrogliosis. They further demonstrated that increased IL-1 expression plays an essential role in regulating age-related CNS inflammation and functional decline. They also demonstrated that the NLRP3 inflammasome promotes ageing-related thymic demise and immunosenescence. Another study, by Camell et al. (2019), revealed the role of the NLRP3-inflammasome in age-related adipose tissue B-cell expansion. They identified unique resident aged adipose B cells that impair the basal role of adipose tissue during ageing. The accumulation of unique B cells and age-induced defects in lipolysis are dependent on the NLRP3 inflammasome and IL-1 signaling. The acetylation status of NLRP3 during ageing was reported by He et al. (2020). These authors showed that NLRP3 is deacetylated during the ageing process owing to decreased SIRT2 expression in macrophages. Deacetylated NLRP3 facilitates hyperactivation of the NLRP3 inflammasome, increasing inflammation during ageing. They provided evidence that the acetylation switch of the inflammasome, regulated by SIRT2, is a physiological factor that regulates age-associated chronic inflammation.

Role of Autophagy in Inflammation

Autophagy is an evolutionarily conserved process that occurs in all eukaryotic cells from yeast to humans. The highly complex autophagy machinery and related signaling pathways have been extensively studied over the last 30 years. The primary function of autophagy is to degrade self-components; once activated, autophagy involves the sequestration of cytosolic components, including damaged

cell organelles, proteins, or other macromolecule nutrients, and provides energy to maintain cell homeostasis. In ageing and age-related diseases, there are significant reductions in these processes that lead to the accumulation of damaged molecules, proteins, DNA, and lipids, leading to the loss of cellular integrity, as defective autophagy has been implicated in various age-associated diseases (Aman et al. 2021).

Understanding the relationship between autophagy and inflammation provides insights into ageing and age-related diseases. Reviewing studies that use animal models and human samples support the important role of autophagy in maintaining tissue homeostasis through inflammation suppression. Impaired autophagy often results in increased inflammation and has been demonstrated as a major driver of age-related tissue damage using evidence obtained from studies on the role of autophagy in modulating the differentiation and metabolic state of inflammatory cells (Aman et al. 2021). It has been shown that immune cell differentiation is dependent on the balance between mTOR and AMPK signaling activation (Riffelmacher et al. 2018). When mTOR is activated, autophagic flux decreases and the cells exhibit proinflammatory phenotypes. In contrast, shifting the balance toward AMPK signaling results in increased autophagic activity with differentiation into non- or anti-inflammatory immune cells. Because overall autophagy responses decrease during ageing, this may be an important mechanism for promoting proinflammatory responses during ageing (Aman et al. 2021).

Senoinflammation Schema: Exacerbation by SASP and Suppression by Calorie Restriction (CR)

Previous literature has extensively shown the role of low-grade inflammation in ageing and age-related diseases. To clarify distinctions among widely used explanations and concepts, a number of terms and perspectives have been proposed. Chronic inflammation associated with ageing has been suggested to occur through a variety of mechanisms, including molecular inflammation, micro-inflammation, pan-inflammation, and gero-inflammation, which describe the actions of chronic inflammation and proinflammatory mediators (Fulop et al. 2017; Chhetri et al. 2018). Despite these attempts, the precise age-related chronic inflammatory processes remain poorly understood and under-characterized.

The senoinflammation (senescent chronic inflammation) schema presented herein (*see* Fig. 3.1) was developed in 2019, based on available data showing the initiation of the chronic inflammatory process triggered by oxidative stress-induced redox imbalance, which is associated with ageing and numerous chronic diseases (Chung et al. 2019). By understanding molecular, cellular, and systemic senoinflammation, one could gain a better understanding of how chronic inflammation exacerbates the age-related functional declines and metabolic alterations that occur.

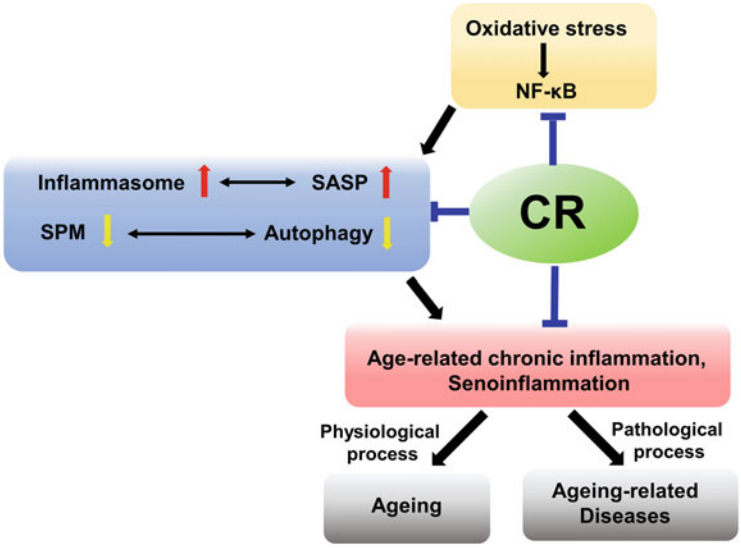


Fig. 3.1 Chronic inflammation as an underlying mechanism of ageing and ageing-related diseases. CR: calorie restriction, SPM: specialized pro-resolving lipid mediators, SASP: senescence-associated secretory phenotype

As described earlier, senescent cells secrete soluble mediators called SASP (Coppe et al. 2010). Recent studies have shown that SASP is a significant pathophysiological risk factor exacerbating the ageing process and metabolic diseases (Chung et al. 2019). The SASP plays a multifaceted role as an active mediator in the senoinflammatory process and metabolic changes associated with ageing (Shakeri et al. 2018). Despite considerable progress in understanding the cellular mechanisms of the SASP, its precise role and contribution to ageing and proinflammatory molecular pathways require further exploration. Reports have shown that senolysis (i.e., senescent cell removal) improves glucose metabolism and β-cell function while reducing SASP and senescent biomarker expression in mice (Aguayo-Mazzucato et al. 2019).

Senescence-associated secretory proinflammatory mediators and SASP are released by senescent cells (Salminen et al. 2012). To remove senescent cells from the secretome, macrophages are recruited by chemotactic factors (Childs et al. 2017). However, the polarized M2 phenotype of senescent macrophages secretes proinflammatory cytokines, exhibits impaired phagocytosis, and is characterized by lower growth rates (Yarbro et al. 2020). In keratinocytes, melanocytes, monocytes, fibroblasts, and epithelial cells, IL-1β, IL-6, and IL-8 are the most potent proinflammatory cytokines secreted by SA-induced stress (Freund et al. 2010). Most senescent cells contain elevated levels of matrix metalloproteases (MMPs), another proinflammatory component of SASP. MMPs regulate the production of cytokines and chemokines that are associated with inflammation (Coppe et al. 2010). Several

recent studies have reported that PTBP1 and HSP90 regulate the SASP (Georgilis et al. 2018; Fuhrmann-Stroissnigg et al. 2018). In summary, SASP and related mediators impose a substantial impact on senoinflamatory process with ageing.

Caloric restriction (CR) has been demonstrated to be effective in combating the ageing process and age-related diseases such as diabetes, obesity, cardiovascular disease, rheumatoid arthritis, and Alzheimer's disease (Chung et al. 2013). It is the only known intervention of ageing that extends not only the mean lifespan but also the maximum life span of experimental animals. Our lab reported the first experimental evidence showing CR's potent and broad anti-inflammatory action by suppressing key proinflammatory NF-κB activation (Kim et al. 2002). Recent work also showed CR's powerful modulation of many proinflammatory factors, including IL-1β, IL-6, TNF, and COX-2 (inducible nitric oxide synthase) (Allen et al. 2019). After two monthly applications of 30% CR to obese mice, cytokines, and chemokines such as IL-6, IL-2, IL1Rα, MCP-1, and CXCL16, which are important components of the SASP (Kurki et al. 2012). The expression of proinflammatory and lipogenic genes, such as MCP-1, SREBPs, and peroxisome proliferator-activated receptor (PPAR)-γ, was significantly suppressed by even mild CR in liver tissue (Park et al. 2017). As a result, CR has been shown to regulate the symptomatic prevalence of senoinflammation, which progresses to pathological conditions such as chronic inflammation, insulin resistance, and low energy metabolism resulting from chronic inflammation (Chung et al. 2019; Johansson et al. 2019).

Researchers have studied the effects of CR on inflammatory and metabolic signaling pathways as well as the relationship between ageing and CR. According to evidence from previous studies, CR modulates nuclear signaling pathways by regulating NF-κB, SIRT, and other nuclear molecules, which results in reduced senoinflammation during ageing. Such evidence strongly supports the notion that CR's unique anti-ageing action may be based on its diversified anti-inflammatory capability.

Conclusion

Considering the available evidence and data on age-related chronic inflammation through biochemical, molecular, and systems biology analyses, we concluded that chronic inflammation is a major factor underlying ageing and age-related disease processes. The proinflammatory cytokines and chemokines that comprise the SASP increase stress on the intracellular signaling network, tissues, organs, and systems, leading to various metabolic disorders and chronic inflammation. Alterations in inflammasome, SPM, and autophagy trigger chronic inflammation, thus leading to accelerated ageing and age-related chronic diseases. Therefore, a better understanding of the molecular mechanisms involved in chronic inflammation may provide a fundamental platform for developing effective interventions that delay

ageing-related dysfunction and prevent age-related proinflammatory, i.e., senoinflammatory diseases.

References

Adler AS, Sinha S, Kawahara TL, Zhang JY, Segal E, Chang HY (2007) Motif module map reveals enforcement of ageing by continual NF-kappaB activity. Genes Dev 21(24):3244–3257. https://doi.org/10.1101/gad.1588507

Aguayo-Mazzucato C, Andle J, Lee TB Jr, Midha A, Talemal L, Chipashvili V et al (2019) Acceleration of beta cell ageing determines diabetes and senolysis improve disease outcomes. Cell Metab 30(1):129–142 e4. https://doi.org/10.1016/j.cmet.2019.05.006

Allen BD, Liao CY, Shu J, Muglia LJ, Majzoub JA, Diaz V et al (2019) Hyperadrenocorticism of calorie restriction contributes to its anti-inflammatory action in mice. Ageing Cell 18(3):e12944. https://doi.org/10.1111/acel.12944

Aman Y, Schmauck-Medina T, Hansen M, Morimoto RI, Simon AK, Bjedov I et al (2021) Autophagy in healthy ageing and disease. Nat Ageing 1(8):634–650. https://doi.org/10.1038/s43587-021-00098-4

Basil MC, Levy BD (2016) Specialized pro-resolving mediators: endogenous regulators of infection and inflammation. Nat Rev Immunol 16(1):51–67. https://doi.org/10.1038/nri.2015.4

Bluher M (2016) Adipose tissue inflammation: a cause or consequence of obesity-related insulin resistance? Clin Sci (Lond) 130(18):1603–1614. https://doi.org/10.1042/CS20160005

Broz P, Dixit VM (2016) Inflammasomes: mechanism of assembly, regulation, and signaling. Nat Rev Immunol 16(7):407–420. https://doi.org/10.1038/nri.2016.58

Cai D, Frantz JD, Tawa NE Jr, Melendez PA, Oh BC, Lidov HG et al (2004) IKKbeta/NF-kappaB activation causes severe muscle wasting in mice. Cell 119(2):285–298. https://doi.org/10.1016/j.cell.2004.09.027

Camell CD, Gunther P, Lee A, Goldberg EL, Spadaro O, Youm YH et al (2019) Ageing induces an Nlrp3 inflammasome-dependent expansion of adipose B cells that impairs metabolic homeostasis. Cell Metab 30(6):1024–1039 e6. https://doi.org/10.1016/j.cmet.2019.10.006

Chen M, Xu H (2015) Parainflammation, chronic inflammation, and age-related macular degeneration. J Leukoc Biol 98(5):713–725. https://doi.org/10.1189/jlb.3RI0615-239R

Chhetri JK, de Souto Barreto P, Fougere B, Rolland Y, Vellas B, Cesari M (2018) Chronic inflammation and sarcopenia: a regenerative cell therapy perspective. Exp Gerontol 103:115–123. https://doi.org/10.1016/j.exger.2017.12.023

Childs BG, Gluscevic M, Baker DJ, Laberge RM, Marquess D, Dananberg J et al (2017) Senescent cells: an emerging target for diseases of ageing. Nat Rev Drug Discov 16(10):718–735. https://doi.org/10.1038/nrd.2017.116

Chung HY, Sung B, Jung KJ, Zou Y, Yu BP (2006) The molecular inflammatory process in ageing. Antioxid Redox Signal 8(3-4):572–581. https://doi.org/10.1089/ars.2006.8.572

Chung KW, Kim DH, Park MH, Choi YJ, Kim ND, Lee J et al (2013) Recent advances in calorie restriction research on ageing. Exp Gerontol 48(10):1049–1053. https://doi.org/10.1016/j.exger.2012.11.007

Chung KW, Lee EK, Kim DH, An HJ, Kim ND, Im DS et al (2015) Age-related sensitivity to endotoxin-induced liver inflammation: implication of inflammasome/IL-1beta for steatohepatitis. Ageing Cell 14(4):524–533. https://doi.org/10.1111/acel.12305

Chung HY, Kim DH, Lee EK, Chung KW, Chung S, Lee B et al (2019) Redefining chronic inflammation in ageing and age-related diseases: proposal of the senoinflammation concept. Ageing Dis 10(2):367–382. https://doi.org/10.14336/AD.2018.0324

Coppe JP, Desprez PY, Krtolica A, Campisi J (2010) The senescence-associated secretory phenotype: the dark side of tumor suppression. Annu Rev Pathol 5:99–118. https://doi.org/10.1146/annurev-pathol-121808-102144

Dunn HC, Ager RR, Baglietto-Vargas D, Cheng D, Kitazawa M, Cribbs DH et al (2015) Restoration of lipoxin A4 signaling reduces Alzheimer's disease-like pathology in the 3xTg-AD mouse model. J Alzheimers Dis 43(3):893–903. https://doi.org/10.3233/JAD-141335

Esparza-Lopez J, Alvarado-Munoz JF, Escobar-Arriaga E, Ulloa-Aguirre A, de Jesus Ibarra-Sanchez M (2019) Metformin reverses mesenchymal phenotype of primary breast cancer cells through STAT3/NF-kappaB pathways. BMC Cancer 19(1):728. https://doi.org/10.1186/s12885-019-5945-1

Fan Y, Mao R, Yang J (2013) NF-kappaB and STAT3 signaling pathways collaboratively link inflammation to cancer. Protein Cell 4(3):176–185. https://doi.org/10.1007/s13238-013-2084-3

Franceschi C, Bonafe M, Valensin S, Olivieri F, De Luca M, Ottaviani E et al (2000) Inflamm-ageing. An evolutionary perspective on immunosenescence. Ann N Y Acad Sci 908:244–254. https://doi.org/10.1111/j.1749-6632.2000.tb06651.x

Freire MO, Van Dyke TE (2013) Natural resolution of inflammation. Periodontol 2000 63(1):149–164. https://doi.org/10.1111/prd.12034

Freund A, Orjalo AV, Desprez PY, Campisi J (2010) Inflammatory networks during cellular senescence: causes and consequences. Trends Mol Med 16(5):238–246. https://doi.org/10.1016/j.molmed.2010.03.003

Fuhrmann-Stroissnigg H, Niedernhofer LJ, Robbins PD (2018) Hsp90 inhibitors as senolytic drugs to extend healthy ageing. Cell Cycle 17(9):1048–1055. https://doi.org/10.1080/15384101.2018.1475828

Fulop T, Larbi A, Dupuis G, Le Page A, Frost EH, Cohen AA et al (2017) Immunosenescence and inflamm-ageing as two sides of the same coin: friends or foes? Front Immunol 8:1960. https://doi.org/10.3389/fimmu.2017.01960

Gangemi S, Pescara L, D'Urbano E, Basile G, Nicita-Mauro V, Davi G et al (2005) Ageing is characterized by a profound reduction in anti-inflammatory lipoxin A4 levels. Exp Gerontol 40(7):612–614. https://doi.org/10.1016/j.exger.2005.04.004

Georgilis A, Klotz S, Hanley CJ, Herranz N, Weirich B, Morancho B et al (2018) PTBP1-mediated alternative splicing regulates the inflammatory secretome and the pro-tumorigenic effects of senescent cells. Cancer Cell 34(1):85–102 e9. https://doi.org/10.1016/j.ccell.2018.06.007

Gritsenko A, Green JP, Brough D, Lopez-Castejon G (2020) Mechanisms of NLRP3 priming in inflammageing and age-related diseases. Cytokine Growth Factor Rev 55:15–25. https://doi.org/10.1016/j.cytogfr.2020.08.003

He M, Chiang HH, Luo H, Zheng Z, Qiao Q, Wang L et al (2020) An acetylation switch of the NLRP3 inflammasome regulates ageing-associated chronic inflammation and insulin resistance. Cell Metab 31(3):580–591 e5. https://doi.org/10.1016/j.cmet.2020.01.009

Helenius M, Hanninen M, Lehtinen SK, Salminen A (1996) Changes associated with ageing and replicative senescence in the regulation of transcription factor nuclear factor-kappa B. Biochem J 318(Pt 2):603–608. https://doi.org/10.1042/bj3180603

Johansson HE, Edholm D, Kullberg J, Rosqvist F, Rudling M, Straniero S et al (2019) Energy restriction in obese women suggests a linear reduction of hepatic fat content and time-dependent metabolic improvements. Nutr Diabetes 9(1):34. https://doi.org/10.1038/s41387-019-0100-2

Kim HJ, Jung KJ, Yu BP, Cho CG, Choi JS, Chung HY (2002) Modulation of redox-sensitive transcription factors by calorie restriction during ageing. Mech Ageing Dev 123(12):1589–1595. https://doi.org/10.1016/s0047-6374(02)00094-5

Kim CH, Lee EK, Choi YJ, An HJ, Jeong HO, Park D et al (2016) Short-term calorie restriction ameliorates genomewide, age-related alterations in DNA methylation. Ageing Cell 15(6):1074–1081. https://doi.org/10.1111/acel.12513

Kohli P, Levy BD (2009) Resolvins and protectins: mediating solutions to inflammation. Br J Pharmacol 158(4):960–971. https://doi.org/10.1111/j.1476-5381.2009.00290.x

Korhonen P, Helenius M, Salminen A (1997) Age-related changes in the regulation of transcription factor NF-kappa B in rat brain. Neurosci Lett 225(1):61–64. https://doi.org/10.1016/s0304-3940(97)00190-0

Kurki E, Shi J, Martonen E, Finckenberg P, Mervaala E (2012) Distinct effects of calorie restriction on adipose tissue cytokine and angiogenesis profiles in obese and lean mice. Nutr Metab (Lond) 9(1):64. https://doi.org/10.1186/1743-7075-9-64

Lee HM, Kim TS, Jo EK (2016) MiR-146 and miR-125 in the regulation of innate immunity and inflammation. BMB Rep 49(6):311–318. https://doi.org/10.5483/bmbrep.2016.49.6.056

Levine SJ (2008) Molecular mechanisms of soluble cytokine receptor generation. J Biol Chem 283(21):14177–14181. https://doi.org/10.1074/jbc.R700052200

Li C, Xu MM, Wang K, Adler AJ, Vella AT, Zhou B (2018) Macrophage polarization and meta-inflammation. Transl Res 191:29–44. https://doi.org/10.1016/j.trsl.2017.10.004

Lopes-Paciencia S, Saint-Germain E, Rowell MC, Ruiz AF, Kalegari P, Ferbeyre G (2019) The senescence-associated secretory phenotype and its regulation. Cytokine 117:15–22. https://doi.org/10.1016/j.cyto.2019.01.013

Lowe JM, Menendez D, Bushel PR, Shatz M, Kirk EL, Troester MA et al (2014) p53 and NF-kappaB coregulate proinflammatory gene responses in human macrophages. Cancer Res 74(8):2182–2192. https://doi.org/10.1158/0008-5472.CAN-13-1070

Oishi Y, Manabe I (2016) Macrophages in age-related chronic inflammatory diseases. NPJ Ageing Mech Dis 2:16018. https://doi.org/10.1038/npjamd.2016.18

Pamplona FA, Vitória G, Ribeiro FC, Moraes CA, Ledur PF, Karmirian K et al (2021) Age-linked suppression of lipoxin A4 mediates cognitive deficits in mice and humans. bioRxiv, 2021.11.12.468379. https://doi.org/10.1101/2021.11.12.468379

Park MH, Hong JT (2016) Roles of NF-kappaB in cancer and inflammatory diseases and their therapeutic approaches. Cells 5(2). https://doi.org/10.3390/cells5020015

Park D, Lee EK, Jang EJ, Jeong HO, Kim BC, Ha YM et al (2013) Identification of the dichotomous role of age-related LCK in calorie restriction revealed by integrative analysis of cDNA microarray and interactome. Age (Dordr) 35(4):1045–1060. https://doi.org/10.1007/s11357-012-9426-6

Park CY, Park S, Kim MS, Kim HK, Han SN (2017) Effects of mild calorie restriction on lipid metabolism and inflammation in liver and adipose tissue. Biochem Biophys Res Commun 490(3):636–642. https://doi.org/10.1016/j.bbrc.2017.06.090

Rea IM, Gibson DS, McGilligan V, McNerlan SE, Alexander HD, Ross OA (2018) Age and age-related diseases: role of inflammation triggers and cytokines. Front Immunol 9:586. https://doi.org/10.3389/fimmu.2018.00586

Riffelmacher T, Richter FC, Simon AK (2018) Autophagy dictates metabolism and differentiation of inflammatory immune cells. Autophagy 14(2):199–206. https://doi.org/10.1080/15548627.2017.1362525

Salminen A, Kauppinen A, Kaarniranta K (2012) Emerging role of NF-kappaB signaling in the induction of senescence-associated secretory phenotype (SASP). Cell Signal 24(4):835–845. https://doi.org/10.1016/j.cellsig.2011.12.006

Schulze J, Vogelgesang A, Dressel A (2014) Catecholamines, steroids and immune alterations in ischemic stroke and other acute diseases. Ageing Dis 5(5):327–339. https://doi.org/10.14336/AD.2014.0500327

Shakeri H, Lemmens K, Gevaert AB, De Meyer GRY, Segers VFM (2018) Cellular senescence links ageing and diabetes in cardiovascular disease. Am J Physiol Heart Circ Physiol 315(3):H448–H462. https://doi.org/10.1152/ajpheart.00287.2018

Tilstra JS, Robinson AR, Wang J, Gregg SQ, Clauson CL, Reay DP et al (2012) NF-kappaB inhibition delays DNA damage-induced senescence and ageing in mice. J Clin Invest 122(7):2601–2612. https://doi.org/10.1172/JCI45785

Wang C, Zhou Z, Song W, Cai Z, Ding Z, Chen D et al (2022) Inhibition of IKKbeta/NF-kappaB signaling facilitates tendinopathy healing by rejuvenating inflammageing induced tendon-

derived stem/progenitor cell senescence. Mol Ther Nucleic Acids 27:562–576. https://doi.org/10.1016/j.omtn.2021.12.026

Wiley CD, Campisi J (2021) The metabolic roots of senescence: mechanisms and opportunities for intervention. Nat Metab 3(10):1290–1301. https://doi.org/10.1038/s42255-021-00483-8

Wyczalkowska-Tomasik A, Czarkowska-Paczek B, Zielenkiewicz M, Paczek L (2016) Inflammatory markers change with age, but do not fall beyond reported normal ranges. Arch Immunol Ther Exp (Warsz) 64(3):249–254. https://doi.org/10.1007/s00005-015-0357-7

Xia Y, Shen S, Verma IM (2014) NF-kappaB, an active player in human cancers. Cancer Immunol Res 2(9):823–830. https://doi.org/10.1158/2326-6066.CIR-14-0112

Yarbro JR, Emmons RS, Pence BD (2020) Macrophage immunometabolism and inflammageing: roles of mitochondrial dysfunction, cellular senescence, CD38, and NAD. Immunometabolism 2(3):e200026. https://doi.org/10.20900/immunometab20200026

Youm YH, Grant RW, McCabe LR, Albarado DC, Nguyen KY, Ravussin A et al (2013) Canonical Nlrp3 inflammasome links systemic low-grade inflammation to functional decline in ageing. Cell Metab 18(4):519–532. https://doi.org/10.1016/j.cmet.2013.09.010

Yu BP (1996) Ageing and oxidative stress: modulation by dietary restriction. Free Radic Biol Med 21(5):651–668. https://doi.org/10.1016/0891-5849(96)00162-1

Zhang G, Li J, Purkayastha S, Tang Y, Zhang H, Yin Y et al (2013) Hypothalamic programming of systemic ageing involving IKK-beta, NF-kappaB and GnRH. Nature 497(7448):211–216. https://doi.org/10.1038/nature12143

Chapter 4
Heart Disease and Ageing: The Roles of Senescence, Mitochondria, and Telomerase in Cardiovascular Disease

Laura K. Booth, Rachael E. Redgrave, Simon Tual-Chalot, Ioakim Spyridopoulos, Helen M. Phillips, and Gavin D. Richardson

Abstract During ageing molecular damage leads to the accumulation of several hallmarks of ageing including mitochondrial dysfunction, cellular senescence, genetic instability and chronic inflammation, which contribute to the development and progression of ageing-associated diseases including cardiovascular disease. Consequently, understanding how these hallmarks of biological ageing interact with the cardiovascular system and each other is fundamental to the pursuit of improving cardiovascular health globally. This review provides an overview of our current understanding of how candidate hallmarks contribute to cardiovascular diseases such as atherosclerosis, coronary artery disease and subsequent myocardial infarction, and age-related heart failure. Further, we consider the evidence that, even in the absence of chronological age, acute cellular stress leading to accelerated biological ageing expedites cardiovascular dysfunction and impacts on cardiovascular health. Finally, we consider the opportunities that modulating hallmarks of ageing offer for the development of novel cardiovascular therapeutics.

Keywords Ageing · Atherosclerosis · Cardiovascular · Heart failure · Inflammation · Remodelling · Senescence · Senolytic

L. K. Booth · I. Spyridopoulos
Translational and Clinical Research Institute, Vascular Biology and Medicine Theme, Newcastle University, Newcastle upon Tyne, UK
e-mail: L.Booth2@newcastle.ac.uk; ioakim.spyridopoulos@newcastle.ac.uk

R. E. Redgrave · S. Tual-Chalot · H. M. Phillips · G. D. Richardson (✉)
Biosciences Institute, Vascular Biology and Medicine Theme, Newcastle University, Newcastle upon Tyne, UK
e-mail: simon.tual-chalot@newcastle.ac.uk; helen.phillips@newcastle.ac.uk; Gavin.richardson@ncl.ac.uk

J. R. Harris, V. I. Korolchuk (eds.), *Biochemistry and Cell Biology of Ageing: Part IV, Clinical Science*, Subcellular Biochemistry 103,
https://doi.org/10.1007/978-3-031-26576-1_4

Introduction: Cardiovascular Ageing

More than 75% of Americans between the ages of 60 and 79, and nearly 90% of those over 80, suffer from cardiovascular disease (CVD) (Virani et al. 2021). It is therefore unsurprising that CVD is the leading cause of death for people over the age of 65 (North and Sinclair 2012). In the decades to come, increased prevalence of age-related CVDs such as atherosclerosis, coronary artery disease, myocardial infarction (MI), thoracic aortic aneurysm, valvular heart disease, and heart failure (HF) will contribute to even greater health and economic burden as the world's population continues to age (Childs et al. 2017; Olivieri et al. 2013). In this review, the mechanisms of cardiovascular ageing concerning several age-related CVDs will be described, with a focus on mitochondrial dysfunction and the non-canonical roles of telomerase.

Cardiovascular Ageing and Atherosclerosis

Atherosclerosis is classed as a disease of ageing and is the leading cause of vascular disease worldwide (Wang and Bennett 2012). Atherosclerosis is initiated by endothelial injury or accumulation of low-density lipoproteins (LDLs) within the arterial wall which leads to the development of lipid and protein-filled "plaques", triggering both the innate and adaptive immune responses (Moriya 2019). Inflammation stimulates necrotic core enlargement, extracellular matrix degeneration and cap thinning, erosion, calcification, and intra-plaque angiogenesis (Stojanović et al. 2020). Ultimately the atherosclerotic plaque can become unstable until rupture triggers thrombus formation, leading to blockage of the artery and thus cessation of blood flow distal to the occlusion leading to severe ischaemic injuries including MI and stroke (Montecucco et al. 2016; Thygesen et al. 2012; Moriya 2019).

Ageing and Heart Failure

An estimated 6.2 million adults in the USA are currently living with clinical HF with approximately 90% of these individuals being over 60 years old (Benjamin et al. 2019). HF is associated with ageing as a result of several pathophysiologies that contribute to impaired myocardial function and the inability of the heart to provide the circulatory efficiency required to meet organ demand.

At a cellular level, myocardial ageing is associated with structural, biochemical, and biomechanical changes including increased arterial stiffness, cardiomyocyte hypertrophy, chronic sterile inflammation, amyloid deposition, and increased interstitial fibrosis (Horn and Trafford 2016). Interestingly, fibrosis can occur in the absence of hypertension (Lin et al. 2008), suggesting that age-related fibrosis is

driven by an independent mechanism. Together these cellular changes affect the heart at the tissue level, leading to myocardial remodelling which is characterised by increased left ventricular (LV) mass, due to the thickening and stiffening of the LV walls and interventricular septum, and a decrease in diastolic functions such as myocardial relaxation and peak contractility (Triposkiadis et al. 2019). The apparent lack of systolic dysfunction with age has led to age-associated HF often being considered as HF with preserved ejection fraction (HFpEF) (Borlaug and Paulus 2011). However, it is becoming evident that with age, the systolic functional reserve is also diminished, and the heart is unable to respond to periods of increased cardiac demand (Norman et al. 2011). Over 50% of hospitalised HF patients exhibit HFpEF and the prevalence is expected to increase at a rate of over 10% per decade with population ageing (Benjamin et al. 2019; Borlaug and Paulus 2011).

As discussed above, atherosclerosis is ageing-associated and therefore there is also an increased prevalence of coronary heart disease (CHD) in the older population. The critical outcome of CHD is plaque rupture, causing artery blockage and myocardial infarction. If the blockage is not rapidly removed and sustained ischemia occurs, extensive cardiomyocyte death and cellular necrosis may take place, impacting cardiac function which can result in death. Following MI, the hearts of surviving patients undergo a rapid maladaptive myocardial remodelling characterised by dilatation, hypertrophy, and the formation of a discrete collagen scar. Additionally, progressive ventricular remodelling can continue for weeks or months following MI (Sutton and Sharpe 2000). Most patients receiving intervention for MI are in the older age range (mean age of 65 years old), and age remains the most important predictor of outcomes following MI, with the older population having increased mortality and poorer functional outcomes than younger individuals. Patients older than 70 account for up to half of those admitted to hospital with MI and 80% of deaths due to MI occur in those aged over 65 years (McMechan and Adgey 1998). Age is associated with an increased risk of developing heart failure: in a study of 896 patients, Torabi et al. observed that in the six years after MI, 50% of patients aged younger than 65 years, 73% of patients aged between 65–75 years, and 87% of patients over 75 years had developed heart failure (Torabi et al. 2014).

Ageing and Cardiothoracic Surgery

Ageing impacts, and is the most important prognostic indicator of cardiothoracic surgical outcome (Duncan et al. 2020). Subsequent to surgery, including that required to treat CVD, older individuals have increased mortality and are at higher risk of developing organ injury (Benedetto et al. 2021; Baquero and Rich 2015) which can lead to the progression of multiple chronic conditions (DiMaria-Ghalili et al. 2014; Matsuura et al. 2020), disability (Hong et al. 2021), and lower quality of life (Koch et al. 2007). The cost of treating organ injury as a result of cardiac surgery alone is >£100M per year in the UK (Sergin et al. 2016).

Senescence as a Driver of Cardiovascular Disease

Cellular senescence is considered a hallmark of ageing (López-Otín et al. 2013) defined as a loss of the division potential of mitotic cells and the production of the senescence-associated secretory phenotype (SASP), a cocktail of proinflammatory cytokines, chemokines, and growth factors. Therefore, senescence contributes to age-related tissue dysfunction through impaired homeostasis and elevated inflammation (Wiley and Campisi 2021). Senescent cells accumulate in most organ tissues with age, as well as in several age-related diseases and can be driven by several interconnected mechanisms including mitochondrial dysfunction, increased reactive oxygen species (ROS), DNA damage and telomere attrition (Kuilman et al. 2010). The identification of senescent cells is difficult as there are currently no known specific markers that unambiguously identify all senescent cells (Sharpless and Sherr 2015). As such, multiple markers including senescence-associated β-galactosidase (SA-β-Gal) activity, expression of cyclin-dependent kinase inhibitors (p21Cip, p16Ink4a, and p53), presence of DNA damage or critically short telomere length, and lack of proliferation, are often used in combination with each other to facilitate the characterisation of senescent cells (González-Gualda et al. 2021; de Magalhaes and Passos 2018). Transgenic mice which allow pharmacogenetic induction of apoptosis in p16-expressing senescent cells have been developed to investigate the contribution of senescence to ageing and age-associated disease (Baker et al. 2011; Demaria et al. 2014). The *p16-INKATTAC* and *p16-3MR* transgenic mice share a similar approach, as they both contain an apoptosis-inducing protein transgene, driven by the $p16^{Ink4a}$ promoter, which is only functional after the administration of pharmacological agents (Baker et al. 2011; Demaria et al. 2014). More recently, senescent cells have been shown to upregulate pro-survival pathways, thus protecting themselves from a hostile microenvironment (Zhu et al. 2015; Wang 1995). This not only aids senescent cell identification but can be exploited scientifically, as inhibiting these pathways causes apoptosis in senescent cells (Zhu et al. 2015). Several compounds that inhibit these pathways, including Bcl-2 family members, p53/p21Cip, ephrins, phosphatidylinositol-4,5-bisphosphate 3-kinase, plasminogen-activated inhibitor-1 and 2 and hypoxia-inducible factor-1α, have now been identified as promoting apoptosis in senescent cells, and have therefore been termed senolytics (Zhu et al. 2015). Together, these tools for senescent cell elimination have been used to demonstrate that in preclinical models, senescence is causal to the pathophysiology of multiple age-related diseases, including several CVDs (Anderson et al. 2019; Dookun et al. 2020; Martin-Ruiz et al. 2020; Walaszczyk et al. 2019; Baker et al. 2016; Demaria et al. 2017; Childs et al. 2016; Roos et al. 2016).

Clinically, there is extensive evidence of the association between vessel wall senescence accumulation and atherosclerosis (Stojanović et al. 2020). Histological analysis of post-mortem tissues has identified that atherosclerotic vessels contain more senescent endothelial and vascular smooth muscle cells than aged-matched healthy arteries (Stojanović et al. 2020). Moreover, expression of p16 in diseased

human coronary arteries positively correlates with plaque instability (Holdt et al. 2011), and the capability of cellular senescence to drive atherosclerosis is indicated by the increased risk of MI in relatively chronologically young patients with human progeria syndromes (Prakash et al. 2018).

Preclinically, LDL receptor-deficient and apolipoprotein E–deficient mouse models of atherosclerosis demonstrate that senescence accumulates in several cell populations in and around the atherogenic plaque during atherogenesis (Childs et al. 2016; Roos et al. 2016). These populations contribute to a proinflammatory environment and plaque development, indicated by the fact that senescent cell elimination reduces the expression of typical proinflammatory SASP proteins including matrix metalloproteinases (MMP) MMP-3, MMP-13, and the inflammatory cytokines IL-1α and tumour necrosis factor-α, and furthermore decreases plaque burden (Childs et al., 2016).

Senescence of immune cells, including the T cell population, is associated with atherogenesis and is a biomarker of CVD risk (Martin-Ruiz et al. 2020). Senescent, terminally differentiated CD8+ T-cells (TEMRA) are found inside unstable plaques and are an independent predictor of all-cause mortality in the elderly (Martin-Ruiz et al. 2020; Nakajima et al. 2002). While the studies of Childs et al. (2016) did not investigate if the elimination of senescent T-cells contributed to a reduced plaque burden, senolytics have been demonstrated to reduce T-cell immunosenescence in aged mice (Martin-Ruiz et al. 2020).

Senescence accumulates in cardiomyocytes, endothelial cells, cardiac fibroblasts and cardiac progenitor cells within the myocardium during ageing and is increased in the myocardium with age-associated CVD (Shimizu and Minamino 2019). Clinically, aged patients presenting with ventricular dysfunction have increased cardiomyocyte expression of p53 and p16, with increased expression being associated with hypertrophy at either an organ or cellular level (Song et al. 1999; Birks et al. 2008; Predmore et al. 2010). Cardiomyocyte senescence has also been linked to an increased risk of ventricular arrhythmias (Chadda et al. 2018). Endothelial cell senescence is observed with HF, particularly in patients suffering from HFpEF (Gevaert et al. 2017) and myocardial fibrosis and myofibroblast differentiation, which are increased with age and associated with HFpEF, are induced by senescent stimuli (Mellone et al. 2016; Zhu et al. 2013).

In vitro, paracrine SASP signalling from senescent myocardial cells, such as cardiomyocytes, endothelial cells, and fibroblasts, causes phenotypic alterations associated with cardiac remodelling. Several independent studies have also demonstrated that senescence promotes cardiac remodelling in preclinical studies: using senomorphic, senolytic, or pharmacogenetic approaches to reduce senescence, it has been demonstrated that attenuation of cardiac senescence decreases inflammation, cardiomyocyte hypertrophy and fibrosis in several different models of cardiovascular ageing (Anderson et al. 2019; Walaszczyk et al. 2019; Lewis-McDougall et al. 2019; Zhu et al. 2015; Baker et al. 2016; Iske et al. 2020; Demaria et al. 2017; Correia-Melo et al. 2019). In particular, elimination of senescent cells from aged mice also reduced LV mass and improved LV function (Walaszczyk et al. 2019; Zhu et al. 2015).

It is well-recognised that biological processes and phenomena associated with increased senescence are also associated with poorer surgical outcomes. Frailty, one of the best indicators of accumulated senescence, is prognostic of surgical outcome and increased age is associated with an increased peri-surgical likelihood of myocardial injury, accelerated progression of chronic conditions, disability, lower quality of life, and death (Koch et al. 2007; DiMaria-Ghalili et al. 2014; Matsuura et al. 2020; Nashef et al. 2012; Sun et al. 2021; Hong et al. 2021; Duncan et al. 2020). Preclinical studies have demonstrated a direct link between increased myocardial senescence, increased mortality and poorer outcomes following surgery (Walaszczyk et al. 2019; Iske et al. 2020). Interestingly, an accumulation of myocardial senescence is also associated with reduced resilience to isoproterenol-induced myocardial stress (Baker et al. 2016). Taken together, it is possible that increased myocardial senescence reduces a patient's resilience to cardiac surgery and contributes to poorer outcomes. If this is the case, blood sampling and SASP quantification prior to surgery may allow for better risk stratification of patients and would also suggest that targeting senescence is a potential therapeutic intervention to improve surgical outcomes.

Mitochondrial Dysfunction and Oxidative Stress

Progressive or chronic alterations in mitochondrial function and bioenergetics such as increased production of ROS, mitochondrial DNA (mtDNA) damage and respiratory chain dysfunction, occur in both the heart and vascular system (Judge et al. 2005; Ungvari et al. 2007; Navarro and Boveris 2007) and are associated with several age-related CVDs (Poznyak et al. 2020). Reduced mitochondrial bioenergetics is suggested to be a key contributor to the progression of heart failure. For example, polymorphisms in the gene peroxisome proliferator-activated receptor-gamma coactivator (*PGC-1α*), responsible for controlling and maintaining mitochondrial content, are linked to an increased risk of hypertrophic cardiomyopathy (Oka et al. 2020). Furthermore, a significant positive relationship exists between myocardial ROS levels and LV contractile dysfunction in failing hearts (Ide et al. 2000). Experimentally, mice deficient in mitochondrial superoxide dismutase, a ROS scavenger, exhibit characteristics of dilated cardiomyopathy (Lebovitz et al. 1996).

Age-related mitochondrial dysfunction has also been proposed as a key driver of the atherogenic processes (Nowak et al. 2017). Oxidative modification of LDL, and its transport into the subendothelial space of the arterial wall, is considered an initiating event for atherosclerosis (Nowak et al. 2017). Increased ROS and oxidative stress also promote endothelial dysfunction and apoptosis, as well as influencing T-cell and vascular smooth muscle cell proliferation and apoptosis (Richardson et al. 2018; Nowak et al. 2017). Together, this increases inflammation and the development of the atherosclerotic plaque, ultimately contributing to plaque rupture (Madamanchi and Runge 2007; Anderson et al. 2018). *PGC-1α* dysfunction leading

to increased ROS production can drive telomere shortening alongside both telomeric and non-telomeric DNA damage, accelerating vascular ageing and promoting atherosclerosis (Xiong et al. 2015). T cell mitochondrial function also declines with increased age (Ron-Harel et al. 2018) and T cells with dysfunctional mitochondria accelerate senescence in mice, leading to a premature ageing phenotype triggered by the induction of proinflammatory cytokines: so-called "inflammageing" (Desdín-Micó et al. 2020).

Similar to senescence, mitochondrial dysfunction is considered a key hallmark of ageing (López-Otín et al. 2013). However, only recently has the intricate and complex relationship between these two processes been identified. It is now clear that mitochondrial dysfunction is both a driver and consequence of cellular senescence (Chapman et al. 2019). Mitochondrial dysfunction, increased ROS production and associated oxidative stress activate the DNA damage response (DDR) and drive senescence (Chapman et al. 2019). ROS can cause DNA damage in the form of either single or double-stranded breaks (DSBs) throughout the genome, and telomeres appear to be particularly sensitive to ROS-induced damage due to their guanine-rich regions which have increased susceptibility to oxidation (Grollman and Moriya 1993). Single-strand DNA damage within telomeric regions can also accelerate telomere shortening due to the low efficiency of single-strand telomeric DNA damage repair (von Zglinicki et al. 2000). Telomeres containing single-strand DNA damage do not fully replicate during cellular division and shorten more in the following cellular division as the sequence beyond the damage is lost (von Zglinicki et al. 2000).

While many senescent cells have an increase in mitochondrial mass, their mitochondria are dysfunctional and demonstrate a decreased mitochondrial membrane potential, an increased proton leak and elevated ROS (Passos et al. 2007). Mitochondrial-derived ROS are a component of the SASP (Nelson et al. 2018), and mitochondria themselves are essential for the expression of SASP proteins (Correia-Melo et al. 2016). Senescent cells with depleted mitochondria lose their proinflammatory and pro-oxidant phenotype as well as the expression of the cyclin-dependent kinase inhibitors p21 and p16, however they remain in cell cycle arrest (Correia-Melo et al. 2016). Mitochondrial-mediated SASP production is at least in part controlled by a ROS–JNK signalling pathway which drives the release of cytoplasmic chromatin fragments, triggering the innate immunity cytosolic DNA-sensing cGAS-STING pathway (Vizioli et al. 2020). This in turn activates NFκB signalling, switching on the transcription of proinflammatory genes and the SASP (Vizioli et al. 2020). It has also been suggested that in senescent cells, increased mitochondrial outer membrane permeability (MOMP) allows the release of mtDNA which also activates cGAS-STING signalling, thus increasing proinflammatory gene expression. Further, the expression of pro-survival pathways may allow for sublethal apoptosis, termed minority MOMP, in which cytochrome c release and caspase activation induce DNA damage in the absence of apoptosis, contributing to genetic instability and perhaps deeper senescence (Birch and Passos 2017).

The mitochondrial genome is relatively sensitive to oxidative stress (Richter et al. 1988), further exacerbating the effects of mitochondrial dysfunction on disease pathophysiology: mitochondrial dysfunction ultimately leads to DNA damage in the mitochondrial genome and further mitochondrial dysfunction (Hollensworth et al. 2000). While the precise mechanisms for this remain unclear, the proximity of the mitochondrial genome to sites of ROS production and the lack of histones to provide protection have all been discussed (Miller et al. 2021). Together, these observations highlight the cyclical interactions between mitochondrial dysfunction, oxidative stress and senescence, and illustrate how the initiation of any of these processes could lead to a downward spiral in tissue function (Fig. 4.1).

In the heart, the cardiomyocytes of aged mice display a decline in expression of most mitochondrial genes, including those involved in the electron transport chain (Anderson et al. 2019). Moreover, and demonstrating that oxidative stress can drive cardiomyocyte senescence, transgenic mice overexpressing the pro-oxidant enzyme monoamine oxidase A (MAO-A), specifically in the cardiomyocytes, show increased myocardial senescence and exhibit myocardial dysfunction (Anderson et al. 2019).

Mitochondria also have mechanistic roles in acute CVD. This is notably seen in the context of ischaemia-reperfusion injury (IRI) which, though not an age-related disease per se, does primarily affect the older population since it is a significant consequence of reperfusion therapies used to return the blood supply to the myocardium following a heart attack (Aversano et al. 2002). IRI is a highly complex and multifactorial process (Neri et al. 2017; Halladin 2015; Hausenloy and Yellon 2013), but mitochondrial ROS generation is key to the pathophysiology driving cardiomyocyte death, endothelial dysfunction and microvascular occlusion, and promotes inflammation in this injury setting (Kalogeris et al. 2012; Neri et al. 2017; Hausenloy and Yellon 2013). As ROS can act as a contributor to mitochondrial Ca^{2+} overload, it can further increase ROS levels, creating a feedback loop whereby a state of Ca^{2+} overload is maintained and ROS generation is increased (Penna et al. 2009; Murphy and Steenbergen 2007; Kaneko et al. 1990). Unfortunately, while it is clear mitochondria and oxidative stress contribute to the pathophysiology of IRI, and several preclinical studies have reported antioxidants to protect against cardiac IRI (Dhalla et al. 2000), these results have failed to transfer clinically (Hausenloy and Yellon 2013; Desmet et al. 2011; Siddiqi et al. 2014; Atar et al. 2015). However, it may be that targeting the downstream effects of oxidative stress such as senescence, rather than oxidative stress itself, is a preferable strategy which may provide a longer treatment window to attenuate maladaptive remodelling post-cardiac IRI. In preclinical models of cardiac IRI, increased senescence has been observed in multiple cell types within the area of myocardium that experienced increased oxidative stress (Dookun et al. 2020). Interestingly, elimination of senescence following cardiac IRI using a senolytic approach improved cellular respiration, attenuated inflammation and remodelling, and enhanced revascularisation, all of which were associated with an improved myocardial function (Dookun et al. 2020). Numerous clinical conditions besides MI including stroke, organ

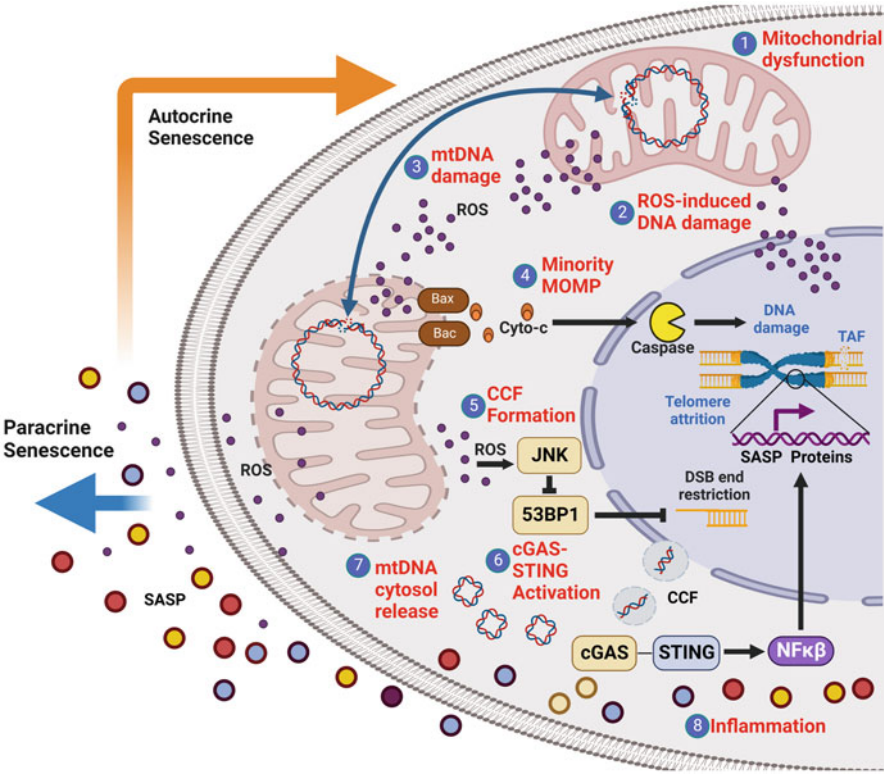


Fig. 4.1 Mitochondrial senescence and the SASP. (1) Mitochondrial dysfunction contributes to increased ROS production. (2) ROS can directly induce DNA damage in the form of double and single-stranded breaks in the genomic and telomeric DNA. DNA damage can also accelerate telomere shortening. Together these induce senescence. (3) Mitochondrial-derived ROS drives autocrine and paracrine mtDNA damage, further contributing to mitochondrial dysfunction. (4) Senescent cells have increased MOMP, allowing the release of caspase-activating cytochrome c. However, due to increased pro-survival protein expression in senescent cells, this release is insufficient to induce apoptosis (minority MOMP) and instead leads to increased mitochondrial and genomic DNA damage. (5) ROS activation of JNK inhibits tumour suppressor P53-binding protein 1 (53BP1) and thereby DSB end restriction, contributing to the release of cytoplasmic chromatin fragments which are detected by the cGAS-STING pathway (6), an innate immune system double-stranded DNA sensor which responds to CCF and (7) mtDNA release, due to increased mitochondrial membrane permeability, with NFκβ activation and expression of proinflammatory SASP genes which increases inflammation (8). These proinflammatory proteins, together with ROS, maintain autocrine cellular senescence but also induce senescence in surrounding tissues (bystander effect), propagating mitochondrial dysfunction and leading to a cascade in mitochondrial dysfunction and senescence

transplantation, and peripheral vascular disease (Widgerow 2014; Kalogeris et al. 2012) are linked to the occurrence of IRI, however, it has yet to be identified if senescence contributes to pathophysiology in these disease settings.

Telomeres and Telomerase in Cardiovascular Disease, Replicative Senescence and Beyond

The association between telomere length and CVD is well-established and reviewed (Hoffmann et al. 2021). Telomerase reporter mice and knockout models, such as those lacking expression of either the telomerase RNA component (TERC) or telomerase reverse transcriptase (TERT), have aided our understanding of how experimentally-induced myocardial senescence can contribute to CVD (Blasco et al. 1997; Leri et al. 2003; Richardson et al. 2012, 2018). However, it is now apparent that telomeres and telomerase interact with CVD beyond their roles in regulating replicative senescence.

It had long been debated as to how predominantly post-mitotic cardiomyocytes, which rarely proliferate and therefore are protected from replicative stress, can acquire a senescent phenotype. Data from recent studies, including our own, have now determined that senescence can also be induced by DNA-damaging agents including oxidative stress, which cause DNA damage foci that are preferentially located within telomeres, termed telomere-associated foci (TAF) (Hewitt et al. 2012; Anderson et al. 2019). During physiological ageing, cardiomyocytes in humans and mice accumulate TAF (Anderson et al. 2019). TAF are persistent (they are not as efficiently repaired as non-telomeric damaged DNA), induce senescence via activation of the p16 and p21 pathways, and occur independently of telomere shortening and proliferation (Anderson et al. 2019) (Fig. 4.2). Pharmacogenetic elimination of senescent cells from aged mice reduces the number of TAF-positive cardiomyocytes but does not affect telomere length (Anderson et al. 2019), suggesting that TAF accumulation is the primary trigger of cardiomyocyte senescence during ageing.

Telomerase activity is protective against senescence: cells stably transfected with human telomerase can divide indefinitely (Bodnar et al. 1998). Conversely, inhibition of either TERT or TERC promotes senescence, reduces lifespan and accelerates ageing in cells and small animal models (Shay and Wright 2004). Clinically, patients suffering from mutations in genes that encode TERT demonstrate an accelerated ageing phenotype that includes an increased prevalence of CVD (Armanios et al. 2005; Vulliamy et al. 2001; Ballew and Savage 2013; Khincha et al. 2017; Lina et al. 2008). While telomere maintenance contributes to these anti-senescence effects, TERT has activity independent of telomere preservation that may also protect against senescence. In particular, mitochondrial-localised TERT expression improves mitochondrial function, reduces mitochondrial ROS production, and protects against DNA damage and instability in both genomic and mitochondrial DNA (Haendeler et al. 2009; Ahmed et al. 2008; Fleisig et al. 2016). Mice which constitutively express TERT but are cancer-resistant (due to enhanced expression of the tumour suppressors p53, p16, and p19ARF) demonstrate decreased ageing-associated pathologies and an increase in median lifespan (Tomás-Loba et al. 2008). Interestingly, this anti-aged phenotype was associated not only with telomere maintenance but also a reduction in both genomic and telomere-associated DNA damage

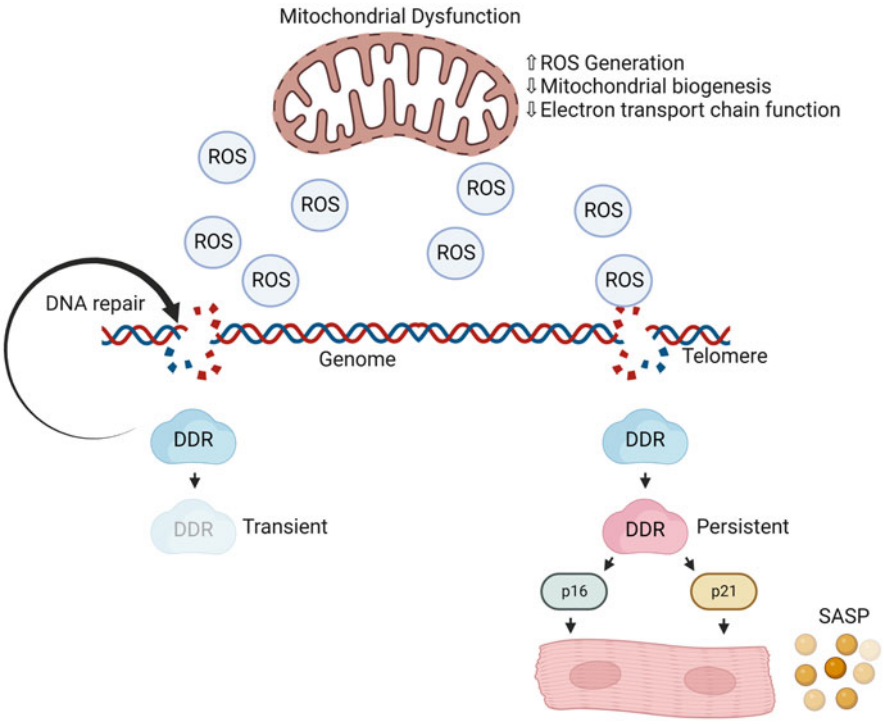


Fig. 4.2 Cardiomyocyte senescence is induced independently of telomere shortening. Mitochondrial dysfunction and increased ROS generation induce telomeric DNA damage in cardiomyocytes. While DNA damage occurs in both genomic and telomeric regions, genomic DNA damage is repaired, resulting in a transient DDR that may not be sufficient for senescence establishment. Alternatively, irreparable and therefore persistent DNA damage at telomeres causes a persistent DDR and cardiomyocyte senescence, which is associated with SASP-mediated inflammation

(Tomás-Loba et al. 2008), supporting a senoprotective role of telomerase that is independent of telomere maintenance.

The senescent phenotype is associated with a reduction in both canonical and non-canonical telomerase function, as p53 attenuates TERT expression via the inhibition of PGC-1α, which upregulates TERT expression in proliferative cells. As such, a crisis point must be reached in conditions of oxidative stress whereby sufficient DDR signalling activates p53, and presumably the senescence pathway, which then inhibits both mitochondrial biogenesis and the defensive functions of TERT. This facilitates escalating ROS generation, DNA damage (both mitochondrial and genomic) and ROS-mediated telomere shortening (Fig. 4.3). Indicating this may contribute to CVD are the observations that, in both cardiomyocytes and T-cells, p53 downregulates PGC-1α, resulting in increased mitochondrial oxidative damage (Villeneuve et al. 2013; Schank et al. 2020).

TERT is increased in multiple cell populations including cardiomyocytes in response to cardiac injury (Richardson et al. 2012). While the functionality of this

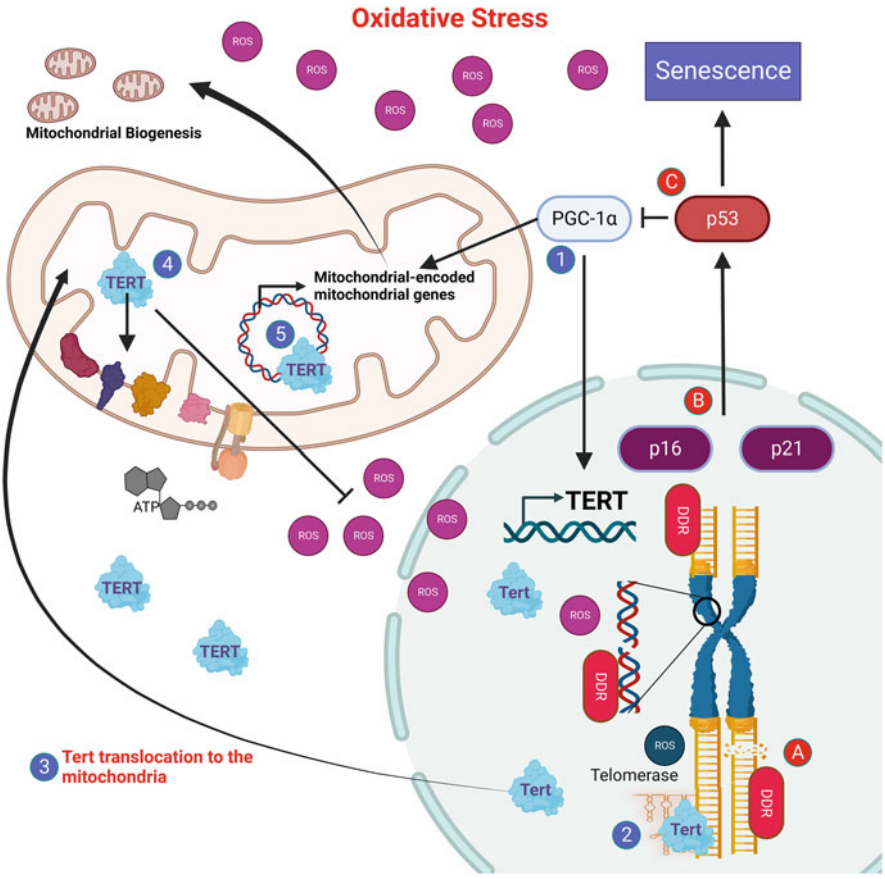


Fig. 4.3 TERT mediated protection from senescence. (1) In conditions of increased oxidative stress, PGC-1α promotes mitochondrial biogenesis and upregulates TERT expression. (2) Through canonical roles and telomerase function, TERT maintains telomeres, and oxidative stress promotes the export of TERT from the nucleus. (3) Through unknown mechanisms TERT translocates into the mitochondria. (4) Increases in mitochondrial-located TERT are associated with improved electron transport chain function and a decrease in ROS, processes which will attenuate DNA damage and telomere shortening. (5) TERT interacts with mtDNA and protects mtDNA from damage, maintaining mitochondrial biogenesis and thereby function. (A) If telomeres reach a crisis point or DNA damage is persistent, activation of the classical senescence pathways that converge in p53 activation (B). (C) p53 inhibits PGC-1α and therefore both mitochondrial biogenesis and TERT expression, preventing the defensive functions of TERT enabling increased oxidative stress, telomere attrition, and further DNA damage

expression is not yet known, given the post-mitotic nature of cardiomyocytes, it is arguably unlikely that TERT is upregulated to maintain telomeres during extensive proliferation. Instead, TERT upregulation could provide a mechanism for the cardiomyocyte, and potentially other cell types, to protect against mitochondrial dysfunction and oxidative stress, which in turn may protect against DNA damage

and senescence/apoptosis. In support of this notion, recent studies have demonstrated that mice which overexpress mitochondrial TERT, but lacked nuclear TERT, had improved mitochondrial respiration, attenuated myocardial remodelling and increased revascularization after they were subjected to cardiac IRI; these factors contributed to improved cardiac function (Ale-Agha et al. 2021). These benefits overlap with the outcome of the studies described earlier, in which senescence was eliminated following cardiac IRI (Dookun et al. 2020).

While the relationship between telomere length, atherosclerosis and the development of CHD has been studied extensively (Harst et al. 2007; Samani et al. 2001; Brouilette et al. 2007), little is known regarding the interactions between non-canonical telomerase activities and these diseases. T-cells express telomerase to maintain telomere length and protect against senescence (Weng et al. 1996). It has been demonstrated that oxidative stress can suppress telomerase activity within T-cells (Callender et al. 2018) which could accelerate T-cell senescence through two mechanisms: (**i**) as a result of replication in the absence of telomerase and (**ii**) through increased mitochondrial dysfunction and ROS generation due to suppression of mitochondrial TERT activity. Given that T-cell senescence is associated with a proinflammatory phenotype, chronic oxidative stress within an atherosclerotic plaque could contribute to disease pathophysiology. Furthermore, subpopulations of T-cells, such as T_{Reg} cells, are anti-atherogenic, and therefore senescence in these populations could also promote atherosclerosis.

Taken together this data suggests that if the pro-tumourigenic activities of TERT can be overcome, enhancing telomerase expression may have therapeutic value for a wide range of CVDs through senescence protection (telomere-dependent and -independent mechanisms), improved mitochondrial function, reduced oxidative stress and reduced inflammation. Interestingly statins, one of the most potent drug families which can slow the progression of atherosclerosis (by delaying age-related inflammatory changes in the arterial vessel wall), have been shown to stimulate telomerase activity (Bennaceur et al. 2014). Although, it remains unknown if stimulation of TERT's non-canonical activities contributes to the benefits associated with statin use.

Mitochondrial Dysfunction of T Lymphocytes as a Potential Mechanism of Enhanced Inflammation Post-myocardial Infarction

In vertebrates, premature ageing of the immune system (termed "immunosenescence") is mainly linked to thymic involution and changes in cellular immunity as a response to pathogens, such as recurrent viral infections, throughout life (Müller et al. 2013). These include a reduction in circulating lymphocytes and naïve T lymphocytes, the loss of stimulatory T lymphocyte co-receptors, the increase of oligoclonal memory cells, and finally, increased levels of proinflammatory

cytokines. Immune ageing has been proven to correlate with higher mortality across different age groups (Strindhall et al. 2007; Alpert et al. 2019). Relative lymphopenia in over 50,000 otherwise healthy middle-aged Americans has recently been identified as a strong predictor of overall mortality as well as cardiovascular mortality (Zidar et al. 2019). In the Newcastle 85+ study, we have previously shown that women exhibited higher lymphocyte counts and a higher frequency of naïve T-cells, paralleled by lower cardiovascular mortality, without differences in non-cardiovascular mortality (Martin-Ruiz et al. 2020; Spyridopoulos et al. 2016). We also found in a different study that MI leads to accelerated immunosenescence and shorter leukocyte telomere length (Hoffmann et al. 2015; Spyridopoulos et al. 2009). Finally, fewer lymphocytes following MI also predicted higher mortality in our patients (Boag et al. 2015; Spray et al. 2021). Lymphocyte proliferation can be enhanced in vitro by activating telomerase (Richardson et al. 2018). We have shown that T lymphocyte proliferation can be induced in a TERT-dependent manner in vitro by the telomerase activator (Richardson et al. 2018), TA-65MD® (T.A. Sciences, New York, USA). Further, TA-65 treatment has been shown to improve the outcome of mice after experimental MI, a function that required the presence of mitochondrial TERT (Ale-Agha et al. 2021; Jabbour et al. 2019). Importantly, mice that express mitochondrial-localised but lack nuclear-localised TERT are phenotypically normal and show no obvious signs of hyperproliferative diseases (Ale-Agha et al. 2021), suggesting that the TA-65-enhanced mitochondrial-TERT expression is not pro-tumourigenic. Our TACTIC (Telomerase Activator to Reverse Immunosenescence in Acute Coronary Syndrome: A Double-Blind, Phase II, Randomised Controlled Trial) study [5] is the first randomised clinical trial using a telomerase activator in patients following myocardial infarction, and will investigate whether treatment reduces inflammation while simultaneously enhancing immunity following MI.

Inflammation Is an Important Residual Risk Post-myocardial Infarction

Following myocardial infarction, secondary prevention for patients nowadays consists of targeting their residual risk, which is thought to be largely attributed to either hypercholesterolemia (which can be targeted with statins and proprotein convertase subtilisin/kexin type 9 inhibitors) or platelet aggregation (which can be treated with dual antiplatelet therapy). Recently inflammation, as quantified by high-sensitivity C-reactive protein (hsCRP), has been added to this. The CANTOS trial has successfully proven that reducing inflammation in CAD patients with elevated hsCRP (>2 mg/L) improves outcomes (Ridker et al. 2017). In a more detailed subanalysis of the CANTOS trial, the authors found that the relative improvement of outcome correlated directly to the magnitude of hsCRP reduction (Ridker et al. 2018). Secondly, adverse remodelling is also propagated by excessive inflammation;

early studies with the IL-1 antagonist Anakinra following MI suggest treatment can reduce the progression to heart failure (Abbate et al. 2020). However, a major limitation of the CANTOS trial was the effect of IL-1β blockade on immunity, as patients in the treatment group had a higher risk for infections as well as a higher risk of dying from sepsis. Clearly, there is a need for anti-inflammatory targets post-MI without compromising immunity; targeting immune ageing directly could present a viable option.

Mitophagy and Age-Related Cardiovascular Disease

In the heart, the mitochondria can metabolically adapt to changes in cardiac stress, ensuring they meet the high energy demand of the heart. However, as previously mentioned, ageing is associated with altered cardiac mitochondrial metabolism and mitochondrial dysfunction (Lesnefsky et al. 2016; Lesnefsky and Hoppel 2006). In order to prevent cardiomyocytes containing damaged mitochondria from undergoing cell death, the adaptive process mitophagy facilitates the efficient removal of dysfunctional and damaged mitochondria within a cell. Thus, the physiological consequences of mitophagy prevent ROS-mediated damage to proteins and DNA, and prevents inflammation. Hence, mitophagy maintains cardiac homeostasis by controlling a dynamic balance between the elimination of mitochondria and mitochondrial biogenesis, maintaining a healthy mitochondrial network. Mitophagy is defined as mitochondrial autophagy and is a selective form of autophagy specifically eliminating dysfunctional mitochondria in cells (Narendra et al. 2008). Autophagy is a key catabolic pathway in cellular quality control. During autophagy, damaged organelles are engulfed by autophagosomes and are subsequently degraded by fusion with lysosomes. There are two known mechanisms for mitophagy: adaptor-mediated and receptor-mediated. The former pathway functions via Phosphatase and Tensin Homolog (PTEN)-induced putative kinase 1 (PINK) and Parkin-mediated mitophagy, and this is the most well-characterised pathway. When a mitochondrion is dysfunctional or damaged, PINK1 accumulates on the outer surface of the mitochondrion (Lazarou et al. 2015). Here, PINK1 phosphorylates ubiquitin and the E3 ubiquitin ligase, Parkin, which ubiquitinates key mitochondrion-associated proteins (Jin et al. 2010; Matsuda et al. 2010; Narendra et al. 2008). These signals are bound by autophagic adaptor proteins such as p62/SQSTM1 that subsequently bind with microtubule-associated protein 1A/1B-light chain 3 (LC3), which is tethered on the phagophore membranes (Gustafsson and Dorn 2019), hence sequestering the ubiquitinated mitochondrion within the autophagosome. This fuses with a lysosome leading to the degradation of the damaged mitochondria (Pankiv et al. 2007; Lazarou et al. 2015). As such, impaired mitophagy leads to an accumulation of "old", defective mitochondria. While it remains unclear if impaired mitophagy contributes to senescence induction, is a result of senescence, or both, there are clear interactions between senescence-inducing pathways, the senescent phenotype and attenuated

mitophagy. The senescence regulator p53 can interact with Parkin by blocking the translocation of Parkin, p53 can suppress mitophagy (Ahmad et al. 2015).

Impaired mitophagy has been implicated in the pathology of various age-related CVDs (Dominic et al. 2014). In healthy young hearts, there is an underlying level of baseline mitophagy essential for maintaining the cellular homeostasis in an energy-efficient heart, and for responding and adapting to stress. Thus, the mitophagy pathways are tightly regulated. However, there is evidence that a decrease in mitophagy is associated with ageing (Zhou et al. 2017) and also with the pathogenesis of the CVD (Taneike et al. 2010), due to the accumulation of dysfunctional mitochondrial which reduces the ability of the heart to adapt to stress. In patients with the late stages of heart disease, a low number of autophagosomes is associated with a poor prognosis (Saito et al. 2016). This accounts for the pathological changes which are observed in the cardiac mitochondria of CVD patients, including the presence of giant megamitochondria, the loss, reorientation, or change in the shape of the cristae, formation of intramitochondrial rods and crystalloids (Hoppel et al. 2009).

In atherosclerosis, destabilisation of the atherosclerotic plaques has been associated with deficient mitophagy which in turn is linked to cell death, cell stress, and ROS accumulation in the plaques (Madamanchi and Runge 2007; Grootaert et al. 2018). Various studies have shown that within atherosclerotic plaques from human samples and mouse models, autophagy is either decreased or dysfunctional, shown by reduced expression of autophagic markers p62 and LC3-II (Razani et al. 2012; Sergin et al. 2016; Swaminathan et al. 2014). Activation of mitophagy has been suggested as a possible mechanism to slow disease progression. Antioxidant therapeutic strategies, such as melatonin treatment (which has anti-inflammatory properties) (Ma et al. 2018), activate mitophagy and as a consequence stabilise atherosclerotic plaques.

One important role of mitophagy is to suppress inflammation, which can lead to myocardial damage and is described as a key feature of CVD. ROS excretion and release of mtDNA from dysfunctional mitochondria activate the NLRP3 inflammasome (Nakahira et al. 2011; Heid et al. 2013) leading to inflammation in the heart. However, mitophagy can remove these damaged mitochondria and thus has an important role in suppressing inflammation. This was highlighted when transgenic mice with cardiac-specific overexpression of *Beclin1* (a key regulator of autophagy) were exposed to lipopolysaccharide-induced sepsis, and an activation of the PINK1/Parkin mitophagy pathway was observed—this was noted alongside reduced inflammation, fibrosis and improved cardiac function in the mice (Sun et al. 2018).

Mouse models which are *Parkin*-deficient or overexpress *Parkin* have illustrated that loss of mitophagy accelerates ageing in the heart (Kubli et al. 2013; Hoshino et al. 2013) or that enhancement of mitophagy delays cardiac ageing (Hoshino et al. 2013; Gao et al. 2021), respectively, highlighting the benefits of promoting Parkin-mediated mitophagy. Furthermore, following MI, cardiac-specific *Parkin*-deficient mice show that mitophagy is essential to reduce cardiac injury. After MI, the *Parkin*-deficient mice had reduced survival and developed larger infarcts: when compared to

the control mice, these infarcts had reduced mitophagy and increased dysfunctional mitochondria in the border zone, accounting for the observed heart failure (Kubli et al. 2013). Hence it has been demonstrated that loss of mitophagy exacerbates cardiac injury, leading to reduced survival.

Myocardial IRI leads to mitochondrial damage accompanied by an initial adaptive autophagic response to cardiac injury (Gustafsson and Gottlieb 2009), which is shown by an increase in mitophagy in both cardiomyocytes and platelets (Zhang et al. 2018). However, this response may be short-lived: Billia and colleagues showed that acute cardiac IRI ultimately leads to a reduction of autophagy flux, as seen in heart tissue from patients with end-stage heart failure (Billia et al. 2011). In the tissue, PINK1 protein levels were reduced compared to control samples, thus leading to the accumulation of damaged mitochondria, severe oxidative stress and apoptosis of cardiomyocytes (Campos et al. 2016). Studies from transgenic mouse models illustrate the initial cardioprotective role of mitophagy. In one example, cardiac-specific deletion of *Drp1* (a mitochondrial fission mediator) leads to an accumulation of elongated and damaged mitochondria, and suppresses mitophagy, thus promoting cardiac dysfunction and increasing susceptibility to IRI (Ikeda et al. 2015). An alternative mitophagy pathway is via the autophagy receptor Fun14 domain-containing protein 1 (FUNDC1), which interacts directly with LC3. In response to IRI-induced hypoxia, the *Fund1* knockout mouse model is unable to protect the heart from IRI due to a lack of mitophagy specifically (Zhang et al. 2017). It was later shown that general autophagy remained unchanged (Xu et al. 2022). Similarly, *Pink1*-deficient mice are more susceptible to IRI (Siddall et al. 2013), a phenotype which is rescued when *Pink1* is overexpressed, as this reduces cell death and decreases infarct size (Wang et al. 2015). Thus, it is the induction of mitophagy that contributes to the cardioprotective effect of attenuating cardiac injury. This has been proposed as a strategy to protect the cardiac environment in CVD, although this needs to be carefully appraised as excessive mitochondrial removal in response to IRI can increase cardiomyocyte death (Lesnefsky et al. 2017).

The association between mitophagy and senescence in CVD is evidenced by two independent studies which demonstrated that restoration of mitophagy prevents age-related CVDs by delaying cellular senescence. D-galactose-induced accelerated ageing mice display a phenotype which, when untreated, leads to increased cardiac cellular senescence and impaired cardiac function: mice were administered acacetin (Cui et al. 2018) or Kanglexin (KLX) (Li et al. 2022) and both treatments improved the cardiac function of these mice. In the former study, the authors found that the underlying mechanism for this improvement was protection from cellular senescence which was attributed to an upregulation of mitophagy (Hong et al. 2021). After treatment with KLX, a similar increase in mitophagy is noted, specifically in senescent cardiomyocytes, which was shown to be due to the enhanced stability of Parkin (Li et al. 2022). Moreover, treatment of a mouse model of accelerated ageing (caused by activation of the major proinflammatory NF-κB pathway) with rapamycin (a known autophagy and mitophagy activator) reduced cellular senescence, prevented age-related frailty and reduced histopathological evidence of age-related disease in several organs including the heart (Correia-Melo et al.

2019). Thus, enhancing mitophagy could represent a useful strategy for targeting age/senescence-related CVD. Manipulation of mitophagy is an attractive model to delay cardiac ageing and disease, however there are challenges as the level of mitophagy must be tightly regulated and is dose-dependent. Increasing autophagy excessively can lead to unnecessary degradation of cellular organelles and proteins leading to counterproductive energy deficiency.

Accelerated Ageing and Cardiovascular Disease: Chemotherapy-Induced Cardiotoxicity

Though cancer survival is improving globally, many anticancer interventions leave survivors with lasting off-target effects. Though radiation therapy has been reported to increase frailty, especially in the context of neurocognitive defects from cranial radiotherapy (Armstrong et al. 2013), pharmacological approaches can also promote an ageing phenotype in various organ systems. Given the increasing population of cancer survivors, understanding the long-term impacts of these off-target effects is a priority for clinicians, and chemotherapy-induced cardiotoxicity (CIC) is a major player in the field, which plagues even newer-generation chemotherapies such as tyrosine kinase inhibitors (Chaar et al. 2018). The anthracycline drug class, which remains a cornerstone of anticancer treatment for countless patients globally, is one of the most well-studied examples of CIC-inducing drugs. Most commonly used for breast cancer and sarcoma treatments, these drugs have long been associated with cardiovascular toxicity, most notoriously in a delayed symptomatic form, whereby cardiovascular phenomena arise many years after therapy conclusion. Typically, patients may present with arrhythmias, reduced LV function or fulminant heart failure, and interventions at this late stage in the disease are often inefficient. In the past, anthracycline-induced cardiotoxicity was categorised as either acute, early-onset, or late-onset. More recently, however, studies by Cardinale et al. have pointed towards anthracycline-induced cardiotoxicity (AIC) being one continuous phenomenon, with the temporal difference in presentation being perhaps due to patient risk factors and cumulative anthracycline dosage (Cardinale et al. 2015). This paradigm shift has facilitated a re-appraisal of how AIC may play out over time mechanistically, and this has allowed for more intuitive parallels to be drawn between progressive AIC and an accelerated ageing phenotype in the cardiac environment (Mitry et al. 2020; Maejima et al. 2008; Rebbaa et al. 2003). There is increasing interest in the concept that anthracycline-induced senescence may contribute to the long-term cardiotoxicity associated with these drugs (Saleh et al. 2020). Senescence induction is a recognised response to chemotherapy (Perkins et al. 2020; Ewald et al. 2010; Wang et al. 2020; Saleh et al. 2020; Wyld et al. 2020) and cancer survivors demonstrate an accelerated ageing phenotype overall, including increased comorbidity manifesting as conditions usually associated with ageing, including increased cardiac events, peripheral neuropathy, a decline in bone health and cognitive decline,

all consistent with an increase in systemic senescence (Cupit-Link et al. 2017). With regards to the heart, the commonly used anthracycline doxorubicin (DOX), for which DNA damage is a primary therapeutic mechanism, can induce fibroblasts and cardiomyocytes to senescence *in vitro* (Zhang et al. 2009; Maejima et al. 2008; Fourie et al. 2019). Murine studies have also implicated the accumulation of senescent cells within the heart as causal to AIC (Demaria et al. 2017). Using a transgenic model that allows the identification and elimination of p16-expressing senescent cells, Demaria et al. demonstrated that following exposure to DOX, senescence is increased, and cardiac function is reduced (Demaria et al. 2017). However, the elimination of senescent cells prevented the functional decline (Demaria et al. 2017). Similarly, elimination of senescence with the senolytic navitoclax reduced markers of cardiotoxicity and restored cardiac function in DOX-treated mice (Lérida-Viso et al. 2022). These data suggest that senescence is an active participant in the progression to myocardial dysfunction and not just a passive bystander. Interestingly, first-generation TERT-knockout mice (which retain long telomeres but lack the non-canonical activities of TERT) are more sensitive to DOX-induced cardiotoxicity than littermates (Werner et al. 2008), further supporting the notion that mitochondrial TERT is senoprotective. Moreover, cardiomyocyte-specific overexpression of TERT also protects from DOX-induced cardiac dysfunction (Chatterjee et al. 2021). Upon DOX exposure, TERT overexpression enhances TERT mitochondrial translocation, which protects against mitochondrial dysfunction and ROS generation (Chatterjee et al. 2021). While this study did not quantify senescence, the obvious DNA-damaging properties of DOX coupled with mitochondrial dysfunction/ROS being drivers of cardiomyocyte senescence prompt the idea that mitochondrial TERT may prevent or delay cardiomyocyte senescence, preventing senescence-induced myocardial remodelling and cardiac dysfunction. To date, most studies regarding CIC have focused on the anthracycline class of drugs. However, due to aforementioned improvements in cancer survivorship, it is now becoming evident that delayed and chronic cardiotoxicity may also be a factor with other classes of chemotherapeutic agents (Florescu et al. 2013; Michel et al. 2019) Interestingly, chemotherapeutics including paclitaxel, temozolomide, and cisplatin can induce senescence in murine skin (Demaria et al. 2017). Despite these studies, it remains unknown if senescence induction underlies the cardiotoxicity of these chemotherapies. Furthermore, as with ageing, interactions between the senescent myocardium and the adaptive immune system may contribute to CIC.

Figure 4.4 summarises how intrinsic and extrinsic stresses may drive mitochondrial dysfunction and oxidative stress leading senescence and CVD.

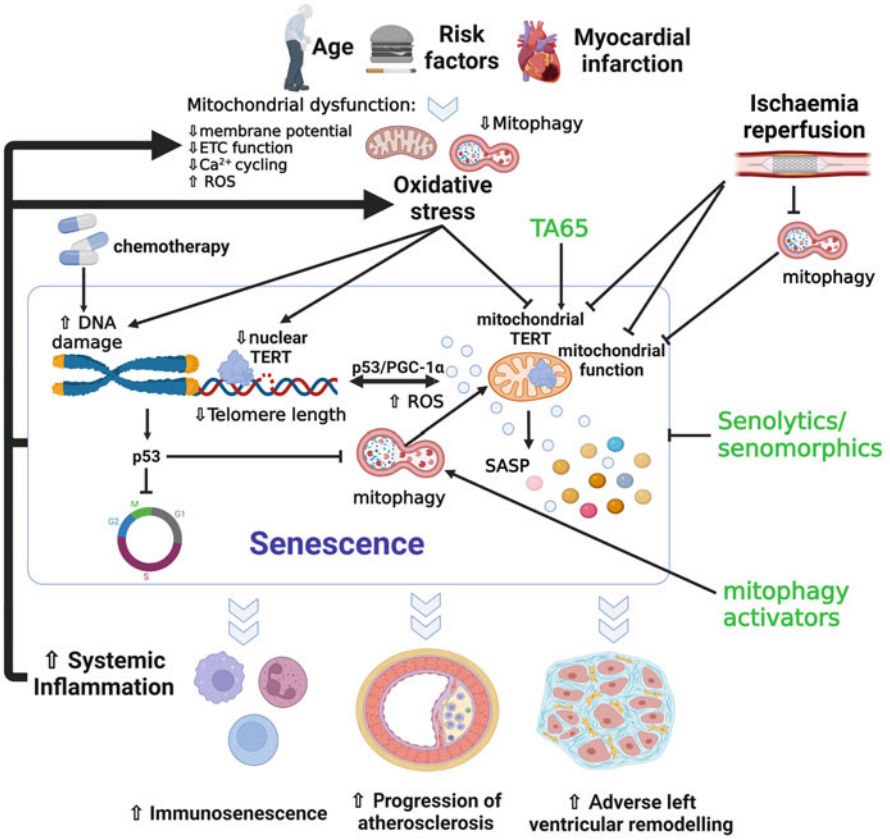


Fig. 4.4 Intrinsic and extrinsic stresses drive mitochondrial dysfunction and oxidative stress. This stress leads to increased *TERT* expression, which is senoprotective. If stress outweighs these protective functions, DNA damage leads to p53 activation, which downregulates *TERT* expression, and expression of p53 targets leads to senescence. Senescence drives local and systemic inflammation via the SASP, leading to age-related disease and a cascade of oxidative stress through a positive feedback loop. Activation of mitochondrial TERT with TA65 or pharmacological elimination of senescent cells reduces oxidative stress at a cellular- and organ-level, attenuating the cascade of oxidative stress. DNA-damaging agents such as anthracyclines may directly induce cellular senescence, contributing to increased systemic inflammation and oxidative stress through the same mechanisms

Viral Infection, Inflammation, Senescence, and Cardiovascular Disease

The presence of senescent immune cells can lead to a reduction in effective immunity, enhanced inflammation (by driving the secretion of inflammatory cytokines via the SASP), and as a result of endothelial and myocardial cell targeting through cytotoxic, pro-apoptotic mediators (granzyme, perforin) as previously reviewed (Liu

et al. 2020). Ageing is related to chronic low-grade sterile inflammation, increased immunosenescence and a high risk for cardiovascular-related mortality. We have observed that viral infection may accelerate immunosenescence as cytomegalovirus (CMV)-seropositive patients demonstrate signs of accelerated immune ageing following myocardial infarction, that seem to link with impaired myocardial healing (Spyridopoulos et al. 2009; Hoffmann et al. 2015). Importantly, in patients with previous CMV infection, where there is a known abundance of virus-specific cytotoxic T lymphocytes, we have found an (**i**) increased Th1 proinflammatory response, (**ii**) enhanced infiltration of the heart with T lymphocytes, and finally (**iii**) adverse cardiac remodelling (Martin-Ruiz et al. 2020; Hoffmann et al. 2015; Spyridopoulos et al. 2016; Spray et al. 2021). Accumulation of T-cell senescence is associated with higher mortality, age-related myocardial decline and a predisposition towards CVDs (Martin-Ruiz et al. 2020; Hoffmann et al. 2015; Spyridopoulos et al. 2016). Together, this suggests that CMV infection mediates immunosenescence, and the associated immune cell dysregulation contributes to excessive inflammation and thereby adverse remodelling following a myocardial infarction.

Acute cardiovascular complications are also associated with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and it is established that age is the greatest risk for mortality and morbidity post-infection (Xie et al. 2022). It is possible that these associations are a result of viral induction of senescence: it has been demonstrated that similar to CMV, albeit in a more acute setting, SARS-CoV-2 infection increases immune cell senescence, compromises cytotoxic T-cell activity, and that the SASP has been implicated in contributing to cytokine storm in coronavirus disease 2019 (COVID-19) patients (Lee et al. 2022). While the interaction between SARS-CoV-2 and senescence is not yet fully understood, the best indication that senescence contributes to COVID-19 are studies which have shown that the elimination of senescent cells improves the survival of aged mice against ß-coronavirus infection (Camell et al. 2021), and those that show that senescence elimination post SARS-CoV-2 infection reduced inflammation and mitigated a COVID-19-reminiscent lung disease in both hamsters and mice (Lee et al. 2021). Studies are underway to explore whether the senolytic Fisetin can reduce the requirement for hospitalisation in a cohort of older COVID-19 patients (Camell et al. 2021). Given the association between senescence and chronic diseases, including those of the cardiovascular system, it is possible that SARS-CoV-2-induced senescence may underlie several post-infection conditions, perhaps including so-called “long-COVID”.

Acknowledgements The authors acknowledge the contribution of past and present members of the laboratory of G.R who have contributed to the data discussed in this review. We acknowledge the funding support of the British Heart Foundation PG/22/10788 and PG/21/10761. Figures were created with BioRender.com.

References

Abbate A, Trankle CR, Buckley LF, Lipinski MJ, Appleton D, Kadariya D, Canada JM, Carbone S, Roberts CS, Abouzaki N, Melchior R, Christopher S, Turlington J, Mueller G, Garnett J, Thomas C, Markley R, Wohlford GF, Puckett L, Chazal HM, Chiabrando JG, Bressi E, Buono MGD, Schatz A, Vo C, Dixon DL, Biondi-Zoccai GG, Kontos MC, Tassell BWV (2020) Interleukin-1 blockade inhibits the acute inflammatory response in patients with ST-Segment–elevation myocardial infarction. J Am Heart Assoc 9(5):e014941. https://doi.org/10.1161/JAHA.119.014941

Ahmad T, Sundar IK, Lerner CA, Gerloff J, Tormos AM, Yao H, Rahman I (2015) Impaired mitophagy leads to cigarette smoke stress-induced cellular senescence: implications for chronic obstructive pulmonary disease. FASEB J 29(7):2912–2929. https://doi.org/10.1096/fj.14-268276

Ahmed S, JoF P, Birket MJ, Beckmann T, Brings S, Peters H, Birch-Machin MA, von Zglinicki T, Saretzki G (2008) Telomerase does not counteract telomere shortening but protects mitochondrial function under oxidative stress. J Cell Sci 121(7):1046–1053. https://doi.org/10.1242/jcs.019372

Ale-Agha N, Jakobs P, Goy C, Zurek M, Rosen J, Dyballa-Rukes N, Metzger S, Greulich J, von Ameln F, Eckermann O, Unfried K, Brack F, Grandoch M, Thielmann M, Kamler M, Gedik N, Kleinbongard P, Heinen A, Heusch G, Gödecke A, Altschmied J, Haendeler J (2021) Mitochondrial telomerase reverse transcriptase protects from myocardial ischemia/reperfusion injury by improving complex I composition and function. Circulation 144(23):1876–1890. https://doi.org/10.1161/circulationaha.120.051923

Alpert A, Pickman Y, Leipold M, Rosenberg-Hasson Y, Ji X, Gaujoux R, Rabani H, Starosvetsky E, Kveler K, Schaffert S, Furman D, Caspi O, Rosenschein U, Khatri P, Dekker CL, Maecker HT, Davis MM, Shen-Orr SS (2019) A clinically meaningful metric of immune age derived from high-dimensional longitudinal monitoring. Nat Med 25(3):487–495. https://doi.org/10.1038/s41591-019-0381-y

Anderson R, Richardson GD, Passos JF (2018) Mechanisms driving the ageing heart. Exp Gerontol 109:5–15. https://doi.org/10.1016/j.exger.2017.10.015

Anderson R, Lagnado A, Maggiorani D, Walaszczyk A, Dookun E, Chapman J, Birch J, Salmonowicz H, Ogrodnik M, Jurk D, Proctor C, Correia-Melo C, Victorelli S, Fielder E, Berlinguer-Palmini R, Owens A, Greaves LC, Kolsky KL, Parini A, Douin-Echinard V, LeBrasseur NK, Arthur HM, Tual-Chalot S, Schafer MJ, Roos CM, Miller JD, Robertson N, Mann J, Adams PD, Tchkonia T, Kirkland JL, Mialet-Perez J, Richardson GD, Passos JF (2019) Length-independent telomere damage drives post-mitotic cardiomyocyte senescence. EMBO J. https://doi.org/10.15252/embj.2018100492

Armanios M, Chen J-L, Chang Y-PC, Brodsky RA, Hawkins A, Griffin CA, Eshleman JR, Cohen AR, Chakravarti A, Hamosh A, Greider CW (2005) Haploinsufficiency of telomerase reverse transcriptase leads to anticipation in autosomal dominant dyskeratosis congenita. Proc Natl Acad Sci U S A 102(44):15960–15964. https://doi.org/10.1073/pnas.0508124102

Armstrong GT, Reddick WE, Petersen RC, Santucci A, Zhang N, Srivastava D, Ogg RJ, Hillenbrand CM, Sabin N, Krasin MJ, Kun L, Pui CH, Hudson MM, Robison LL, Krull KR (2013) Evaluation of memory impairment in aging adult survivors of childhood acute lymphoblastic leukemia treated with cranial radiotherapy. J Natl Cancer Inst 105(12):899–907. https://doi.org/10.1093/jnci/djt089

Atar D, Arheden H, Berdeaux A, Bonnet JL, Carlsson M, Clemmensen P, Cuvier V, Danchin N, Dubois-Rande JL, Engblom H, Erlinge D, Firat H, Halvorsen S, Hansen HS, Hauke W, Heiberg E, Koul S, Larsen AI, Le Corvoisier P, Nordrehaug JE, Paganelli F, Pruss RM, Rousseau H, Schaller S, Sonou G, Tuseth V, Veys J, Vicaut E, Jensen SE (2015) Effect of intravenous TRO40303 as an adjunct to primary percutaneous coronary intervention for acute ST-elevation myocardial infarction: MITOCARE study results. Eur Heart J 36(2):112–119. https://doi.org/10.1093/eurheartj/ehu331

Aversano T, Aversano LT, Passamani E, Knatterud GL, Terrin ML, Williams DO, Forman SA (2002) Thrombolytic therapy vs primary percutaneous coronary intervention for myocardial infarction in patients presenting to hospitals without on-site cardiac surgery: a randomized controlled trial. JAMA 287(15):1943–1951. https://doi.org/10.1001/jama.287.15.1943

Baker DJ, Wijshake T, Tchkonia T, LeBrasseur NK, Childs BG, van de Sluis B, Kirkland JL, van Deursen JM (2011) Clearance of p16Ink4a-positive senescent cells delays ageing-associated disorders. Nature 479(7372):232–236. https://doi.org/10.1038/nature10600

Baker DJ, Childs BG, Durik M, Wijers ME, Sieben CJ, Zhong J, Saltness RA, Jeganathan KB, Verzosa GC, Pezeshki A, Khazaie K, Miller JD, van Deursen JM (2016) Naturally occurring p16Ink4a-positive cells shorten healthy lifespan. Nature 530(7589):184–189. https://doi.org/10.1038/nature16932

Ballew BJ, Savage SA (2013) Updates on the biology and management of dyskeratosis congenita and related telomere biology disorders. Expert Rev Hematol 6(3):327–337. https://doi.org/10.1586/ehm.13.23

Baquero GA, Rich MW (2015) Perioperative care in older adults. J Geriatr Cardiol 12(5):465–469. https://doi.org/10.11909/j.issn.1671-5411.2015.05.018

Benedetto U, Sinha S, Dimagli A, Cooper G, Mariscalco G, Uppal R, Moorjani N, Krasopoulos G, Kaura A, Field M, Trivedi U, Kendall S, Angelini GD, Akowuah EF, Tsang G (2021) Decade-long trends in surgery for acute Type A aortic dissection in England: a retrospective cohort study. Lancet Reg Health - Europe 7:100131. https://doi.org/10.1016/j.lanepe.2021.100131

Benjamin EJ, Muntner P, Alonso A, Bittencourt MS, Callaway CW, Carson AP, Chamberlain AM, Chang AR, Cheng S, Das SR et al (2019) Heart Disease and Stroke Statistics-2019 Update: a report from the American Heart Association. Circulation 139(10):e56–e528. https://doi.org/10.1161/CIR.0000000000000659

Bennaceur K, Atwill M, Al Zhrany N, Hoffmann J, Keavney B, Breault D, Richardson G, von Zglinicki T, Saretzki G, Spyridopoulos I (2014) Atorvastatin induces T cell proliferation by a telomerase reverse transcriptase (TERT) mediated mechanism. Atherosclerosis 236(2):312–320. https://doi.org/10.1016/j.atherosclerosis.2014.07.020

Billia F, Hauck L, Konecny F, Rao V, Shen J, Mak TW (2011) PTEN-inducible kinase 1 (PINK1)/Park6 is indispensable for normal heart function. Proc Natl Acad Sci U S A 108(23):9572–9577. https://doi.org/10.1073/pnas.1106291108

Birch J, Passos JF (2017) Targeting the SASP to combat ageing: mitochondria as possible intracellular allies? BioEssays 39(5):1600235. https://doi.org/10.1002/bies.201600235

Birks EJ, Latif N, Enesa K, Folkvang T, Luong LA, Sarathchandra P, Khan M, Ovaa H, Terracciano CM, Barton PJR, Yacoub MH, Evans PC (2008) Elevated p53 expression is associated with dysregulation of the ubiquitin-proteasome system in dilated cardiomyopathy. Cardiovasc Res 79(3):472–480. https://doi.org/10.1093/cvr/cvn083

Blasco MA, Lee HW, Hande MP, Samper E, Lansdorp PM, DePinho RA, Greider CW (1997) Telomere shortening and tumour formation by mouse cells lacking telomerase RNA. Cell 91(1):25–34. https://doi.org/10.1016/s0092-8674(01)80006-4

Boag SE, Das R, Shmeleva EV, Bagnall A, Egred M, Howard N, Bennaceur K, Zaman A, Keavney B, Spyridopoulos I (2015) T lymphocytes and fractalkine contribute to myocardial ischemia/reperfusion injury in patients. J Clin Invest 125(8):3063–3076. https://doi.org/10.1172/jci80055

Bodnar AG, Ouellette M, Frolkis M, Holt SE, Chiu CP, Morin GB, Harley CB, Shay JW, Lichtsteiner S, Wright WE (1998) Extension of life-span by introduction of telomerase into normal human cells. Science 279(5349):349–352. https://doi.org/10.1126/science.279.5349.349

Borlaug BA, Paulus WJ (2011) Heart failure with preserved ejection fraction: pathophysiology, diagnosis, and treatment. Eur Heart J 32(6):670. https://doi.org/10.1093/eurheartj/ehq426

Brouilette SW, Moore JS, McMahon AD, Thompson JR, Ford I, Shepherd J, Packard CJ, Samani NJ (2007) Telomere length, risk of coronary heart disease, and statin treatment in the West of

Scotland Primary Prevention Study: a nested case-control study. Lancet 369(9556):107–114. https://doi.org/10.1016/s0140-6736(07)60071-3

Callender LA, Carroll EC, Beal RWJ, Chambers ES, Nourshargh S, Akbar AN, Henson SM (2018) Human CD8+ EMRA T cells display a senescence-associated secretory phenotype regulated by p38 MAPK. Aging Cell 17(1):e12675. https://doi.org/10.1111/acel.12675

Camell CD, Yousefzadeh MJ, Zhu Y, Prata LGPL, Huggins MA, Pierson M, Zhang L, O'Kelly RD, Pirtskhalava T, Xun P, Ejima K, Xue A, Tripathi U, Espindola-Netto JM, Giorgadze N, Atkinson EJ, Inman CL, Johnson KO, Cholensky SH, Carlson TW, LeBrasseur NK, Khosla S, O'Sullivan MG, Allison DB, Jameson SC, Meves A, Li M, Prakash YS, Chiarella SE, Hamilton SE, Tchkonia T, Niedernhofer LJ, Kirkland JL, Robbins PD (2021) Senolytics reduce coronavirus-related mortality in old mice. Science 373(6552):eabe4832. https://doi.org/10.1126/science.abe4832

Campos JC, Bozi LH, Bechara LR, Lima VM, Ferreira JC (2016) Mitochondrial quality control in cardiac diseases. Front Physiol 7:479. https://doi.org/10.3389/fphys.2016.00479

Cardinale D, Colombo A, Bacchiani G, Tedeschi I, Meroni CA, Veglia F, Civelli M, Lamantia G, Colombo N, Curigliano G, Fiorentini C, Cipolla CM (2015) Early detection of anthracycline cardiotoxicity and improvement with heart failure therapy. Circulation 131(22):1981–1988. https://doi.org/10.1161/CIRCULATIONAHA.114.013777

Chaar M, Kamta J, Ait-Oudhia S (2018) Mechanisms, monitoring, and management of tyrosine kinase inhibitors-associated cardiovascular toxicities. Onco Targets Ther 11:6227–6237. https://doi.org/10.2147/OTT.S170138

Chadda KR, Ajijola OA, Vaseghi M, Shivkumar K, Huang CL, Jeevaratnam K (2018) Ageing, the autonomic nervous system and arrhythmia: from brain to heart. Ageing Res Rev 48:40–50. https://doi.org/10.1016/j.arr.2018.09.005

Chapman J, Fielder E, Passos JF (2019) Mitochondrial dysfunction and cell senescence: deciphering a complex relationship. FEBS Lett 593(13):1566–1579. https://doi.org/10.1002/1873-3468.13498

Chatterjee S, Hofer T, Costa A, Lu D, Batkai S, Gupta SK, Bolesani E, Zweigerdt R, Megias D, Streckfuss-Bömeke K, Brandenberger C, Thum T, Bär C (2021) Telomerase therapy attenuates cardiotoxic effects of doxorubicin. Mol Ther 29(4):1395–1410. https://doi.org/10.1016/j.ymthe.2020.12.035

Childs BG, Baker DJ, Wijshake T, Conover CA, Campisi J, van Deursen JM (2016) Senescent intimal foam cells are deleterious at all stages of atherosclerosis. Science 354(6311):472–477. https://doi.org/10.1126/science.aaf6659

Childs BG, Gluscevic M, Baker DJ, Laberge RM, Marquess D, Dananberg J, van Deursen JM (2017) Senescent cells: an emerging target for diseases of ageing. Nat Rev Drug Discov 16(10):718–735. https://doi.org/10.1038/nrd.2017.116

Correia-Melo C, Marques FD, Anderson R, Hewitt G, Hewitt R, Cole J, Carroll BM, Miwa S, Birch J, Merz A, Rushton MD, Charles M, Jurk D, Tait SW, Czapiewski R, Greaves L, Nelson G, Bohlooly YM, Rodriguez-Cuenca S, Vidal-Puig A, Mann D, Saretzki G, Quarato G, Green DR, Adams PD, von Zglinicki T, Korolchuk VI, Passos JF (2016) Mitochondria are required for pro-ageing features of the senescent phenotype. EMBO J 35(7):724–742. https://doi.org/10.15252/embj.201592862

Correia-Melo C, Birch J, Fielder E, Rahmatika D, Taylor J, Chapman J, Lagnado A, Carroll BM, Miwa S, Richardson G, Jurk D, Oakley F, Mann J, Mann DA, Korolchuk VI, Passos JF (2019) Rapamycin improves healthspan but not inflammaging in nfκb1−/− mice. Aging Cell 18(1):e12882. https://doi.org/10.1111/acel.12882

Cui S, Xue L, Yang F, Dai S, Han Z, Liu K, Liu B, Yuan Q, Cui Z, Zhang Y, Xu F, Chen Y (2018) Postinfarction hearts are protected by premature senescent cardiomyocytes via GATA4-dependent CCN1 secretion. J Am Heart Assoc 7(18):e009111. https://doi.org/10.1161/JAHA.118.009111

Cupit-Link MC, Kirkland JL, Ness KK, Armstrong GT, Tchkonia T, LeBrasseur NK, Armenian SH, Ruddy KJ, Hashmi SK (2017) Biology of premature ageing in survivors of cancer. ESMO Open 2(5):e000250. https://doi.org/10.1136/esmoopen-2017-000250

de Magalhaes JP, Passos JF (2018) Stress, cell senescence and organismal ageing. Mech Ageing Dev 170:2–9. https://doi.org/10.1016/j.mad.2017.07.001

Demaria M, Ohtani N, Youssef Sameh A, Rodier F, Toussaint W, Mitchell James R, Laberge R-M, Vijg J, Van Steeg H, Dollé Martijn ET, Hoeijmakers Jan HJ, de Bruin A, Hara E, Campisi J (2014) An essential role for senescent cells in optimal wound healing through secretion of PDGF-AA. Dev Cell 31(6):722–733. https://doi.org/10.1016/j.devcel.2014.11.012

Demaria M, O'Leary MN, Chang J, Shao L, Liu S, Alimirah F, Koenig K, Le C, Mitin N, Deal AM, Alston S, Academia EC, Kilmarx S, Valdovinos A, Wang B, de Bruin A, Kennedy BK, Melov S, Zhou D, Sharpless NE, Muss H, Campisi J (2017) Cellular senescence promotes adverse effects of chemotherapy and cancer relapse. Cancer Discov 7(2):165–176. https://doi.org/10.1158/2159-8290.Cd-16-0241

Desdín-Micó G, Soto-Heredero G, Aranda JF, Oller J, Carrasco E, Gabandé-Rodríguez E, Blanco EM, Alfranca A, Cussó L, Desco M, Ibañez B, Gortazar AR, Fernández-Marcos P, Navarro MN, Hernaez B, Alcamí A, Baixauli F, Mittelbrunn M (2020) T cells with dysfunctional mitochondria induce multimorbidity and premature senescence. Science 368(6497):1371–1376. https://doi.org/10.1126/science.aax0860

Desmet W, Bogaert J, Dubois C, Sinnaeve P, Adriaenssens T, Pappas C, Ganame J, Dymarkowski S, Janssens S, Belmans A, Van de Werf F (2011) High-dose intracoronary adenosine for myocardial salvage in patients with acute ST-segment elevation myocardial infarction. Eur Heart J 32(7):867–877. https://doi.org/10.1093/eurheartj/ehq492

Dhalla NS, Elmoselhi AB, Hata T, Makino N (2000) Status of myocardial antioxidants in ischemia–reperfusion injury. Cardiovasc Res 47(3):446–456. https://doi.org/10.1016/s0008-6363(00)00078-x

DiMaria-Ghalili RA, Sullivan-Marx EM, Compher C (2014) Inflammation, functional status, and weight loss during recovery from cardiac surgery in older adults: a pilot study. Biol Res Nurs 16(3):344–352. https://doi.org/10.1177/1099800413503489

Dominic EA, Ramezani A, Anker SD, Verma M, Mehta N, Rao M (2014) Mitochondrial cytopathies and cardiovascular disease. Heart 100(8):611–618. https://doi.org/10.1136/heartjnl-2013-304657

Dookun E, Walaszczyk A, Redgrave R, Palmowski P, Tual-Chalot S, Suwana A, Chapman J, Jirkovsky E, Donastorg Sosa L, Gill E, Yausep OE, Santin Y, Mialet-Perez J, Andrew Owens W, Grieve D, Spyridopoulos I, Taggart M, Arthur HM, Passos JF, Richardson GD (2020) Clearance of senescent cells during cardiac ischemia-reperfusion injury improves recovery. Aging Cell 19(10):e13249. https://doi.org/10.1111/acel.13249

Duncan AE, Kartashov A, Robinson SB, Randall D, Zhang K, Luber J, James RA, Halvorson S, Bokesch P (2020) Risk factors, resource use, and cost of postoperative low cardiac output syndrome. J Thorac Cardiovasc Surg. https://doi.org/10.1016/j.jtcvs.2020.06.125

Ewald JA, Desotelle JA, Wilding G, Jarrard DF (2010) Therapy-induced senescence in cancer. J Natl Cancer Inst 102(20):1536–1546. https://doi.org/10.1093/jnci/djq364

Fleisig HB, Hukezalie KR, Thompson CA, Au-Yeung TT, Ludlow AT, Zhao CR, Wong JM (2016) Telomerase reverse transcriptase expression protects transformed human cells against DNA-damaging agents, and increases tolerance to chromosomal instability. Oncogene 35(2):218–227. https://doi.org/10.1038/onc.2015.75

Florescu M, Cinteza M, Vinereanu D (2013) Chemotherapy-induced cardiotoxicity. Maedica (Bucur) 8(1):59–67

Fourie C, Davis T, Kriel J, Engelbrecht AM (2019) The paracrine effects of fibroblasts on Doxorubicin-treated breast cancer cells. Exp Cell Res 381(2):280–287. https://doi.org/10.1016/j.yexcr.2019.05.020

Gao B, Yu W, Lv P, Liang X, Sun S, Zhang Y (2021) Parkin overexpression alleviates cardiac aging through facilitating K63-polyubiquitination of TBK1 to facilitate mitophagy. Biochim Biophys Acta Mol Basis Dis 1867(1):165997. https://doi.org/10.1016/j.bbadis.2020.165997

Gevaert AB, Shakeri H, Leloup AJ, Van Hove CE, De Meyer GRY, Vrints CJ, Lemmens K, Van Craenenbroeck EM (2017) Endothelial senescence contributes to heart failure with preserved ejection fraction in an aging mouse model. Circ Heart Fail 10(6). https://doi.org/10.1161/circheartfailure.116.003806

González-Gualda E, Baker AG, Fruk L, Muñoz-Espín D (2021) A guide to assessing cellular senescence in vitro and in vivo. FEBS J 288(1):56–80. https://doi.org/10.1111/febs.15570

Grollman AP, Moriya M (1993) Mutagenesis by 8-oxoguanine: an enemy within. Trends Genet 9(7):246–249. https://doi.org/10.1016/0168-9525(93)90089-Z

Grootaert MOJ, Roth L, Schrijvers DM, De Meyer GRY, Martinet W (2018) Defective autophagy in atherosclerosis: to die or to senesce? Oxid Med Cell Longev 2018:7687083. https://doi.org/10.1155/2018/7687083

Gustafsson AB, Dorn GW 2nd (2019) Evolving and expanding the roles of mitophagy as a homeostatic and pathogenic process. Physiol Rev 99(1):853–892. https://doi.org/10.1152/physrev.00005.2018

Gustafsson AB, Gottlieb RA (2009) Autophagy in ischemic heart disease. Circ Res 104(2):150–158. https://doi.org/10.1161/CIRCRESAHA.108.187427

Haendeler J, Dröse S, Büchner N, Jakob S, Altschmied J, Goy C, Spyridopoulos I, Zeiher AM, Brandt U, Dimmeler S (2009) Mitochondrial telomerase reverse transcriptase binds to and protects mitochondrial DNA and function from damage. Arterioscler Thromb Vasc Biol 29(6):929–935. https://doi.org/10.1161/ATVBAHA.109.185546

Halladin N (2015) Oxidative and inflammatory biomarkers of ischemia and reperfusion injuries. Danish Med J 62(4):B5054

Harst P, Steege G, Boer RA, Voors AA, Hall AS, Mulder MJ, Gilst WH, Veldhuisen DJ (2007) Telomere length of circulating leukocytes is decreased in patients with chronic heart failure. J Am Coll Cardiol 49(13):1459–1464. https://doi.org/10.1016/j.jacc.2007.01.027

Hausenloy DJ, Yellon DM (2013) Myocardial ischemia-reperfusion injury: a neglected therapeutic target. J Clin Invest 123(1):92–100. https://doi.org/10.1172/JCI62874

Heid ME, Keyel PA, Kamga C, Shiva S, Watkins SC, Salter RD (2013) Mitochondrial reactive oxygen species induces NLRP3-dependent lysosomal damage and inflammasome activation. J Immunol 191(10):5230–5238. https://doi.org/10.4049/jimmunol.1301490

Hewitt G, Jurk D, Marques FDM, Correia-Melo C, Hardy T, Gackowska A, Anderson R, Taschuk M, Mann J, Passos JF (2012) Telomeres are favoured targets of a persistent DNA damage response in ageing and stress-induced senescence. Nat Commun 3:708

Hoffmann J, Shmeleva EV, Boag SE, Fiser K, Bagnall A, Murali S, Dimmick I, Pircher H, Martin-Ruiz C, Egred M, Keavney B, von Zglinicki T, Das R, Todryk S, Spyridopoulos I (2015) Myocardial ischemia and reperfusion leads to transient CD8 immune deficiency and accelerated immunosenescence in CMV-seropositive patients. Circ Res 116(1):87–98. https://doi.org/10.1161/circresaha.116.304393

Hoffmann J, Richardson G, Haendeler J, Altschmied J, Andrés V, Spyridopoulos I (2021) Telomerase as a therapeutic target in cardiovascular disease. Arterioscler Thromb Vasc Biol 41(3):1047–1061. https://doi.org/10.1161/ATVBAHA.120.315695

Holdt LM, Sass K, Gäbel G, Bergert H, Thiery J, Teupser D (2011) Expression of Chr9p21 genes CDKN2B (p15(INK4b)), CDKN2A (p16(INK4a), p14(ARF)) and MTAP in human atherosclerotic plaque. Atherosclerosis 214(2):264–270. https://doi.org/10.1016/j.atherosclerosis.2010.06.029

Hollensworth SB, Shen C, Sim JE, Spitz DR, Wilson GL, LeDoux SP (2000) Glial cell type-specific responses to menadione-induced oxidative stress. Free Radic Biol Med 28(8):1161–1174. https://doi.org/10.1016/s0891-5849(00)00214-8

Hong YX, Wu WY, Song F, Wu C, Li GR, Wang Y (2021) Cardiac senescence is alleviated by the natural flavone acacetin via enhancing mitophagy. Aging (Albany NY) 13(12):16381–16403. https://doi.org/10.18632/aging.203163

Hoppel CL, Tandler B, Fujioka H, Riva A (2009) Dynamic organization of mitochondria in human heart and in myocardial disease. Int J Biochem Cell Biol 41(10):1949–1956. https://doi.org/10.1016/j.biocel.2009.05.004

Horn MA, Trafford AW (2016) Aging and the cardiac collagen matrix: novel mediators of fibrotic remodelling. J Mol Cell Cardiol 93:175–185. https://doi.org/10.1016/j.yjmcc.2015.11.005

Hoshino A, Mita Y, Okawa Y, Ariyoshi M, Iwai-Kanai E, Ueyama T, Ikeda K, Ogata T, Matoba S (2013) Cytosolic p53 inhibits Parkin-mediated mitophagy and promotes mitochondrial dysfunction in the mouse heart. Nat Commun 4:2308. https://doi.org/10.1038/ncomms3308

Ide T, Tsutsui H, Kinugawa S, Suematsu N, Hayashidani S, Ichikawa K, Utsumi H, Machida Y, Egashira K, Takeshita A (2000) Direct evidence for increased hydroxyl radicals originating from superoxide in the failing myocardium. Circ Res 86(2):152–157. https://doi.org/10.1161/01.RES.86.2.152

Ikeda Y, Shirakabe A, Maejima Y, Zhai P, Sciarretta S, Toli J, Nomura M, Mihara K, Egashira K, Ohishi M, Abdellatif M, Sadoshima J (2015) Endogenous Drp1 mediates mitochondrial autophagy and protects the heart against energy stress. Circ Res 116(2):264–278. https://doi.org/10.1161/CIRCRESAHA.116.303356

Iske J, Seyda M, Heinbokel T, Maenosono R, Minami K, Nian Y, Quante M, Falk CS, Azuma H, Martin F, Passos JF, Niemann CU, Tchkonia T, Kirkland JL, Elkhal A, Tullius SG (2020) Senolytics prevent mt-DNA-induced inflammation and promote the survival of aged organs following transplantation. Nat Commun 11(1):4289. https://doi.org/10.1038/s41467-020-18039-x

Jabbour E, Mullighan CG, Pullarkat VA, Lacayo NJ, Bajel A, Rubnitz J, Leonard J, Khaw SL, Vear SI, Alexander T, Norris R, Salem A, Schmidt M, Tong B, Zhou Y, Ross JA, Bensman L, Jacobson A, Stock W (2019) Combination BCL-2 inhibitor therapy with venetoclax and navitoclax in patients with relapsed/refractory acute lymphoblastic leukemia and lymphoblastic lymphoma. Clin Lymphoma Myeloma Leuk 19:S184–S185. https://doi.org/10.1016/j.clml.2019.07.022

Jin SM, Lazarou M, Wang C, Kane LA, Narendra DP, Youle RJ (2010) Mitochondrial membrane potential regulates PINK1 import and proteolytic destabilization by PARL. J Cell Biol 191(5):933–942. https://doi.org/10.1083/jcb.201008084

Judge S, Jang YM, Smith A, Hagen T, Leeuwenburgh C (2005) Age-associated increases in oxidative stress and antioxidant enzyme activities in cardiac interfibrillar mitochondria: implications for the mitochondrial theory of aging. FASEB J 19(3):1–21. https://doi.org/10.1096/fj.04-2622fje

Kalogeris T, Baines CP, Krenz M, Korthuis RJ (2012) Cell biology of ischemia/reperfusion injury. Int Rev Cell Mol Biol 298:229–317. https://doi.org/10.1016/B978-0-12-394309-5.00006-7

Kaneko M, Singal PK, Dhalla NS (1990) Alterations in heart sarcolemmal Ca2+-ATPase and Ca2+-binding activities due to oxygen free radicals. Basic Res Cardiol 85(1):45–54. https://doi.org/10.1007/bf01907013

Khincha PP, Bertuch AA, Agarwal S, Townsley DM, Young NS, Keel S, Shimamura A, Boulad F, Simoneau T, Justino H, Kuo C, Artandi S, McCaslin C, Cox DW, Chaffee S, Collins BF, Giri N, Alter BP, Raghu G, Savage SA (2017) Pulmonary arteriovenous malformations: an uncharacterised phenotype of dyskeratosis congenita and related telomere biology disorders. Eur Respir J 49(1):1601640. https://doi.org/10.1183/13993003.01640-2016

Koch CG, Li L, Lauer M, Sabik J, Starr NJ, Blackstone EH (2007) Effect of functional health-related quality of life on long-term survival after cardiac surgery. Circulation 115(6):692–699. https://doi.org/10.1161/CIRCULATIONAHA.106.640573

Kubli DA, Quinsay MN, Gustafsson AB (2013) Parkin deficiency results in accumulation of abnormal mitochondria in aging myocytes. Commun Integr Biol 6(4):e24511. https://doi.org/10.4161/cib.24511

Kuilman T, Michaloglou C, Mooi WJ, Peeper DS (2010) The essence of senescence. Genes Dev 24(22):2463–2479. https://doi.org/10.1101/gad.1971610

Lazarou M, Sliter DA, Kane LA, Sarraf SA, Wang C, Burman JL, Sideris DP, Fogel AI, Youle RJ (2015) The ubiquitin kinase PINK1 recruits autophagy receptors to induce mitophagy. Nature 524(7565):309–314. https://doi.org/10.1038/nature14893

Lebovitz RM, Zhang H, Vogel H, Cartwright J, Dionne L, Lu N, Huang S, Matzuk MM (1996) Neurodegeneration, myocardial injury, and perinatal death in mitochondrial superoxide dismutase-deficient mice. Proc Natl Acad Sci 93(18):9782–9787. https://doi.org/10.1073/pnas.93.18.9782

Lee S, Yu Y, Trimpert J, Benthani F, Mairhofer M, Richter-Pechanska P, Wyler E, Belenki D, Kaltenbrunner S, Pammer M et al (2021) Virus-induced senescence is a driver and therapeutic target in COVID-19. Nature 599(7884):283–289. https://doi.org/10.1038/s41586-021-03995-1

Lee KA, Flores RR, Jang IH, Saathoff A, Robbins PD (2022) Immune senescence, immunosenescence and aging. Front Aging 3:900028. https://doi.org/10.3389/fragi.2022.900028

Leri A, Franco S, Zacheo A, Barlucchi L, Chimenti S, Limana F, Nadal-Ginard B, Kajstura J, Anversa P, Blasco MA (2003) Ablation of telomerase and telomere loss leads to cardiac dilatation and heart failure associated with p53 upregulation. EMBO J 22(1):131–139. https://doi.org/10.1093/emboj/cdg013

Lérida-Viso A, Estepa-Fernández A, Morellá-Aucejo Á, Lozano-Torres B, Alfonso M, Blandez JF, Bisbal V, Sepúlveda P, García-Fernández A, Orzáez M, Martínez-Máñez R (2022) Pharmacological senolysis reduces doxorubicin-induced cardiotoxicity and improves cardiac function in mice. Pharmacol Res:106356. https://doi.org/10.1016/j.phrs.2022.106356

Lesnefsky EJ, Hoppel CL (2006) Oxidative phosphorylation and aging. Ageing Res Rev 5(4):402–433. https://doi.org/10.1016/j.arr.2006.04.001

Lesnefsky EJ, Chen Q, Hoppel CL (2016) Mitochondrial metabolism in aging heart. Circ Res 118(10):1593–1611. https://doi.org/10.1161/CIRCRESAHA.116.307505

Lesnefsky EJ, Chen Q, Tandler B, Hoppel CL (2017) Mitochondrial dysfunction and myocardial ischemia-reperfusion: implications for novel therapies. Annu Rev Pharmacol Toxicol 57:535–565. https://doi.org/10.1146/annurev-pharmtox-010715-103335

Lewis-McDougall FC, Ruchaya PJ, Domenjo-Vila E, Shin Teoh T, Prata L, Cottle BJ, Clark JE, Punjabi PP, Awad W, Torella D, Tchkonia T, Kirkland JL, Ellison-Hughes GM (2019) Aged-senescent cells contribute to impaired heart regeneration. Aging Cell 18(3):e12931. https://doi.org/10.1111/acel.12931

Li HM, Liu X, Meng ZY, Wang L, Zhao LM, Chen H, Wang ZX, Cui H, Tang XQ, Li XH, Han WN, Bai X, Lin Y, Liu H, Zhang Y, Yang BF (2022) Kanglexin delays heart aging by promoting mitophagy. Acta Pharmacol Sin 43(3):613–623. https://doi.org/10.1038/s41401-021-00686-5

Lin J, Lopez EF, Jin Y, Van Remmen H, Bauch T, Han H-C, Lindsey ML (2008) Age-related cardiac muscle sarcopenia: combining experimental and mathematical modeling to identify mechanisms. Exp Gerontol 43(4):296–306. https://doi.org/10.1016/j.exger.2007.12.005

Lina B-V, Inderjeet D, Hannah T, Abraham A, Ben Zion G, Alexander V, Tom V (2008) Expanding the clinical phenotype of autosomal dominant dyskeratosis congenita caused by TERT mutations. Haematologica 93(6):943–944. https://doi.org/10.3324/haematol.12317

Liu D, Richardson G, Benli FM, Park C, de Souza JV, Bronowska AK, Spyridopoulos I (2020) Inflammageing in the cardiovascular system: mechanisms, emerging targets, and novel therapeutic strategies. Clin Sci (Lond) 134(17):2243–2262. https://doi.org/10.1042/cs20191213

López-Otín C, Blasco MA, Partridge L, Serrano M, Kroemer G (2013) The hallmarks of aging. Cell 153(6):1194–1217. https://doi.org/10.1016/j.cell.2013.05.039

Ma S, Chen J, Feng J, Zhang R, Fan M, Han D, Li X, Li C, Ren J, Wang Y, Cao F (2018) Melatonin ameliorates the progression of atherosclerosis via mitophagy activation and NLRP3 inflammasome inhibition. Oxid Med Cell Longev 2018:9286458. https://doi.org/10.1155/2018/9286458

Madamanchi NR, Runge MS (2007) Mitochondrial dysfunction in atherosclerosis. Circ Res 100(4): 460–473. https://doi.org/10.1161/01.RES.0000258450.44413.96

Maejima Y, Adachi S, Ito H, Hirao K, Isobe M (2008) Induction of premature senescence in cardiomyocytes by doxorubicin as a novel mechanism of myocardial damage. Aging Cell 7(2): 125–136. https://doi.org/10.1111/j.1474-9726.2007.00358.x

Martin-Ruiz C, Hoffmann J, Shmeleva E, Zglinicki TV, Richardson G, Draganova L, Redgrave R, Collerton J, Arthur H, Keavney B, Spyridopoulos I (2020) CMV-independent increase in CD27-CD28+ CD8+ EMRA T cells is inversely related to mortality in octogenarians. NPJ Aging Mech Dis 6:3. https://doi.org/10.1038/s41514-019-0041-y

Matsuda N, Sato S, Shiba K, Okatsu K, Saisho K, Gautier CA, Sou YS, Saiki S, Kawajiri S, Sato F, Kimura M, Komatsu M, Hattori N, Tanaka K (2010) PINK1 stabilized by mitochondrial depolarization recruits Parkin to damaged mitochondria and activates latent Parkin for mitophagy. J Cell Biol 189(2):211–221. https://doi.org/10.1083/jcb.200910140

Matsuura R, Iwagami M, Moriya H, Ohtake T, Hamasaki Y, Nangaku M, Doi K, Kobayashi S, Noiri E (2020) The clinical course of acute kidney disease after cardiac surgery: a retrospective observational study. Sci Rep 10(1):6490. https://doi.org/10.1038/s41598-020-62981-1

McMechan SR, Adgey AA (1998) Age related outcome in acute myocardial infarction. Elderly people benefit from thrombolysis and should be included in trials. BMJ 317(7169):1334–1335. https://doi.org/10.1136/bmj.317.7169.1334

Mellone M, Hanley CJ, Thirdborough S, Mellows T, Garcia E, Woo J, Tod J, Frampton S, Jenei V, Moutasim KA, Kabir TD, Brennan PA, Venturi G, Ford K, Herranz N, Lim KP, Clarke J, Lambert DW, Prime SS, Underwood TJ, Vijayanand P, Eliceiri KW, Woelk C, King EV, Gil J, Ottensmeier CH, Thomas GJ (2016) Induction of fibroblast senescence generates a non-fibrogenic myofibroblast phenotype that differentially impacts on cancer prognosis. Aging (Albany NY) 9(1):114–132. https://doi.org/10.18632/aging.101127

Michel L, Rassaf T, Totzeck M (2019) Cardiotoxicity from immune checkpoint inhibitors. Int J Cardiol Heart Vasc 25:100420. https://doi.org/10.1016/j.ijcha.2019.100420

Miller KN, Dasgupta N, Liu T, Adams PD, Vizioli MG (2021) Cytoplasmic chromatin fragments-from mechanisms to therapeutic potential. Elife 10. https://doi.org/10.7554/eLife.63728

Mitry MA, Laurent D, Keith BL, Sira E, Eisenberg CA, Eisenberg LM, Joshi S, Gupte S, Edwards JG (2020) Accelerated cardiomyocyte senescence contributes to late-onset doxorubicin-induced cardiotoxicity. Am J Physiol Cell Physiol 318(2):C380–C391. https://doi.org/10.1152/ajpcell.00073.2019

Montecucco F, Carbone F, Schindler TH (2016) Pathophysiology of ST-segment elevation myocardial infarction: novel mechanisms and treatments. Eur Heart J 37(16):1268–1283. https://doi.org/10.1093/eurheartj/ehv592

Moriya J (2019) Critical roles of inflammation in atherosclerosis. J Cardiol 73(1):22–27. https://doi.org/10.1016/j.jjcc.2018.05.010

Müller L, Fülöp T, Pawelec G (2013) Immunosenescence in vertebrates and invertebrates. Immun Ageing 10(1):12. https://doi.org/10.1186/1742-4933-10-12

Murphy E, Steenbergen C (2007) Preconditioning: the mitochondrial connection. Annu Rev Physiol 69(1):51–67. https://doi.org/10.1146/annurev.physiol.69.031905.163645

Nakahira K, Haspel JA, Rathinam VA, Lee SJ, Dolinay T, Lam HC, Englert JA, Rabinovitch M, Cernadas M, Kim HP, Fitzgerald KA, Ryter SW, Choi AM (2011) Autophagy proteins regulate innate immune responses by inhibiting the release of mitochondrial DNA mediated by the NALP3 inflammasome. Nat Immunol 12(3):222–230. https://doi.org/10.1038/ni.1980

Nakajima T, Schulte S, Warrington KJ, Kopecky SL, Frye RL, Goronzy JJ, Weyand CM (2002) T-cell-mediated lysis of endothelial cells in acute coronary syndromes. Circulation 105(5): 570–575. https://doi.org/10.1161/hc0502.103348

Narendra D, Tanaka A, Suen DF, Youle RJ (2008) Parkin is recruited selectively to impaired mitochondria and promotes their autophagy. J Cell Biol 183(5):795–803. https://doi.org/10.1083/jcb.200809125

Nashef SA, Roques F, Sharples LD, Nilsson J, Smith C, Goldstone AR, Lockowandt U (2012) EuroSCORE II. Eur J Cardiothorac Surg 41(4):734–744; discussion 744-735. https://doi.org/10.1093/ejcts/ezs043

Navarro A, Boveris A (2007) The mitochondrial energy transduction system and the aging process. Am J Physiol-Cell Physiol 292(2):C670–C686. https://doi.org/10.1152/ajpcell.00213.2006

Nelson G, Kucheryavenko O, Wordsworth J, von Zglinicki T (2018) The senescent bystander effect is caused by ROS-activated NF-κB signalling. Mech Ageing Dev 170:30–36. https://doi.org/10.1016/j.mad.2017.08.005

Neri M, Riezzo I, Pascale N, Pomara C, Turillazzi E (2017) Ischemia/reperfusion injury following acute myocardial infarction: a critical issue for clinicians and forensic pathologists. Mediators Inflamm 2017:7018393. https://doi.org/10.1155/2017/7018393

Norman HS, Oujiri J, Larue SJ, Chapman CB, Margulies KB, Sweitzer NK (2011) Decreased cardiac functional reserve in heart failure with preserved systolic function. J Card Fail 17(4):301–308. https://doi.org/10.1016/j.cardfail.2010.11.004

North BJ, Sinclair DA (2012) The intersection between aging and cardiovascular disease. Circ Res 110(8):1097–1108. https://doi.org/10.1161/circresaha.111.246876

Nowak WN, Deng J, Ruan XZ, Xu Q (2017) Reactive oxygen species generation and atherosclerosis. Arterioscler Thromb Vasc Biol 37(5):e41–e52. https://doi.org/10.1161/ATVBAHA.117.309228

Oka SI, Sabry AD, Cawley KM, Warren JS (2020) Multiple levels of PGC-1α dysregulation in heart failure. Front Cardiovasc Med 7:2. https://doi.org/10.3389/fcvm.2020.00002

Olivieri F, Recchioni R, Marcheselli F, Abbatecola AM, Santini G, Borghetti G, Antonicelli R, Procopio AD (2013) Cellular senescence in cardiovascular diseases: potential age-related mechanisms and implications for treatment. Curr Pharm Des 19(9):1710–1719

Pankiv S, Clausen TH, Lamark T, Brech A, Bruun JA, Outzen H, Overvatn A, Bjorkoy G, Johansen T (2007) p62/SQSTM1 binds directly to Atg8/LC3 to facilitate degradation of ubiquitinated protein aggregates by autophagy. J Biol Chem 282(33):24131–24145. https://doi.org/10.1074/jbc.M702824200

Passos J, Saretzki G, Ahmed S, Nelson G, Richter T, Peters H, Wappler I, Birket M, Harold G, Schaeuble K, Birch-Machin M, Kirkwood T, von Zglinicki T (2007) Mitochondrial dysfunction accounts for the stochastic heterogeneity in telomere-dependent senescence. PLoS Biol 5(5). https://doi.org/10.1371/journal.pbio.0050110

Penna C, Mancardi D, Rastaldo R, Pagliaro P (2009) Cardioprotection: a radical view: free radicals in pre and postconditioning. Biochim Biophys Acta (BBA) - Bioenergetics 1787(7):781–793. https://doi.org/10.1016/j.bbabio.2009.02.008

Perkins DW, Haider S, Robertson D, Buus R, O'Leary L, Isacke CM (2020) Therapy-induced senescence in normal tissue promotes breast cancer metastasis. bioRxiv:2020.2010.2017.343590. https://doi.org/10.1101/2020.10.17.343590

Poznyak AV, Ivanova EA, Sobenin IA, Yet SF, Orekhov AN (2020) The role of mitochondria in cardiovascular diseases. Biology (Basel) 9(6). https://doi.org/10.3390/biology9060137

Prakash A, Gordon LB, Kleinman ME, Gurary EB, Massaro J, D'Agostino R Sr, Kieran MW, Gerhard-Herman M, Smoot L (2018) Cardiac abnormalities in patients with Hutchinson-Gilford Progeria Syndrome. JAMA Cardiol 3(4):326–334. https://doi.org/10.1001/jamacardio.2017.5235

Predmore JM, Wang P, Davis F, Bartolone S, Westfall MV, Dyke DB, Pagani F, Powell SR, Day SM (2010) Ubiquitin proteasome dysfunction in human hypertrophic and dilated cardiomyopathies. Circulation 121(8):997–1004. https://doi.org/10.1161/CIRCULATIONAHA.109.904557

Razani B, Feng C, Coleman T, Emanuel R, Wen H, Hwang S, Ting JP, Virgin HW, Kastan MB, Semenkovich CF (2012) Autophagy links inflammasomes to atherosclerotic progression. Cell Metab 15(4):534–544. https://doi.org/10.1016/j.cmet.2012.02.011

Rebbaa A, Zheng X, Chou PM, Mirkin BL (2003) Caspase inhibition switches doxorubicin-induced apoptosis to senescence. Oncogene 22(18):2805–2811. https://doi.org/10.1038/sj.onc.1206366

Richardson GD, Breault D, Horrocks G, Cormack S, Hole N, Owens WA (2012) Telomerase expression in the mammalian heart. FASEB J 26(12):4832–4840. https://doi.org/10.1096/fj.12-208843

Richardson GD, Sage A, Bennaceur K, Al Zhrany N, Coelho-Lima J, Dookun E, Draganova L, Saretzki G, Breault DT, Mallat Z, Spyridopoulos I (2018) Telomerase mediates lymphocyte proliferation but not the atherosclerosis-suppressive potential of regulatory T-cells. Arterioscler Thromb Vasc Biol 38(6):1283–1296. https://doi.org/10.1161/ATVBAHA.117.309940

Richter C, Park JW, Ames BN (1988) Normal oxidative damage to mitochondrial and nuclear DNA is extensive. Proc Natl Acad Sci 85(17):6465–6467. https://doi.org/10.1073/pnas.85.17.6465

Ridker PM, Everett BM, Thuren T, MacFadyen JG, Chang WH, Ballantyne C, Fonseca F, Nicolau J, Koenig W, Anker SD, Kastelein JJP, Cornel JH, Pais P, Pella D, Genest J, Cifkova R, Lorenzatti A, Forster T, Kobalava Z, Vida-Simiti L, Flather M, Shimokawa H, Ogawa H, Dellborg M, Rossi PRF, Troquay RPT, Libby P, Glynn RJ (2017) Antiinflammatory therapy with canakinumab for atherosclerotic disease. N Engl J Med 377(12):1119–1131. https://doi.org/10.1056/NEJMoa1707914

Ridker PM, MacFadyen JG, Everett BM, Libby P, Thuren T, Glynn RJ, Ridker PM, MacFadyen JG, Everett BM, Libby P et al (2018) Relationship of C-reactive protein reduction to cardiovascular event reduction following treatment with canakinumab: a secondary analysis from the CANTOS randomised controlled trial. Lancet 391(10118):319–328. https://doi.org/10.1016/S0140-6736(17)32814-3

Ron-Harel N, Notarangelo G, Ghergurovich JM, Paulo JA, Sage PT, Santos D, Satterstrom FK, Gygi SP, Rabinowitz JD, Sharpe AH, Haigis MC (2018) Defective respiration and one-carbon metabolism contribute to impaired naïve T cell activation in aged mice. Proc Natl Acad Sci U S A 115(52):13347–13352. https://doi.org/10.1073/pnas.1804149115

Roos CM, Zhang B, Palmer AK, Ogrodnik MB, Pirtskhalava T, Thalji NM, Hagler M, Jurk D, Smith LA, Casaclang-Verzosa G, Zhu Y, Schafer MJ, Tchkonia T, Kirkland JL, Miller JD (2016) Chronic senolytic treatment alleviates established vasomotor dysfunction in aged or atherosclerotic mice. Aging Cell 15(5):973–977. https://doi.org/10.1111/acel.12458

Saito T, Asai K, Sato S, Hayashi M, Adachi A, Sasaki Y, Takano H, Mizuno K, Shimizu W (2016) Autophagic vacuoles in cardiomyocytes of dilated cardiomyopathy with initially decompensated heart failure predict improved prognosis. Autophagy 12(3):579–587. https://doi.org/10.1080/15548627.2016.1145326

Saleh T, Bloukh S, Carpenter VJ, Alwohoush E, Bakeer J, Darwish S, Azab B, Gewirtz DA (2020) Therapy-induced senescence: an "old" friend becomes the enemy. Cancers (Basel) 12(4). https://doi.org/10.3390/cancers12040822

Samani NJ, Boultby R, Butler R, Thompson JR, Goodall AH (2001) Telomere shortening in atherosclerosis. Lancet 358(9280):472–473. https://doi.org/10.1016/s0140-6736(01)05633-1

Schank M, Zhao J, Wang L, Li Z, Cao D, Nguyen LN, Dang X, Khanal S, Nguyen LNT, Thakuri BKC, Ogbu SC, Lu Z, Zhang J, Wu XY, Morrison ZD, El Gazzar M, Ning S, Moorman JP, Yao ZQ (2020) Telomeric injury by KML001 in human T cells induces mitochondrial dysfunction through the p53-PGC-1α pathway. Cell Death Dis 11(12):1030. https://doi.org/10.1038/s41419-020-03238-7

Sergin I, Bhattacharya S, Emanuel R, Esen E, Stokes CJ, Evans TD, Arif B, Curci JA, Razani B (2016) Inclusion bodies enriched for p62 and polyubiquitinated proteins in macrophages protect against atherosclerosis. Sci Signal 9(409):ra2. https://doi.org/10.1126/scisignal.aad5614

Sharpless NE, Sherr CJ (2015) Forging a signature of in vivo senescence. Nat Rev Cancer 15(7):397–408. https://doi.org/10.1038/nrc3960

Shay JW, Wright WE (2004) Senescence and immortalization: role of telomeres and telomerase. Carcinogenesis 26(5):867–874. https://doi.org/10.1093/carcin/bgh296

Shimizu I, Minamino T (2019) Cellular senescence in cardiac diseases. J Cardiol 74(4):313–319. https://doi.org/10.1016/j.jjcc.2019.05.002

Siddall HK, Yellon DM, Ong SB, Mukherjee UA, Burke N, Hall AR, Angelova PR, Ludtmann MH, Deas E, Davidson SM, Mocanu MM, Hausenloy DJ (2013) Loss of PINK1 increases the heart's vulnerability to ischemia-reperfusion injury. PLoS One 8(4):e62400. https://doi.org/10.1371/journal.pone.0062400

Siddiqi N, Neil C, Bruce M, MacLennan G, Cotton S, Papadopoulou S, Feelisch M, Bunce N, Lim PO, Hildick-Smith D, Horowitz J, Madhani M, Boon N, Dawson D, Kaski JC, Frenneaux M, investigators N (2014) Intravenous sodium nitrite in acute ST-elevation myocardial infarction: a randomized controlled trial (NIAMI). Eur Heart J 35(19):1255–1262. https://doi.org/10.1093/eurheartj/ehu096

Song H, Conte JV, Foster AH, McLaughlin JS, Wei C (1999) Increased p53 protein expression in human failing myocardium. J Heart Lung Transplant 18(8):744–749. https://doi.org/10.1016/S1053-2498(98)00039-4

Spray L, Park C, Cormack S, Mohammed A, Panahi P, Boag S, Bennaceur K, Sopova K, Richardson G, Stangl VM, Rech L, Rainer PP, Ramos GC, Hofmann U, Stellos K, Spyridopoulos I (2021) The Fractalkine Receptor CX(3)CR1 links lymphocyte kinetics in CMV-seropositive patients and acute myocardial infarction with adverse left ventricular remodeling. Front Immunol 12:605857–605857. https://doi.org/10.3389/fimmu.2021.605857

Spyridopoulos I, Hoffmann J, Aicher A, Brümmendorf TH, Doerr HW, Zeiher AM, Dimmeler S (2009) Accelerated telomere shortening in leukocyte subpopulations of patients with coronary heart disease: role of cytomegalovirus seropositivity. Circulation 120(14):1364–1372. https://doi.org/10.1161/circulationaha.109.854299

Spyridopoulos I, Martin-Ruiz C, Hilkens C, Yadegarfar ME, Isaacs J, Jagger C, Kirkwood T, von Zglinicki T (2016) CMV seropositivity and T-cell senescence predict increased cardiovascular mortality in octogenarians: results from the Newcastle 85+ study. Aging Cell 15(2):389–392. https://doi.org/10.1111/acel.12430

Stojanović SD, Fiedler J, Bauersachs J, Thum T, Sedding DG (2020) Senescence-induced inflammation: an important player and key therapeutic target in atherosclerosis. Eur Heart J 41(31):2983–2996. https://doi.org/10.1093/eurheartj/ehz919

Strindhall J, Nilsson BO, Löfgren S, Ernerudh J, Pawelec G, Johansson B, Wikby A (2007) No Immune Risk Profile among individuals who reach 100 years of age: findings from the Swedish NONA immune longitudinal study. Exp Gerontol 42(8):753–761. https://doi.org/10.1016/j.exger.2007.05.001

Sun Y, Yao X, Zhang QJ, Zhu M, Liu ZP, Ci B, Xie Y, Carlson D, Rothermel BA, Sun Y, Levine B, Hill JA, Wolf SE, Minei JP, Zang QS (2018) Beclin-1-dependent autophagy protects the heart during sepsis. Circulation 138(20):2247–2262. https://doi.org/10.1161/CIRCULATIONAHA.117.032821

Sun LY, Eddeen AB, Mesana TG (2021) Disability-free survival after major cardiac surgery: a population-based retrospective cohort study. CMAJ Open 9(2):E384–E393. https://doi.org/10.9778/cmajo.20200096

Sutton MGSJ, Sharpe N (2000) Left ventricular remodeling after myocardial infarction. Circulation 101(25):2981–2988. https://doi.org/10.1161/01.CIR.101.25.2981

Swaminathan B, Goikuria H, Vega R, Rodriguez-Antiguedad A, Lopez Medina A, Freijo Mdel M, Vandenbroeck K, Alloza I (2014) Autophagic marker MAP1LC3B expression levels are associated with carotid atherosclerosis symptomatology. PLoS One 9(12):e115176. https://doi.org/10.1371/journal.pone.0115176

Taneike M, Yamaguchi O, Nakai A, Hikoso S, Takeda T, Mizote I, Oka T, Tamai T, Oyabu J, Murakawa T, Nishida K, Shimizu T, Hori M, Komuro I, Takuji Shirasawa TS, Mizushima N, Otsu K (2010) Inhibition of autophagy in the heart induces age-related cardiomyopathy. Autophagy 6(5):600–606. https://doi.org/10.4161/auto.6.5.11947

Thygesen K, Alpert JS, Jaffe AS, Simoons ML, Chaitman BR, White HD (2012) Third universal definition of myocardial infarction. J Am Coll Cardiol 60(16):1581–1598. https://doi.org/10.1016/j.jacc.2012.08.001

Tomás-Loba A, Flores I, Fernández-Marcos PJ, Cayuela ML, Maraver A, Tejera A, Borrás C, Matheu A, Klatt P, Flores JM, Viña J, Serrano M, Blasco MA (2008) Telomerase reverse transcriptase delays aging in cancer-resistant mice. Cell 135(4):609–622. https://doi.org/10.1016/j.cell.2008.09.034

Torabi A, Cleland JG, Rigby AS, Sherwi N (2014) Development and course of heart failure after a myocardial infarction in younger and older people. J Geriatr Cardiol 11(1):1–12. https://doi.org/10.3969/j.issn.1671-5411.2014.01.002

Triposkiadis F, Xanthopoulos A, Butler J (2019) Cardiovascular aging and heart failure: JACC review topic of the week. J Am Coll Cardiol 74(6):804–813. https://doi.org/10.1016/j.jacc.2019.06.053

Ungvari Z, Orosz Z, Labinskyy N, Rivera A, Xiangmin Z, Smith K, Csiszar A (2007) Increased mitochondrial H2O2 production promotes endothelial NF-κB activation in aged rat arteries. Am J Physiol-Heart C 293(1):H37–H47. https://doi.org/10.1152/ajpheart.01346.2006

Villeneuve C, Guilbeau-Frugier C, Sicard P, Lairez O, Ordener C, Duparc T, De Paulis D, Couderc B, Spreux-Varoquaux O, Tortosa F, Garnier A, Knauf C, Valet P, Borchi E, Nediani C, Gharib A, Ovize M, Delisle MB, Parini A, Mialet-Perez J (2013) p53-PGC-1α pathway mediates oxidative mitochondrial damage and cardiomyocyte necrosis induced by monoamine oxidase-A upregulation: role in chronic left ventricular dysfunction in mice. Antioxid Redox Signal 18(1):5–18. https://doi.org/10.1089/ars.2011.4373

Virani SS, Alonso A, Aparicio HJ, Benjamin EJ, Bittencourt MS, Callaway CW, Carson AP, Chamberlain AM, Cheng S, Delling FN et al (2021) Heart Disease and Stroke Statistics-2021 Update. Circulation 143(8):e254–e743. https://doi.org/10.1161/CIR.0000000000000950

Vizioli MG, Liu T, Miller KN, Robertson NA, Gilroy K, Lagnado AB, Perez-Garcia A, Kiourtis C, Dasgupta N, Lei X, Kruger PJ, Nixon C, Clark W, Jurk D, Bird TG, Passos JF, Berger SL, Dou Z, Adams PD (2020) Mitochondria-to-nucleus retrograde signaling drives formation of cytoplasmic chromatin and inflammation in senescence. Genes Dev 34(5-6):428–445. https://doi.org/10.1101/gad.331272.119

von Zglinicki T, Pilger R, Sitte N (2000) Accumulation of single-strand breaks is the major cause of telomere shortening in human fibroblasts. Free Radic Biol Med 28(1):64–74. https://doi.org/10.1016/s0891-5849(99)00207-5

Vulliamy T, Marrone A, Goldman F, Dearlove A, Bessler M, Mason PJ, Dokal I (2001) The RNA component of telomerase is mutated in autosomal dominant dyskeratosis congenita. Nature 413(6854):432–435. https://doi.org/10.1038/35096585

Walaszczyk A, Dookun E, Redgrave R, Tual-Chalot S, Victorelli S, Spyridopoulos I, Owens A, Arthur HM, Passos JF, Richardson GD (2019) Pharmacological clearance of senescent cells improves survival and recovery in aged mice following acute myocardial infarction. Aging Cell: e12945. https://doi.org/10.1111/acel.12945

Wang E (1995) Senescent human fibroblasts resist programmed cell death, and failure to suppress bcl2 is involved. Cancer Res 55(11):2284–2292

Wang JC, Bennett M (2012) Aging and atherosclerosis. Circ Res 111(2):245–259. https://doi.org/10.1161/CIRCRESAHA.111.261388

Wang K, Zhou LY, Wang JX, Wang Y, Sun T, Zhao B, Yang YJ, An T, Long B, Li N, Liu CY, Gong Y, Gao JN, Dong YH, Zhang J, Li PF (2015) E2F1-dependent miR-421 regulates mitochondrial fragmentation and myocardial infarction by targeting Pink1. Nat Commun 6:7619. https://doi.org/10.1038/ncomms8619

Wang B, Kohli J, Demaria M (2020) Senescent cells in cancer therapy: friends or foes? Trends Cancer 6(10):838–857. https://doi.org/10.1016/j.trecan.2020.05.004

Weng NP, Levine BL, June CH, Hodes RJ (1996) Regulated expression of telomerase activity in human T lymphocyte development and activation. J Exp Med 183(6):2471–2479. https://doi.org/10.1084/jem.183.6.2471

Werner C, Hanhoun M, Widmann T, Kazakov A, Semenov A, Pöss J, Bauersachs J, Thum T, Pfreundschuh M, Müller P, Haendeler J, Böhm M, Laufs U (2008) Effects of physical exercise on myocardial telomere-regulating proteins, survival pathways, and apoptosis. J Am Coll Cardiol 52(6):470–482. https://doi.org/10.1016/j.jacc.2008.04.034

Widgerow AD (2014) Ischemia-reperfusion injury: influencing the microcirculatory and cellular environment. Ann Plast Surg 72(2):253–260. https://doi.org/10.1097/SAP.0b013e31825c089c

Wiley CD, Campisi J (2021) The metabolic roots of senescence: mechanisms and opportunities for intervention. Nat Metab 3(10):1290–1301. https://doi.org/10.1038/s42255-021-00483-8

Wyld L, Bellantuono I, Tchkonia T, Morgan J, Turner O, Foss F, George J, Danson S, Kirkland JL (2020) Senescence and cancer: a review of clinical implications of senescence and senotherapies. Cancers (Basel) 12(8). https://doi.org/10.3390/cancers12082134

Xie Y, Xu E, Bowe B, Al-Aly Z (2022) Long-term cardiovascular outcomes of COVID-19. Nat Med 28(3):583–590. https://doi.org/10.1038/s41591-022-01689-3

Xiong S, Patrushev N, Forouzandeh F, Hilenski L, Alexander RW (2015) PGC-1α modulates telomere function and DNA damage in protecting against aging-related chronic diseases. Cell Rep 12(9):1391–1399. https://doi.org/10.1016/j.celrep.2015.07.047

Xu C, Cao Y, Liu R, Liu L, Zhang W, Fang X, Jia S, Ye J, Liu Y, Weng L, Qiao X, Li B, Zheng M (2022) Mitophagy-regulated mitochondrial health strongly protects the heart against cardiac dysfunction after acute myocardial infarction. J Cell Mol Med 26(4):1315–1326. https://doi.org/10.1111/jcmm.17190

Zhang Y-W, Shi J, Li Y-J, Wei L (2009) Cardiomyocyte death in doxorubicin-induced cardiotoxicity. Archivum Immunologiae et Therapiae Experimentalis 57(6):435–445. https://doi.org/10.1007/s00005-009-0051-8

Zhang W, Siraj S, Zhang R, Chen Q (2017) Mitophagy receptor FUNDC1 regulates mitochondrial homeostasis and protects the heart from I/R injury. Autophagy 13(6):1080–1081. https://doi.org/10.1080/15548627.2017.1300224

Zhang W, Chen C, Wang J, Liu L, He Y, Chen Q (2018) Mitophagy in cardiomyocytes and in platelets: a major mechanism of cardioprotection against ischemia/reperfusion injury. Physiology (Bethesda) 33(2):86–98. https://doi.org/10.1152/physiol.00030.2017

Zhou J, Chong SY, Lim A, Singh BK, Sinha RA, Salmon AB, Yen PM (2017) Changes in macroautophagy, chaperone-mediated autophagy, and mitochondrial metabolism in murine skeletal and cardiac muscle during aging. Aging (Albany NY) 9(2):583–599. https://doi.org/10.18632/aging.101181

Zhu F, Li Y, Zhang J, Piao C, Liu T, Li HH, Du J (2013) Senescent cardiac fibroblast is critical for cardiac fibrosis after myocardial infarction. PLoS One 8(9):e74535. https://doi.org/10.1371/journal.pone.0074535

Zhu Y, Tchkonia T, Pirtskhalava T, Gower AC, Ding H, Giorgadze N, Palmer AK, Ikeno Y, Hubbard GB, Lenburg M, O'Hara SP, LaRusso NF, Miller JD, Roos CM, Verzosa GC, LeBrasseur NK, Wren JD, Farr JN, Khosla S, Stout MB, McGowan SJ, Fuhrmann-Stroissnigg-H, Gurkar AU, Zhao J, Colangelo D, Dorronsoro A, Ling YY, Barghouthy AS, Navarro DC, Sano T, Robbins PD, Niedernhofer LJ, Kirkland JL (2015) The Achilles' heel of senescent cells: from transcriptome to senolytic drugs. Aging Cell 14(4):644–658. https://doi.org/10.1111/acel.12344

Zidar DA, Al-Kindi SG, Liu Y, Krieger NI, Perzynski AT, Osnard M, Nmai C, Anthony DD, Lederman MM, Freeman ML, Bonomo RA, Simon DI, Dalton JE (2019) Association of lymphopenia with risk of mortality among adults in the US general population. JAMA Network Open 2(12):e1916526–e1916526. https://doi.org/10.1001/jamanetworkopen.2019.16526

Chapter 5
Chronic Kidney Disease and the Exposome of Ageing

Paul Shiels, Ngoc Tran, Jen McCavitt, Ognian Neytchev, and Peter Stenvinkel

Abstract The gap between improvements in lifespan and age-related health is widening. Globally, the demographic of ageing is increasing and there has emerged a '*diseasome of ageing*', typified by a range of non-communicable diseases which share a common underlying component of a dysregulated ageing process. Within this, chronic kidney disease is an emerging global epidemic.

The extensive inter-individual variation displayed in how people age and how their diseasome manifests and progresses, has required a renewed focus on their life course exposures and the interplay between the environment and the (epi)genome. Termed the exposome, life course abiotic and biotic factors have a significant impact on renal health.

We explore how the exposome of renal ageing can predispose and affect CKD progression. We discuss how the kidney can be used as a model to understand the impact of the exposome in health and chronic kidney disease and how this might be manipulated to improve health span.

Notably, we discuss the manipulation of the foodome to mitigate acceleration of ageing processes by phosphate and to explore use of emerging senotherapies. A range of senotherapies, for removing senescent cells, diminishing inflammatory burden and either directly targeting Nrf2, or manipulating it indirectly via modification of the microbiome are discussed.

Keywords Kidney · Phosphate · Ageing · Exposome · Senotherapy

P. Shiels (✉) · N. Tran · J. McCavitt · O. Neytchev
SoMBS, Davidson Building, University of Glasgow, Glasgow, UK
e-mail: paul.shiels@glasgow.ac.uk; Ngoc.Tran@glasgow.ac.uk; 2394527M@student.gla.ac.uk; o.neytchev.1@research.gla.ac.uk

P. Stenvinkel
Division of Renal Medicine, Department of Clinical Science, Intervention and Technology, Karolinska Institutet, Stockholm, Sweden
e-mail: peter.stenvinkel@ki.sejen

J. R. Harris, V. I. Korolchuk (eds.), *Biochemistry and Cell Biology of Ageing: Part IV, Clinical Science*, Subcellular Biochemistry 103,
https://doi.org/10.1007/978-3-031-26576-1_5

Introduction

Improvement in human life expectancy over the preceding 150 years has not been matched by a similar improvement in health span (i.e., years of healthy living). Consequently, there has been a significant shift in the global demographic of ageing, with the >60s predicted to outnumber the <15 year olds by 2050 (United Nations 2018).

This situation is compounded by the sequiturs of (i) multi-morbidity among the aged as they develop a '*diseasome of ageing*' (ii) the staggering cost of addressing this, imposed on global social and health care systems (estimated pre-Covid pandemic at ~$47 trillion between 2010–2030) (Chen et al. 2018), (iii) low quality of life and (iv) the amplification of its effects by social deprivation (Shiels et al. 2021). Understanding the extrinsic factors underlying these health disparities is critical if we are to develop and apply suitable mitigation strategies to improve health and wellbeing. It is thus crucial that we have a better understanding of how we age at both a mechanistic biological level and an environmental/planetary level.

Ageing Is a Process

Ageing is a process starting pre-conception and ending in death. It is segmental, occurring at different rates in different organ systems and exhibiting significant inter-individual variation (Shiels et al. 2017; Stenvinkel and Shiels 2019). Its phenotype can be hallmarked with a series of features that are common across taxa (Stenvinkel and Shiels 2019). These comprise genomic instability, epigenetic dysregulation, telomere attrition, increasing cellular senescence, stem cell exhaustion, loss of proteostasis, dysregulated nutrient-sensing, mitochondrial dysfunction, and altered intercellular communication (López-Otín et al. 2013). In mammals, these are interfaced with diminished expression of the master cytoprotective regulator nuclear factor erythroid 2-related factor 2 (Nrf2), the chronic sterile inflammatory burden of 'inflammageing', alongside derepression of genomic retrotransposons and microbial dysbiosis (De Cecco et al. 2019; Simon et al. 2019; Mafra et al. 2022c, d). Whether these hallmarks act independently, cumulatively, or synergistically, remains to be determined (Shiels et al. 2019).

A Geroscience Approach to Age-Related Disease

Ageing is malleable and ill health in late life is not inevitable. Unequivocal evidence has shown that ageing can be forestalled by interventions that mitigate age-related deterioration of physiological function. The '*diseasome of ageing*' comprises age-related non-communicable diseases where chronological age is a major risk

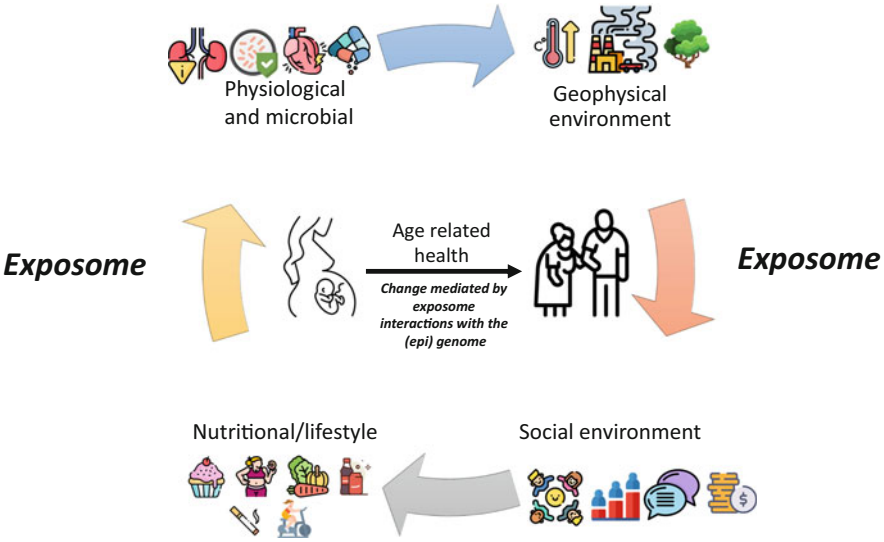


Fig. 5.1 The exposome of ageing. The totality of biotic and abiotic environmental exposures over the life course has a dramatic impact on health and is reflected in substantial inter-individual variation in health and both physical and physiological capability. Exposome effects are mediated by interaction with the (epi)genome

factor. These include cancer, neurodegenerative diseases, osteoporosis, arthritis, non-alcoholic steatohepatitis, type 2 diabetes, and chronic kidney disease (CKD) (Shiels et al. 2021). They all, however, share a common underpinning feature of a dysregulated ageing process, in concert with inflammageing (chronic sterile inflammatory burden), phosphate toxicity, diminished systemic Nrf2 expression, and microbial dysbiosis (Ebert et al. 2020). The emergent phenotype of ageing reflects an interplay between the (epi)genome and the exposome (Fig. 5.1). The exposome constitutes the sum of all environmental exposures across the life course (Wild 2005). This entails interplay between biotic and abiotic environmental factors and the (epi)genome (Horton et al. 2014). Notably, only three basic exposome factors, comprising air pollution, tobacco smoke, and diet, are thought to be responsible for ~50% of annual global deaths (Lim et al. 2012).

Conventionally, the individual disease modalities within the '*diseasome of ageing*' are treated separately, typically resulting in a short improvement in health span. This is pertinent to the prediction that curing cancer or heart disease in a typical 50-year-old woman would only add 2–3 years of extra health span, while treating the underpinning component of ageing may add up to 25 years (Burch et al. 2014). Geroscientists have thus been characterizing basic mechanisms of ageing across a range of species to develop candidate anti-ageing therapies (e.g., nutritional, lifestyle, and pharmacological interventional strategies) that are now tested in human trials. This approach is not always as straightforward, or intuitive, as it first appears. Extrapolation of data from standard laboratory pre-clinical models to human

interventions is often fraught. A consistent feature of this is the failure of drug therapies in late-stage clinical trials after successful pre-clinical testing. Additionally, many standard laboratory models are metabolically morbid (Stenvinkel et al. 2021a) and neglect to test for the effects of antagonistic pleiotropy (i.e., what is good for you when young, is not necessarily good for you when you are old and vice versa) (Williams 1957). In this instance, cellular senescence is a case in point, being onco-protective at older age, but diminishing physiological capability and increasing inflammatory burden at younger age. This is important when determining when and where in the life course any interventions need to be instigated, as these may engender substantially different effects. As such, age-related physical and physiological decline can be ameliorated differentially. To compensate for this, it may be better to consider ageing as a systemic burden of 'wear and tear'. In this respect, we have modelled ageing in the kidney to explore how this might be used to mitigate decline in age-related health span (Kooman et al. 2014; Mafra et al. 2021).

Modelling Ageing Using the Kidney

Studies of patients with kidney failure have presented a unique opportunity to compare the ageing process in terms of both normative and dysregulated ageing. While analysis of renal transplants as a source of healthy tissue has been used to track normative age-related renal function (Shiels et al. 2017), CKD represents the flip side of the coin. CKD is a progressive and deteriorating health condition that poses a major social and economic challenge to the global population (Carney 2020). As the ageing population continues to grow around the globe, more people are presenting with CKD, either independently or together with other co-morbidities within the '*diseasome of ageing*'. Emerging data show that changes in the environment, such as air pollution and global warming, increase the risk of kidney disease (Stenvinkel et al. 2020). CKD is characterized by a gradual decline in the physiological functions of the kidney over time. As the disease progresses, the kidneys are unable to remove harmful substances and excess fluids from the bloodstream, leading to the build-up of toxins, water, and electrolytes in the body. This eventually causes kidney failure in patients and earlier age-related mortality. Compared with the general population, CKD patients have an approximately 20–70% reduction in overall life expectancy depending on their levels of kidney function and the age of onset (Bikbov et al. 2020).

The estimated global prevalence of CKD is around 10–12% with the all-cause mortality rate increasing by >40% within 27 years (1990–2017). The growing burden of CKD is directly proportional to the dominant demographic of ageing, regional socioeconomic disparities, as well as associated risk factors, including hypertension and diabetes. These contributing factors were estimated to be responsible for more than half of the CKD-associated mortality rate in 2017 (Bikbov et al. 2020). CKD-associated premature ageing has been linked with a range of biochemical features, including the excessive accumulation of uremic toxins,

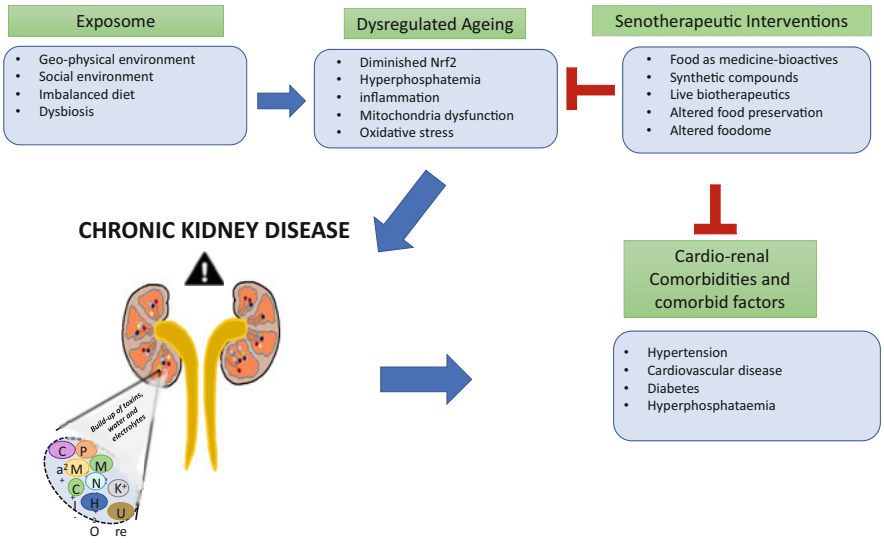


Fig. 5.2 Causes and interventions in CKD. Exposome factors contribute to dysregulation of ageing and the onset and progression of CKD and associated co-morbidities. Senotherapies and modulation of the exposome offer routes to mitigate these effects and ensure better renal function and associated health span

hyperphosphatemia, and mitochondrial dysfunction/oxidative stress (Kooman et al. 2014). The impact of accumulated levels of uremic toxins on the phenotype has been described in depth elsewhere (Ebert et al. 2020). Presently, >150 uremic solutes have been reported (Falconi et al. 2021). Many of these uremic toxins including indoxyl sulfate and p-cresyl sulfate cause extreme and irreversible damage to the kidney and surrounding organs (e.g., heart, liver, brain, or lung) (Vanholder et al. 2014).

Potential underlying mechanisms (Fig. 5.2) include accelerating cellular senescence, impairing Nrf2-mediated stress responses, increasing oxidative stress, and increasing inflammatory burden (Lisowska-Myjak 2014). A clear evidence base indicates that uremia shares underpinning molecular processes with the ageing process, including the hallmarks of cumulative cellular senescence, telomere attrition, post-translational protein modification, stem cell exhaustion, epigenetic dysregulation, and mitochondrial dysfunction (Kooman et al. 2014; Ebert et al. 2022).

Akin to chronic exposure to uremic toxins, CKD patients also develop immunosenescence and vascular senescence, due to dialysis bio-incompatibility with complement activation (Losappio et al. 2020). Overactivation of both immune cells (particularly macrophages and neutrophils) and the renin-angiotensin system result from this (Losappio et al. 2020). As Nrf2 expression diminishes in the course of CKD, cytoprotection via antioxidant factors also systemically diminishes (Stenvinkel and Shiels 2021). Even early-stage CKD patients experience this

systemic loss of redox homeostasis and display increased catabolic metabolism. These events trigger an increase in ATP consumption, thus forming a vicious cycle of mitochondrial dysfunction-oxidative stress-inflammation as CKD progresses (Peter 2021).

CKD also reflects a substantial relationship between the exposome and age-related renal health. Patients with CKD are predominantly reported in low- and middle-income nations (78% of the total patients), as well as less-developed regions in richer countries, where a significant health disparity exists (Mills et al. 2015). Exposome factors, such as socioeconomic position (SEP) and imbalanced diet are significant drivers of this health disparity (Craven et al. 2021). The mechanistic basis of this is not fully determined, but recent research from the National Longitudinal Study of Adolescent to Adult Health has indicated that the influence of SEP can be tracked from the late third decade of life using transcriptional signatures that can predict later life disease risk (Shanahan et al. 2022). These data have highlighted SEP-based inequalities in immune, inflammatory, and metabolic pathways which play key roles in the ageing process. Other exposome factors, such as microbial dysbiosis and hyperphosphataemia have already been linked directly to poorer renal function and increased disease risk among those at lower SEP, correlated with an imbalanced diet (McClelland et al. 2016; Craven et al. 2021). This pattern has also been confirmed by the mechanistic correlation between global DNA hypermethylation and renal dysfunction (Shiels et al. 2017). Further exposome effects on renal dysfunction can be gauged by the impact of the Covid pandemic (Stenvinkel et al. 2021b), global warming, and air pollution which have had a disproportionate effect on the renal health of the elderly (Stenvinkel et al. 2020; Avesani et al. 2022).

These environmental risk factors trigger an inflammatory response that accelerates the ageing process in CKD patients (Kooman et al. 2017). Consistent with this, global warming negatively affects the diversity of microbiota composition (Bestion et al. 2017). This condition might be worsened by long-term exposure to air pollution, which increases the risk of CKD and CVD. It has been shown that exposure to traffic-related air pollution for one year increases CKD incidence and risk of CKD development in older individuals (Kuźma et al. 2021). A more tractable exposome factor affecting renal health is the diet. In this respect, phosphate biology is highly pertinent.

Phosphate Biology

Serum phosphate (PO_4, often denoted as P_i for 'inorganic phosphate') levels correlate strongly with lifespan in mammals (Kuro-o 2010a; Stenvinkel et al. 2018). In man, high serum P_i levels correlate with accelerated biological ageing and poorer renal function (McClelland et al. 2016), as well as all-cause and cardiovascular mortality risk (Chang et al. 2014). Additionally, in the general population higher P_i levels are associated with accelerated vascular ageing (Yoo et al. 2016). In CKD,

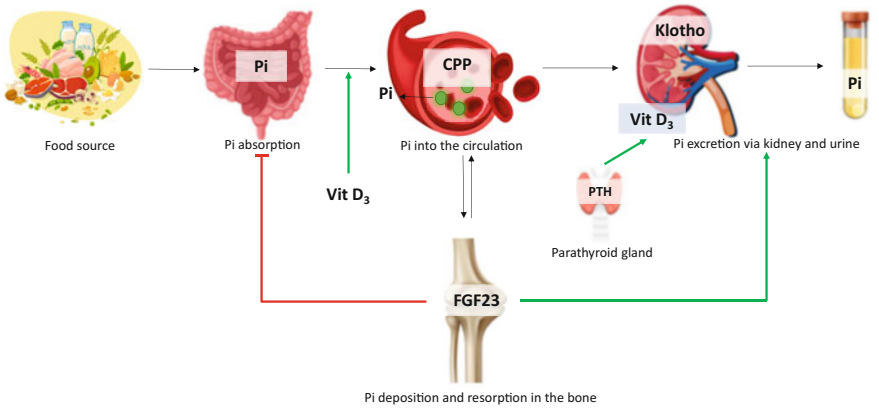


Fig. 5.3 Regulation of phosphate. Schematic representation of the regulation of nutritionally acquired P_i via Vit D, PTH and FGF23

phosphate is also considered a uremic toxin which provides a direct mechanistic link with the exposome of ageing. The source of phosphate, which mostly comes from protein-rich food consumption such as meat, fish, and eggs, is naturally regulated by vitamin D, parathyroid hormone and FGF-23/Klotho (Buchanan et al. 2020). Food additives contain phosphate (Ritz et al. 2012). Dietary-induced hyperphosphatemia contributes to poorer health in the general population while acting as a key driver of premature ageing in CKD patients (McClelland et al. 2016).

Phosphorus is one of the most abundant atoms in our bodies and an essential element for all known forms of life (Penido and Alon 2012). Phosphate has numerous vital biological functions: it is present in nucleic acids (DNA and RNA), energy-storing molecules such as ATP and GTP, the phospholipids that make up cellular and organellar bio-membranes, and mineralised tissues such as bone and teeth (Penido and Alon 2012). The addition or removal of P_i groups from proteins (phosphorylation and dephosphorylation), carried out by kinases, phosphatases, and phosphorylases, is a key mechanism of post-translational regulation of protein activity (Johnson 1997). Thus, it is of paramount importance for organisms to ensure adequate availability of this molecule. Indeed, one of the functions of mineralised bone is to serve as a reservoir of calcium and P_i and to buffer the levels of these ions in the blood (Copp and Shim 1963).

Serum phosphate levels are regulated by a bone-kidney endocrine axis that includes fibroblast growth factor-23 (FGF-23), Klotho, vitamin D, and parathyroid hormone (PTH) (Fig. 5.3) (Ebert et al. 2020). When the P_i level is high, FGF-23 secreted from the bone stimulates P_i excretion in the kidneys by binding to the FGF-23 receptor (and the transmembrane form of Klotho as obligate co-receptor) and reduces absorption in the intestine by lowering serum vitamin D levels (Kuro-o 2010b). Vitamin D, on the other hand, is transformed into the active 1,25-dihydroxyvitamin D_3 in the kidneys and promotes P_i absorption in the intestine and release from the bone (Kuro-o 2010b). Parathyroid hormone stimulates vitamin

D synthesis, but also has a parallel effect that induces P_i excretion, thus leading to an increase in blood calcium concentration without a parallel increase in blood P_i concentration (Kuro-o 2010b).

Interestingly, Klotho also has a soluble form, whose role in P_i homeostasis is still poorly understood (Tan et al. 2017; Batlahally et al. 2020). Soluble Klotho concentration decreases with age and negatively correlates with morbidity and mortality risk (Tan et al. 2017; Batlahally et al. 2020). The FGF23-Klotho pathway is dysregulated in CKD, leading to hyperphosphatemia, upregulation of the pro-inflammatory NF-κB signalling, systemic inflammation, and premature ageing (Ebert et al. 2020). Mice with a defective Klotho gene display a progeroid phenotype and have a greatly reduced lifespan (2–3 months) compared to wild-type (wt) mice (2–3 years), as well as increased serum P_i concentration (~50% higher than wt mice) (Kuro-o 2010a).

The normal serum calcium and P_i concentration ranges in humans are 2.2–2.5 mM (8.8–10.2 mg/dL) and 0.8–1.5 mM (2.5–4.5 mg/dL), respectively (Kuro-o 2010a). These physiological concentrations are high enough for the spontaneous formation of calcium phosphate (CaP) crystals (Brylka and Jahnen-Dechent 2013). CaP crystals are also toxic to cells, especially in the vasculature, and cause cellular damage, oxidative stress, and ectopic calcification (Ewence et al. 2008; Montezano et al. 2010). One of the main mechanisms of toxicity is thought to be the endocytosis of CaP particles by vascular smooth muscle cells (VSMCs), the dissolution of the crystals, and the release of calcium ions, which thus disrupt cytoplasmic calcium signalling (Ewence et al. 2008). This in turn can trigger cell death through apoptosis, osteogenic differentiation, deposition of extracellular CaP crystals (and thus ectopic vascular calcification), and inflammation (Ewence et al. 2008; Montezano et al. 2010). Magnesium deficiency has been shown to increase calcification and osteogenic differentiation of VSMCs, while magnesium supplementation has a protective effect (Montezano et al. 2010).

Several defence mechanisms have evolved to prevent the growth of CaP crystals. The plasma protein fetuin-A has a key role in preventing calcification by binding to and sequestering circulating CaP crystals into calciprotein particles (CPP) and preventing their further growth (Brylka and Jahnen-Dechent 2013). Indeed, fetuin-A deficiency has been associated with ectopic calcification (Brylka and Jahnen-Dechent 2013). Similarly, an increase in calcium and P_i concentration in the blood, commonly observed in CKD patients due to the diminished clearance capacity of the kidneys, can overwhelm the body's protective capacity and lead to calcification (Kuro-o 2013; Ebert et al. 2020). For this reason, a diet low in P_i is recommended for CKD patients (Kuro-o 2013). Indeed, fetuin-A functions as a circulating inhibitor of vascular calcification and its levels have been associated with accelerated ageing in CKD (Carrero et al. 2008).

The fundamental importance of phosphate to the ageing process can also be gauged from the observation that its progeric phenotype in the Klotho mouse can be reversed by knock-out of the NaPi2a transporter (a P_i transporter) when the animal is fed a normal diet. However, when these animals are fed a high P_i diet, the phenotype reappears, indicating that elevated P_i is driving ageing in these

animals (Ohnishi et al. 2009; Ohnishi and Razzaque 2010). The mechanistic basis of this is well understood. High concentrations of extracellular phosphate are toxic to cells. Calciprotein particles have the potential to induce extra-osteogenic transformation of vascular smooth muscle cells and cause cell death when endocytosed as a consequence of intra-cellular calcium release resulting in elevated oxidative stress and mitochondrial dysfunction (Buchanan et al. 2020). Conversely, high intake of dietary P_i can shorten life span in Klotho-deficient mice via activation of the AKT/mammalian target of rapamycin complex 1 (mTORC1) (Kawai et al. 2016).

Modulating the Exposome to Treat CKD

Diet has been considered the single easiest lever with which to leverage improved renal health. The Global Burden of Disease Study 2017 has indicated that 22% of global deaths were attributable to poor diet in 2017 (Willett et al. 2019; Afshin et al. 2019). Modulation of the diet to reduce intake of inorganic phosphate, maintain a salutogenic microbiome, and promote Nrf2-mediated cytoprotective responses is readily achievable. This firmly falls within the concept of Food as Medicine (Mafra et al. 2021). In this respect, increased intake of more plant-based protein, while still maintaining an omnivorous diet, offers renal health benefits. This approach has already shown some success, as it has been demonstrated to decrease CKD risk and progression and to improve on comorbid burden (Carrero et al. 2020; Avesani et al. 2022). Importantly, it has been applied successfully to the treatment of patients undergoing haemodialysis, where use of resistant starch cookies to support growth of saccharolytic salutobionts resulted in reduction of inflammatory burden and enhanced Nrf2 expression (Esgalhado et al. 2020; Kemp et al. 2021; Mafra et al. 2022b).

A positive sequitur from this strategy of eating more plant protein and reducing red meat consumption, is that it offers the possibility to alter food production systems to reduce factory farming, industrial mono-culture farming, and the impact of beef production (Stenvinkel et al. 2020), and thus improve Planetary health (Avesani et al. 2022). Additionally, a shift from a Western diet containing high levels of Ultra Processed Foods (UPFs) would have profound benefits, again mediated through supporting a salutogenic microbiome and better maintenance of Nrf2 agonism. The Western diet supports an industrialized human microbiome, that has outpaced its natural symbiotic evolution with humans and thus presents as a potentially dangerous unknown for human health. Its link to the prevalence of mild to moderate CKD (Craven et al. 2021; Chen et al. 2019) is already indicative of this.

Nutritional modulation of P_i intake can also radically affect age-related renal health, including through modulation of vitamin D metabolism and renal Klotho expression. Low P_i diets can be challenging and difficult to adhere to, as P_i and protein content in food tend to be positively correlated. While low protein intake can help slow down progression of CKD, it can also lead to malnutrition and adverse health outcomes (Buchanan et al. 2020). A high-fat diet (HFD) has been shown to

alter renal Klotho expression in older mice and impair the balance between dietary P_i absorption and vitamin D (Yoshikawa et al. 2018).

Iron deficiency and anaemia upregulate FGF23 and are commonly observed in CKD patients. Thus, iron-based phosphate binders such as ferric citrate have been successfully used to decrease FGF23 expression, and ameliorate both hyperphosphatemia and iron deficiency, and slow CKD progression in mice (Courbon et al. 2020). At the same time, caution is warranted, as excess iron intake has been associated with increased oxidative stress and risk of accelerated ageing (Arruda et al. 2013; Tian et al. 2022). The role of iron in ageing is also supported by a recent multivariate genomic scan (Timmers et al. 2020). A diet high in magnesium can also reduce vascular calcification, but it can also lead to lower bone mineral density and carries a risk of osteomalacia (Buchanan et al. 2020).

Notably, poor quality diets, often observed in lower SEP strata, are typically characterized by high phosphate intake (including from additives), high fat, low vitamin D, and limited quantities of fruits and vegetables rich in polyphenolic and other bioactive compounds (Buchanan et al. 2020). This contributes to an increased risk of CKD and poor health outcomes in people of lower socioeconomic status or who experience food insecurity, at least in part mediated by overexpression of FGF23. Encouragingly, this demographic appears to be also more amenable to improvement upon dietary supplementation, e.g., with vitamin D (Buchanan et al. 2020).

Dietary fibre intake and supplementation with pre- and probiotics are also parameters of interest in the context of CKD, as they are powerful tools for promoting a healthy gut microbiome and avoiding CKD-associated gut dysbiosis (Buchanan et al. 2020; Kemp et al. 2021, p. 2021). Salutogenic intestinal microbes can produce Nrf2-activating compounds, modulate vitamin D/FGF23 homeostasis, produce short-chain fatty acids (SCFA) that promote the health of the intestinal epithelium, maintain integrity of the intestinal barrier, and reduce inflammation (Buchanan et al. 2020; Kemp et al. 2021, p. 2021).

Future Treatment Strategies

Changing medical interventions and tackling exposome factors concurrently may have lasting beneficial effects for health span. An emerging treatment category, termed senotherapy, tackles cumulative cellular senescence and its senescence-associated secretory phenotype (SASP); these often manifest in old age as inflammation and diminished cellular stress responses (Kooman et al. 2014). Senotherapeutics is a broad term which encompasses both pharmaceutical and bioactive agents which affect physiological senescence, often by directly targeting senescent cells (SCs) and their secretome. These agents can have specific effects. For example, senolytics induce SC apoptosis, geroprotectors inhibit or reverse senescence, whilst senomorphics (also known as senostatics) target products of the SASP and its by-products (Tchkonia et al. 2013; Mafra et al. 2021). These treatments can

be applied to improve the dysregulation of ageing in CKD, thus pre-empting the development of the disease or mitigating its effects.

Examples of senotherapeutic interventions include use of Rapamycin and metformin, two repurposed clinical agents targeting the mTOR pathway. These attempt to switch cellular metabolism from catabolic to anabolic, and thus reduce the SASP phenotype (Nayeri Rad et al. 2022). Senolytic drugs, including dasatinib, in combination with quercetin, fisetin, navitoclax, or piperlongumine can induce apoptosis pathways in SCs, which are naturally resistant to apoptosis by nature of their senescent state of growth arrest, thus driving removal of SCs from tissues and organs (Nayeri Rad et al. 2022). However, dual approaches are also beneficial; the first in-human CKD trial using senotherapy, produced encouraging results, demonstrating improvement in renal function with the combination of dasatinib (a repurposed clinical chemotherapeutic agent) and quercetin (a bioactive agent), which complement each other in creating a senolytic effect (Nayeri Rad et al. 2022).

A further strategic development has been the adoption of a biomimetic approach to understand and treat diseases of ageing (Stenvinkel et al. 2018). The application of biomimetics to human health takes advantage of evolution by natural selection to produce, from within the natural world, solutions to human health problems (Stenvinkel et al. 2021a). One example of the insight such an approach can provide has been the identification and subsequent therapeutic targeting of Nrf2. Nrf2 responds to cellular stressors by upregulating over 350 cytoprotective genes which act in concert to reduce oxidative stress and damage, inflammatory burden, as well as modulating energy metabolism (Shiels et al. 2017, 2021; Stenvinkel et al. 2018). Additionally, it has provided a nexus for the re-envisionment of the Hippocratic concept of 'Food as Medicine'. Foods, particularly fruits and vegetables, are rich in phenolic acids broken down by the gut microbiota to generate alkyl catechols, which are potent agonists for Nrf2, thus improving cytoprotection in response to stress (Shiels et al. 2021; Mafra et al. 2022a). A direct sequitur of this approach is a reduction in the number of SCs and thus the effects of the SASP. Notably, within the animal kingdom, Nrf2 forms the molecular basis of stress responses, particularly in long-lived animals, such as the naked mole rat and animals living under extremes of environmental stress, such as the ocean Quahog (Stenvinkel and Shiels 2019). A further benefit of this approach is the reduction in the use of inorganic phosphate preservatives within food stuffs and a return to fermentation of food stuffs to preserve them. This enhances the maintenance of a salutogenic microbiome and promotes renal health (Esgalhado et al. 2020; Kemp et al. 2021; Mafra et al. 2022b).

Similarly, this approach has also identified several bioactive molecules with promise as senotherapeutic agents. One of these, sulforaphane, found naturally in cruciferous vegetables, is a candidate senotherapeutic of promise, exhibiting both geroprotective and senolytic properties and already in a range of human trials (Cardozo et al. 2021). Another natural compound, fisetin, has also been shown to have a senolytic effect, improving both health and life span in a range of pre-clinical testing (Shiels et al. 2021). Whilst both bioactive and pharmaceutical agents have been identified as improving organ function, it is also important to consider that the promotion of cytoprotective effects and impairment of senescence may have adverse

consequences, possibly increasing the risk of diseases such as B cell lymphoma (Franzin et al. 2021; Chaib et al. 2022).

As senolytics remove senescent cells which must be replaced to maintain tissue and organ homeostasis, it may lead to replicative exhaustion and dysfunction. Clearance of senescent pancreatic β-cells using senolytics has already been shown to lead to diabetes in mice (Helman et al. 2016). Additionally, senomorphic or senostatic drugs cannot eliminate SASP sources permanently, requiring repeated administration to ensure efficacy. This may result in suppression of other essential pathways and disturbance in tissue homeostasis, due to blocking of the SASP. Another concern is the lack of information regarding the optimal time points of administration of senolytic or senomorphic agents within the life course, pertinent considering antagonistic pleiotropy. Therapeutics may exert either beneficial, neutral, or negative impacts on different organs at a specific point over the life course. It remains unclear whether they provide protection and improve future health span when administered early in life or should only be administered at middle age or later. Indeed, it remains to be seen if, used in combination, they work synergistically, independently, cumulatively, or competitively. Furthermore, cryptic side effects need to be considered. Already, such effects have been identified while using senotherapeutic drugs. For instance, sulforaphane significantly reduces the water intake in young mice (Bose et al. 2020), which resonates with fluid retention issues observed clinically with the original use of Bardoxolone (Bose et al. 2020). To minimize the risks, further research is needed to determine the safety of these approaches.

Despite these concerns, it would be churlish not to recognize the genuine benefits and promise that senotherapies offer to the treatment of renal disease and the '*diseasome of ageing*' in general. Their regular clinical use is now much anticipated.

Acknowledgements PSh is a Scientific reviewer for Mars UK Ltd and funded by an IPP award between Constant Pharma and UoG.

PSt is funded by Swedish Medical Research Council, CIMED, ALF and The Heart and Lung Foundation.

ON is funded by an IPP award to PS between Constant Pharma and UoG.

NT is supported by MINDSHIFT—H2020-MSCA-ITN-2020, Grant Agreement number: 954798.

References

Afshin A, Sur PJ, Fay KA et al (2019) Health effects of dietary risks in 195 countries, 1990–2017: a systematic analysis for the Global Burden of Disease Study 2017. Lancet 393:1958–1972. https://doi.org/10.1016/S0140-6736(19)30041-8

Arruda LF, Arruda SF, Campos NA et al (2013) Dietary iron concentration may influence aging process by altering oxidative stress in tissues of adult rats. PLoS ONE 8:e61058. https://doi.org/10.1371/journal.pone.0061058

Avesani CM, Cardozo LFMF, Yee-Moon Wang A et al (2022) Planetary health, nutrition and chronic kidney disease: connecting the dots for a sustainable future. J Ren Nutr. S1051227622001613. https://doi.org/10.1053/j.jrn.2022.09.003

Batlahally S, Franklin A, Damianos A et al (2020) Soluble Klotho, a biomarker and therapeutic strategy to reduce bronchopulmonary dysplasia and pulmonary hypertension in preterm infants. Sci Rep 10:12368. https://doi.org/10.1038/s41598-020-69296-1

Bestion E, Jacob S, Zinger L et al (2017) Climate warming reduces gut microbiota diversity in a vertebrate ectotherm. Nat Ecol Evol 1:0161. https://doi.org/10.1038/s41559-017-0161

Bikbov B, Purcell CA, Levey AS et al (2020) Global, regional, and national burden of chronic kidney disease, 1990–2017: a systematic analysis for the Global Burden of Disease Study 2017. Lancet 395:709–733. https://doi.org/10.1016/S0140-6736(20)30045-3

Bose C, Alves I, Singh P et al (2020) Sulforaphane prevents age-associated cardiac and muscular dysfunction through Nrf2 signaling. Aging Cell 19. https://doi.org/10.1111/acel.13261

Brylka L, Jahnen-Dechent W (2013) The role of Fetuin-A in physiological and pathological mineralization. Calcif Tissue Int 93:355–364. https://doi.org/10.1007/s00223-012-9690-6

Buchanan S, Combet E, Stenvinkel P, Shiels PG (2020) Klotho, aging, and the failing kidney. Front Endocrinol 11:560. https://doi.org/10.3389/fendo.2020.00560

Burch JB, Augustine AD, Frieden LA et al (2014) Advances in geroscience: impact on healthspan and chronic disease. J Gerontol A Biol Sci Med Sci 69:S1–S3. https://doi.org/10.1093/gerona/glu041

Cardozo LFMF, Alvarenga LA, Ribeiro M et al (2021) Cruciferous vegetables: rationale for exploring potential salutary effects of sulforaphane-rich foods in patients with chronic kidney disease. Nutr Rev 79:1204–1224. https://doi.org/10.1093/nutrit/nuaa129

Carney EF (2020) The impact of chronic kidney disease on global health. Nat Rev Nephrol 16:251–251. https://doi.org/10.1038/s41581-020-0268-7

Carrero JJ, Stenvinkel P, Fellström B et al (2008) Telomere attrition is associated with inflammation, low fetuin-A levels and high mortality in prevalent haemodialysis patients. J Intern Med 263:302–312. https://doi.org/10.1111/j.1365-2796.2007.01890.x

Carrero JJ, González-Ortiz A, Avesani CM et al (2020) Plant-based diets to manage the risks and complications of chronic kidney disease. Nat Rev Nephrol 16:525–542. https://doi.org/10.1038/s41581-020-0297-2

Chaib S, Tchkonia T, Kirkland JL (2022) Cellular senescence and senolytics: the path to the clinic. Nat Med 28:1556–1568. https://doi.org/10.1038/s41591-022-01923-y

Chang AR, Lazo M, Appel LJ et al (2014) High dietary phosphorus intake is associated with all-cause mortality: results from NHANES III. Am J Clin Nutr 99:320–327. https://doi.org/10.3945/ajcn.113.073148

Chen S, Kuhn M, Prettner K, Bloom DE (2018) The macroeconomic burden of noncommunicable diseases in the United States: estimates and projections. PLoS ONE 13:e0206702. https://doi.org/10.1371/journal.pone.0206702

Chen Y-Y, Chen D-Q, Chen L et al (2019) Microbiome–metabolome reveals the contribution of gut–kidney axis on kidney disease. J Transl Med 17:5. https://doi.org/10.1186/s12967-018-1756-4

Copp DH, Shim SS (1963) The homeostatic function of bone as a mineral reservoir. Oral Surg Oral Med Oral Pathol 16:738–744. https://doi.org/10.1016/0030-4220(63)90081-1

Courbon G, Martinez-Calle M, David V (2020) Simultaneous management of disordered phosphate and iron homeostasis to correct fibroblast growth factor 23 and associated outcomes in chronic kidney disease. Curr Opin Nephrol Hypertens 29:359–366. https://doi.org/10.1097/MNH.0000000000000614

Craven H, McGuinness D, Buchanan S et al (2021) Socioeconomic position links circulatory microbiota differences with biological age. Sci Rep 11:12629. https://doi.org/10.1038/s41598-021-92042-0

De Cecco M, Ito T, Petrashen AP et al (2019) L1 drives IFN in senescent cells and promotes age-associated inflammation. Nature 566:73–78. https://doi.org/10.1038/s41586-018-0784-9

Ebert T, Pawelzik S-C, Witasp A et al (2020) Inflammation and premature ageing in chronic kidney disease. Toxins 12:227. https://doi.org/10.3390/toxins12040227

Ebert T, Tran N, Schurgers L et al (2022) Ageing – oxidative stress, PTMs and disease. Mol Aspects Med 86:101099. https://doi.org/10.1016/j.mam.2022.101099

Esgalhado M, Kemp JA, de Paiva BR et al (2020) Resistant starch type-2 enriched cookies modulate uremic toxins and inflammation in hemodialysis patients: a randomized, double-blind, crossover and placebo-controlled trial. Food Funct 11:2617–2625. https://doi.org/10.1039/C9FO02939G

Ewence AE, Bootman M, Roderick HL et al (2008) Calcium phosphate crystals induce cell death in human vascular smooth muscle cells: a potential mechanism in atherosclerotic plaque destabilization. Circ Res 103. https://doi.org/10.1161/CIRCRESAHA.108.181305

Falconi CA, da Cruz Junho CV, Fogaça-Ruiz F et al (2021) Uremic toxins: an alarming danger concerning the cardiovascular system. Front Physiol 12:686249. https://doi.org/10.3389/fphys.2021.686249

Franzin R, Stasi A, Ranieri E et al (2021) Targeting premature renal aging: from molecular mechanisms of cellular senescence to senolytic trials. Front Pharmacol 12:630419. https://doi.org/10.3389/fphar.2021.630419

Helman A, Klochendler A, Azazmeh N et al (2016) p16Ink4a-induced senescence of pancreatic beta cells enhances insulin secretion. Nat Med 22:412–420. https://doi.org/10.1038/nm.4054

Horton R, Beaglehole R, Bonita R et al (2014) From public to planetary health: a manifesto. Lancet 383:847. https://doi.org/10.1016/S0140-6736(14)60409-8

Johnson LN (1997) From phosphorylase to phosphorylase kinase. In: Heilmeyer L (ed) Interacting protein domains. Springer, Berlin, Heidelberg, pp 9–14

Kawai M, Kinoshita S, Ozono K, Michigami T (2016) Inorganic phosphate activates the AKT/mTORC1 pathway and shortens the life span of an α -Klotho–deficient model. J Am Soc Nephrol 27:2810–2824. https://doi.org/10.1681/ASN.2015040446

Kemp JA, Regis B, Fragoso dos Santos H, Emiliano de Jesus H, Craven H, Ijaz UZ, Shiels PG, Mafra D (2021) The impact of enriched resistant starch type-2 cookies on the gut microbiome in hemodialysis patients. Mol Nutr Food Res 65:e2100374. https://doi.org/10.1002/mnfr.202100374

Kooman JP, Kotanko P, Schols AMWJ et al (2014) Chronic kidney disease and premature ageing. Nat Rev Nephrol 10:732–742. https://doi.org/10.1038/nrneph.2014.185

Kooman JP, Dekker MJ, Usvyat LA et al (2017) Inflammation and premature aging in advanced chronic kidney disease. Am J Physiol-Ren Physiol 313:F938–F950. https://doi.org/10.1152/ajprenal.00256.2017

Kuro-o M (2010a) A potential link between phosphate and aging—lessons from Klotho-deficient mice. Mech Ageing Dev 131:270–275. https://doi.org/10.1016/j.mad.2010.02.008

Kuro-o M (2010b) Overview of the FGF23-Klotho axis. Pediatr Nephrol 25:583–590. https://doi.org/10.1007/s00467-009-1260-4

Kuro-o M (2013) A phosphate-centric paradigm for pathophysiology and therapy of chronic kidney disease. Kidney Int Suppl 3:420–426. https://doi.org/10.1038/kisup.2013.88

Kuźma Ł, Małyszko J, Bachórzewska-Gajewska H et al (2021) Exposure to air pollution and renal function. Sci Rep 11:11419. https://doi.org/10.1038/s41598-021-91000-0

Lim SS, Vos T, Flaxman AD et al (2012) A comparative risk assessment of burden of disease and injury attributable to 67 risk factors and risk factor clusters in 21 regions, 1990–2010: a systematic analysis for the Global Burden of Disease Study 2010. Lancet 380:2224–2260. https://doi.org/10.1016/S0140-6736(12)61766-8

Lisowska-Myjak B (2014) Uremic toxins and their effects on multiple organ systems. Nephron Clin Pract 128:303–311. https://doi.org/10.1159/000369817

López-Otín C, Blasco MA, Partridge L et al (2013) The hallmarks of aging. Cell 153:1194–1217. https://doi.org/10.1016/j.cell.2013.05.039

Losappio V, Franzin R, Infante B et al (2020) Molecular mechanisms of premature aging in hemodialysis: the complex interplay between innate and adaptive immune dysfunction. Int J Mol Sci 21:3422. https://doi.org/10.3390/ijms21103422

Mafra D, Borges NA, Lindholm B et al (2021) Food as medicine: targeting the uraemic phenotype in chronic kidney disease. Nat Rev Nephrol 17:153–171. https://doi.org/10.1038/s41581-020-00345-8

Mafra D, Borges NA, Alvarenga L et al (2022a) Fermented food: should patients with cardiometabolic diseases go back to an early neolithic diet? Crit Rev Food Sci Nutr:1–24. https://doi.org/10.1080/10408398.2022.2077300

Mafra D, Cardozo L, Ribeiro-Alves M et al (2022b) Short report: choline plasma levels are related to Nrf2 transcriptional expression in chronic kidney disease? Clin Nutr ESPEN 50:318–321. https://doi.org/10.1016/j.clnesp.2022.06.008

Mafra D, Ribeiro M, Fonseca L et al (2022c) Archaea from the gut microbiota of humans: could be linked to chronic diseases? Anaerobe 77:102629. https://doi.org/10.1016/j.anaerobe.2022.102629

Mafra D, Ugochukwu SA, Borges NA et al (2022d) Food for healthier aging: power on your plate. Crit Rev Food Sci Nutr:1–14. https://doi.org/10.1080/10408398.2022.2107611

McClelland R, Christensen K, Mohammed S et al (2016) Accelerated ageing and renal dysfunction links lower socioeconomic status and dietary phosphate intake. Aging 8:1135–1149. https://doi.org/10.18632/aging.100948

Mills KT, Xu Y, Zhang W et al (2015) A systematic analysis of worldwide population-based data on the global burden of chronic kidney disease in 2010. Kidney Int 88:950–957. https://doi.org/10.1038/ki.2015.230

Montezano AC, Zimmerman D, Yusuf H et al (2010) Vascular smooth muscle cell differentiation to an osteogenic phenotype involves TRPM7 modulation by magnesium. Hypertension 56:453–462. https://doi.org/10.1161/HYPERTENSIONAHA.110.152058

Nayeri Rad A, Shams G, Avelar RA et al (2022) Potential senotherapeutic candidates and their combinations derived from transcriptional connectivity and network measures. Inform Med Unlocked 30:100920. https://doi.org/10.1016/j.imu.2022.100920

Ohnishi M, Razzaque MS (2010) Dietary and genetic evidence for phosphate toxicity accelerating mammalian aging. FASEB J 24:3562–3571. https://doi.org/10.1096/fj.09-152488

Ohnishi M, Nakatani T, Lanske B, Razzaque MS (2009) In vivo genetic evidence for suppressing vascular and soft-tissue calcification through the reduction of serum phosphate levels, even in the presence of high serum calcium and 1,25-dihydroxyvitamin D levels. Circ Cardiovasc Genet 2:583–590. https://doi.org/10.1161/CIRCGENETICS.108.847814

Penido MGMG, Alon US (2012) Phosphate homeostasis and its role in bone health. Pediatr Nephrol 27:2039–2048. https://doi.org/10.1007/s00467-012-2175-z

Peter S (2021) Mitochondrial dysfunction as part of an inflammatory intermediate phenotype that drives premature ageing. J Intern Med 290:231–234. https://doi.org/10.1111/joim.13243

Ritz E, Hahn K, Ketteler M et al (2012) Phosphate additives in food. Dtsch Ärztebl Int. https://doi.org/10.3238/arztebl.2012.0049

Shanahan MJ, Cole SW, Ravi S et al (2022) Socioeconomic inequalities in molecular risk for chronic diseases observed in young adulthood. Proc Natl Acad Sci 119:e2103088119. https://doi.org/10.1073/pnas.2103088119

Shiels PG, McGuinness D, Eriksson M et al (2017) The role of epigenetics in renal ageing. Nat Rev Nephrol 13:471–482. https://doi.org/10.1038/nrneph.2017.78

Shiels PG, Buchanan S, Selman C, Stenvinkel P (2019) Allostatic load and ageing: linking the microbiome and nutrition with age-related health. Biochem Soc Trans 47:1165–1172. https://doi.org/10.1042/BST20190110

Shiels PG, Painer J, Natterson-Horowitz B et al (2021) Manipulating the exposome to enable better ageing. Biochem J 478:2889–2898. https://doi.org/10.1042/BCJ20200958

Simon M, Van Meter M, Ablaeva J et al (2019) LINE1 derepression in aged wild-type and SIRT6-deficient mice drives inflammation. Cell Metab 29:871–885.e5. https://doi.org/10.1016/j.cmet.2019.02.014

Stenvinkel P, Shiels PG (2019) Long-lived animals with negligible senescence: clues for ageing research. Biochem Soc Trans 47:1157–1164. https://doi.org/10.1042/BST20190105

Stenvinkel P, Shiels PG (2021) Metabolic syndrome in combination with chronic kidney disease—it's a gut feeling. J Intern Med 290:1108–1111. https://doi.org/10.1111/joim.13363
Stenvinkel P, Painer J, Kuro-o M et al (2018) Novel treatment strategies for chronic kidney disease: insights from the animal kingdom. Nat Rev Nephrol 14:265–284. https://doi.org/10.1038/nrneph.2017.169
Stenvinkel P, Shiels PG, Painer J et al (2020) A planetary health perspective for kidney disease. Kidney Int 98:261–265. https://doi.org/10.1016/j.kint.2020.03.024
Stenvinkel P, Avesani CM, Gordon LJ et al (2021a) Biomimetics provides lessons from nature for contemporary ways to improve human health. J Clin Transl Sci 5:e128. https://doi.org/10.1017/cts.2021.790
Stenvinkel P, Painer J, Shiels PG et al (2021b) SARS-COV-2 and biomimetics: what saves the planet will save our health. J Intern Med 289:244–246. https://doi.org/10.1111/joim.13128
Tan S-J, Smith ER, Holt SG et al (2017) Soluble klotho may be a marker of phosphate reabsorption. Clin Kidney J 10:397–404. https://doi.org/10.1093/ckj/sfw146
Tchkonia T, Zhu Y, van Deursen J et al (2013) Cellular senescence and the senescent secretory phenotype: therapeutic opportunities. J Clin Invest 123:966–972. https://doi.org/10.1172/JCI64098
Tian Y, Tian Y, Yuan Z et al (2022) Iron metabolism in aging and age-related diseases. Int J Mol Sci 23:3612. https://doi.org/10.3390/ijms23073612
Timmers PRHJ, Wilson JF, Joshi PK, Deelen J (2020) Multivariate genomic scan implicates novel loci and haem metabolism in human ageing. Nat Commun 11:3570. https://doi.org/10.1038/s41467-020-17312-3
United Nations (2018) World Population Ageing 2017: Highlights. United Nations
Vanholder R, Schepers E, Pletinck A et al (2014) The uremic toxicity of indoxyl sulfate and p-cresyl sulfate: a systematic review. J Am Soc Nephrol 25:1897–1907. https://doi.org/10.1681/ASN.2013101062
Wild CP (2005) Complementing the genome with an "exposome": the outstanding challenge of environmental exposure measurement in molecular epidemiology. Cancer Epidemiol Biomarkers Prev 14:1847–1850. https://doi.org/10.1158/1055-9965.EPI-05-0456
Willett W, Rockström J, Loken B et al (2019) Food in the anthropocene: the EAT–Lancet Commission on healthy diets from sustainable food systems. Lancet 393:447–492. https://doi.org/10.1016/S0140-6736(18)31788-4
Williams GC (1957) Pleiotropy, natural selection, and the evolution of senescence. Evolution 11:398. https://doi.org/10.2307/2406060
Yoo KD, Kang S, Choi Y et al (2016) Sex, age, and the association of serum phosphorus with all-cause mortality in adults with normal kidney function. Am J Kidney Dis 67:79–88. https://doi.org/10.1053/j.ajkd.2015.06.027
Yoshikawa R, Yamamoto H, Nakahashi O et al (2018) The age-related changes of dietary phosphate responsiveness in plasma 1,25-dihydroxyvitamin D levels and renal Cyp27b1 and Cyp24a1 gene expression is associated with renal α-Klotho gene expression in mice. J Clin Biochem Nutr 62:68–74. https://doi.org/10.3164/jcbn.17-20

Chapter 6
Sarcopenia and Ageing

Keith Yu-Kin Cheng, Zhengyuan Bao, Yufeng Long, Chaoran Liu, Tao Huang, Can Cui, Simon Kwoon-Ho Chow, Ronald Man Yeung Wong, and Wing-Hoi Cheung

Abstract Musculoskeletal ageing is a major health challenge as muscles and bones constitute around 55–60% of body weight. Ageing muscles will result in sarcopenia that is characterized by progressive and generalized loss of skeletal muscle mass and strength with a risk of adverse outcomes. In recent years, a few consensus panels provide new definitions for sarcopenia. It was officially recognized as a disease in 2016 with an ICD-10-CM disease code, M62.84, in the International Classification of Diseases (ICD). With the new definitions, there are many studies emerging to investigate the pathogenesis of sarcopenia, exploring new interventions to treat sarcopenia and evaluating the efficacy of combination treatments for sarcopenia. The scope of this chapter is to summarize and appraise the evidence in terms of (1) clinical signs, symptoms, screening, and diagnosis, (2) pathogenesis of sarcopenia with emphasis on mitochondrial dysfunction, intramuscular fat

K. Y.-K. Cheng · Y. Long · C. Liu · T. Huang · C. Cui · R. M. Y. Wong · W.-H. Cheung (✉)
Department of Orthopaedics and Traumatology, The Chinese University of Hong Kong, Hong Kong, China
e-mail: keithykcheng@link.cuhk.edu.hk; longyufeng@link.cuhk.edu.hk; liuchaoran927@link.cuhk.edu.hk; huangtao@link.cuhk.edu.hk; cuican@link.cuhk.edu.hk; ronald.wong@cuhk.edu.hk; louischeung@cuhk.edu.hk

Z. Bao
Department of Orthopaedics and Traumatology, The Chinese University of Hong Kong, Hong Kong, China

Division of Sports Medicine and Adult Reconstructive Surgery, Department of Orthopedic Surgery, Nanjing Drum Tower Hospital, The Affiliated Hospital of Nanjing University Medical School, Nanjing, China
e-mail: brandonbao@link.cuhk.edu.hk

S. K.-H. Chow
Department of Orthopaedics and Traumatology, The Chinese University of Hong Kong, Hong Kong, China

Department of Orthopaedic Surgery, Stanford University, Stanford, CA, USA
e-mail: skhchow@cuhk.edu.hk

J. R. Harris, V. I. Korolchuk (eds.), *Biochemistry and Cell Biology of Ageing: Part IV, Clinical Science*, Subcellular Biochemistry 103,
https://doi.org/10.1007/978-3-031-26576-1_6

infiltration and neuromuscular junction deterioration, and (3) current treatments with regard to physical exercises and nutritional supplement.

Keywords Sarcopenia · Muscle mass · Muscle strength · Mitochondrial dysfunction · Intramuscular fat infiltration · Myosteatosis · Neuromuscular junction · Physical exercise · Nutrition

Ageing

The ageing population is a global phenomenon and countries are experiencing a growth in both size and proportion of older persons in their population. In 2019, there were over 700 million older people aged ≥65. This older population was projected to double to 1.5 billion in 2050, with one in six people in the world aged ≥65 (United Nations, Department of Economic and Social Affairs, Population Division (2019). World Population Ageing 2019: Highlights). Despite life expectancy increasing globally, a significant proportion of these additional years is lived with disability (Scuteri et al. 2016). An ageing population inevitably leads to an increase in the prevalence of age-related morbidities where the musculoskeletal system is heavily involved, such as sarcopenia, osteoporosis, or osteosarcopenia.

Musculoskeletal ageing is one of the most common problems with ageing, such as the loss of bone mineral density with microarchitectural deterioration known as osteoporosis that directly increases the risk of fractures (Novotny et al. 2015); the reduction in muscle mass and muscle functional performance known as sarcopenia (Chen et al. 2020). Both of these conditions share some common risk factors and pathogenesis (Kirk et al. 2019). Sarcopenia is associated with a range of adverse health outcomes (Landi et al. 2012, 2013; Tanimoto et al. 2013) which incur a high socioeconomic burden.

Sarcopenia

Sarcopenia is generally defined as low appendicular skeletal muscle mass with either reduced muscle strength or muscle performance. Primary sarcopenia is age-related with no other cause evident, while secondary sarcopenia is related to other causes, such as activity-related, disease-related, and nutrition-related (Cruz-Jentoft et al. 2010). Over the past decade, several consensus panels have announced the latest operational sarcopenia definitions and their diagnostic criteria. These consensus panels (summarized in Table 6.1) mainly include the European Working Group on Sarcopenia in Older People (EWGSOP) first in 2010 (Cruz-Jentoft et al. 2010) and updated in 2019 (Cruz-Jentoft et al. 2019), the International Working Group on Sarcopenia (IWGS) in 2011 (Fielding et al. 2011), the Asian Working Group for Sarcopenia (AWGS) first in 2014 (Chen et al. 2014) and updated in 2019 (Chen et al. 2020), and the Foundation for the National Institutes of Health (FNIH) in 2014

Table 6.1 Summary of different sarcopenia consensus panels and their respective diagnostic criteria

Sarcopenia definition			Latest diagnostic criteria and cutoff points		
Consensus panel	First published	Updated	ASMI[a] (ASM/height^2 or ASM/BMI[b])	HS (kg)	GS (m/s)
EWGSOP	2010	2019	M < 7.0	M < 27	≤0.8
			F < 5.5	F < 16	
IWGS	2011	–	M ≤ 7.23	–	<1.0
			F ≤ 5.67		
AWGS	2014	2019	M < 7.0	M < 28	<1.0
			F < 5.4	F < 18	
FNIH[b]	2014	–	M < 0.789	M < 26	≤0.8
			F < 0.512	F < 16	

ASMI, appendicular skeletal muscle index; ASM, appendicular skeletal muscle mass; BMI, body mass index; HS, handgrip strength; GS, gait speed; M, male; F, female
[a] Muscle mass assessed by DXA; ASM measured in kg and height in m
[b] ASMI is defined by ASM/BMI for FNIH only

(Studenski et al. 2014). Sarcopenia was officially considered a disease in 2016 when it received an ICD-10-CM disease code, M62.84, in the International Classification of Diseases (ICD) (Vellas et al. 2018), facilitating the progress of international sarcopenia research and eventually clinical management.

Clinical Signs, Symptoms, and Screening

Skeletal muscles undergo physiological and morphological changes as age increases. Fibrous and adipose tissues infiltrate muscles (Marcus et al. 2010), while the size and number of skeletal muscle fibres decrease with advancing age (Walston 2012; Lexell 1995). Symptoms or signs of sarcopenia include falling, muscle weakness, slow walking speed, difficulty rising from a chair or weight loss (Cruz-Jentoft et al. 2019). The screening of sarcopenia is currently not yet a common practice in typical clinical settings, as the public awareness of sarcopenia is not high enough and diagnosis requires a series of tests to confirm. Due to the complexity of the screening tests, EWGSOP recommends using SARC-F questionnaire while AWGS also recommends the measurement of calf circumference as an alternative for case finding or confirming clinical suspicion. SARC-F is a screening tool in the form of a questionnaire that assesses five components, namely "Strength", "Assistance in walking" "Rising from a chair", "Climbing stairs" and "Fall", hence the acronym SARC-F. Each parameter is scored between 0 and 2, with 2 being a worse score. A SARC-F score total ≥4 or calf circumference <34 cm for male or <33 cm for female (AWGS 2019 criteria) are considered as probable sarcopenia for follow-up. SARC-F has a high specificity but only a low-to-moderate sensitivity, limiting the ability to detect non-severe cases (Cruz-Jentoft et al. 2019) or those at the early

onset of sarcopenia. Both case finding methods are simple that can be introduced into clinical practice to facilitate sarcopenia screening and diagnosis.

Diagnosis

The diagnosis of sarcopenia requires separate assessments to measure muscle mass, strength, and performance. Individuals with low muscle mass, with either low muscle strength or muscle performance are considered to have sarcopenia. Those who meet all three criteria have severe sarcopenia. For individual diagnostic cutoff points for various sarcopenia definitions, please refer to Table 6.1.

Dual-energy x-ray absorptiometry (DXA) is the recommended method for the assessment of muscle mass. DXA is a non-invasive imaging technique that is one of the gold standards for body composition measurements, but it is not portable for use in community for sarcopenia screening. Bioelectrical impedance analysis (BIA) is another method recommended by some such as AWGS. BIA estimates fat mass and muscle mass using electrical impedance and is portable with high scanning speed. However, prediction models used by BIA to evaluate muscle mass are mostly relevant only in the populations in which they have been derived (Janssen et al. 2000; Cheng et al. 2021) and the equations used are usually not disclosed, potentially leading to inconsistency in the measurements across various BIA machines by different manufacturers (Yamada et al. 2017). Regardless, AWGS recommended a BIA cutoff for low ASMI at 7.0 kg/m^2 and 5.7 kg/m^2 for male and female respectively. Establishing local reference values and adjustments have been found to improve the accuracy of muscle mass assessment with BIA (Alkahtani 2017; Cheng et al. 2021).

Handgrip strength is the recommended assessment for muscle strength using dynamometer. Grip strength has been found to be associated with mortality and poor quality of life (Leong et al. 2015). It is a convenient and representative measure of strength as it has been proven to correlate with strength in other body parts (Bohannon et al. 2012).

Muscle performance is commonly assessed by testing gait speed in a 6-m walk test. Gait speed is a quick and inexpensive measure of functional performance, which has been found to reliably predict adverse outcomes such as disability, cognitive impairment, institutionalization, falls and/or mortality (Abellan van Kan et al. 2009; Peel et al. 2013; Studenski et al. 2011).

Prevalence

A recent systematic review reported a global sarcopenia prevalence of 8% to 36% in people <60 years and 10% to 27% in people ≥60 years of age, while the prevalence of severe sarcopenia was 2% to 9% (Petermann-Rocha et al. 2022). The prevalence

of sarcopenia varies greatly depending on the sarcopenia definition used (Woo et al. 2015; Mayhew et al. 2019). Sarcopenia prevalence also differs dramatically in different settings, such as in nursing homes with 51% men and 31% women, in hospitalization with 23% men and 24% women or in community settings with 11% men and 9% women with sarcopenia (Papadopoulou et al. 2020). Certain morbidities, such as cardiovascular disease, dementia, diabetes mellitus and respiratory disease (Pacifico et al. 2020), are the risk factors that increase sarcopenia prevalence.

Pathogenesis of Sarcopenia

Pathogenesis of sarcopenia is well known to be multifactorial, including inactivity, mitochondrial dysfunction, apoptosis, low growth hormone, neuromuscular junction deterioration, inflammation, vascular problem, malnutrition, etc. Here we look into the following three major areas: mitochondrial dysfunction, intramuscular fat infiltration and neuromuscular junction deterioration.

Mitochondrial Dysfunction

The mitochondrion is an important organelle and crucial integrator of intermediary metabolism in various metabolic pathways, such as oxidative phosphorylation (Gorman et al. 2016). Normally, to prevent the damage from daily produced mitochondrial reactive oxidative stress (mtROS), mitochondria have developed a quality control mechanism to maintain mitochondrial dynamics. Mitochondrial quality control involves the dynamic processes of biogenesis, fission, fusion, and mitophagy (Anzell et al. 2018; Pickles et al. 2018; Song et al. 2021). Once mitochondrial quality control is impaired, mtROS will be accumulated, resulting in further mitochondrial dysfunction in skeletal muscles and hence sarcopenia.

Mitochondrial biogenesis is an important biological process to maintain the homeostasis of mitochondria, in which peroxisome proliferative activated receptor gamma coactivator 1 alpha (PGC-1α) is a major regulator. The expression level of PGC-1α is prominently enhanced in organs or tissues with high energy demands such as skeletal muscle (Wenz 2009). In these organs or tissues, PGC-1α can be activated by specific stimuli (such as physical exercise) and the activated PGC-1α can induce the initiation of several transcription factors, such as nuclear respiratory factors (NRF-1 and 2), and mitochondrial transcription factor A (Tfam) (Bhatti et al. 2017). The decrease of PGC-1α was observed during the progression of sarcopenia (Muhammad and Allam 2018; Liang et al. 2021; Liu et al. 2021b; Aoki et al. 2020), accompanied by the mitochondrial dysfunction and muscle atrophy. Additionally, low mitochondrial function and muscle performance were observed after knocking out PGC-1α in mice (Derbré et al. 2012). These results provided evidence to support that low level of mitochondrial biogenesis could lead to sarcopenia.

Mitochondrial fusion and fission are two critical processes to maintain mitochondrial dynamic balance and prevent the mitochondrial dysfunction due to the damaged mitochondria or mistranslated proteins in mitochondria. Longer and fused mitochondria are optimal for ATP generation, whereas fission of mitochondria promotes mitophagy and cell division (Farmer et al. 2018). Mitofusin 1 and mitofusin 2 (Mfn1 and Mfn2) are two mitochondrial membrane proteins that control the mitochondrial fusion of the outer mitochondrial membrane, while fusion of the inner mitochondrial membrane requires the membrane-bound protein of optic atrophy 1 (OPA1) (Farmer et al. 2018). Mfn2 is a critical factor in mitochondrial dysfunction in skeletal muscles and the progressive reduction in Mfn2 is reported to associate with ageing (Sebastián et al. 2016). Previous studies showed that the process of mitochondrial fusion was compromised during ageing, then ATP production and transfer of both mitochondrial mtDNA and protein were impaired resulting in the accumulation of mtDNA mutation and hence mitochondrial-related disease such as sarcopenia. Mitochondrial fission is mainly responsible for segregating dysfunctional mitochondria that contain damaged proteins, destabilized membranes, mutated or damaged mtDNA for mitochondrial remodelling to maintain mitochondrial dynamic equilibrium (Pagliuso et al. 2018). Dynamin-related protein 1 (DRP1) plays a crucial role in fission. Briefly, DRP1 in the cytoplasm can be recruited by the DRP1 receptor on mitochondrial membrane to form oligomers around the constriction site, which will constrict the "marked" mitochondrial membrane. In addition, Kleele et al. reported that there were two functionally and mechanistically distinct types of fission, and both are mediated by DRP1 (Kleele et al. 2021). One was the division at the periphery which enabled damaged or mistranslated proteins in mitochondria to be shelled into smaller mitochondria for mitophagy; another one was the division in the middle of mitochondria which is regarded as one of the ways to increase mitochondrial mass. Several studies showed that the functions of fusion and fission decreased during ageing which finally weakened skeletal muscle performance (Zeng et al. 2020; Liu et al. 2021b). Additionally, current studies suggested that the decreased levels of mitochondrial fusion and fission were related to the progression of sarcopenia (Favaro et al. 2019; Liu et al. 2021a; Romanello and Sandri 2021).

Mitophagy is a specific autophagic elimination of mitochondria that is an important mechanism in preserving mitochondria when severe mitochondrial damage occurs. PINK1-Parkin-mediated mitophagy pathway is one of the most significant pathways to maintain mitochondrial homeostasis. PTEN-induced putative protein kinase 1 (PINK1) is a mitochondrial serine or threonine kinase. Normally, PINK1 is easily cleaved in mitochondria (Jin et al. 2010), and Parkin is a kind of cytosolic E3 ubiquitin ligase in cytoplasm (Ni et al. 2015). During ageing, PINK1 is stabilized on the outer membrane of damaged mitochondria where it recruits cytosolic Parkin (Ashrafi and Schwarz 2013; Onishi et al. 2021). Stable PINK1 can phosphorylate Parkin to promote Parkin E3 ligase activity to trigger mitophagy and facilitate the clearance of damaged mitochondria. Parkin ubiquitylates mitochondrial proteins and causes mitochondria to become autophagosome by isolation membranes that fuse with lysosomes for mitochondrial degradation (Ni et al. 2015; Ashrafi and Schwarz

2013). Several studies showed that decrease of PINK1 and Parkin led to a decrease of muscle performances that is accompanied with an increase of mtROS and decreased muscle strength (Gao et al. 2021; Liang et al. 2021; Liu et al. 2021b). Studies have suggested that mitophagy plays an important role in maintaining mitochondrial quality in skeletal muscle by selectively removing dysfunctional and depolarized mitochondria, while decreased level of mitophagy could lead to an accumulation of mitochondrial dysfunctions and hence sarcopenia (Henríquez-Olguin and Knudsen 2019; Wrighton et al. 2021).

Intramuscular Fat Infiltration

Body composition is altered markedly in older people, as reflected in the increased proportion of body fat mass and declined proportion of muscle mass (Macek et al. 2020). Meanwhile, more adipose tissues will be accumulated in skeletal muscles with advancing age (Hioki et al. 2020). Human muscle transcriptome profile showed that lipid metabolism might play an important role in age-related muscle impairment, such as sarcopenia (Tumasian et al. 2021). Satellite cells derived from old animal muscle had higher potential to differentiate into adipocytes. Also, higher levels of muscular adipokines, adipose tissues and lipogenic regulators were found in aged animals (Yada et al. 2006; Tazawa et al. 2019; Zhu et al. 2019; Rivas et al. 2011).

Fat deposition impacts muscle homeostasis in various molecular pathways, including protein synthesis, muscular mitochondrial function, metabolism, and inflammation, thus leading to sarcopenia. Diet-induced obesity (DIO), glycerin-injected and transgenic animals, such as db/db and ob/ob mice, were utilized to investigate the underlying mechanisms on how adipose tissues affected skeletal muscles (Bollheimer et al. 2012; Lukjanenko et al. 2013; Zhu et al. 2019; Choi et al. 2021). Similar to older individuals, increased muscular fat infiltration and triglyceride were also found in obese animal models (Correa-de-Araujo et al. 2020; Huang et al. 2019). The phenomena of the negative correlations between muscular fat infiltration and muscle mass, strength, as well as mobility were observed in humans and can be verified in obese animal models (Xie et al. 2021). Obese mice had 20% lower lean mass percentage compared to control mice (Rivas et al. 2016). Absolute hindlimb muscle mass was also reduced in obese mice, which was significant in aged but not younger groups (Tardif et al. 2014). The myofiber size in old DIO mice was a third less than that in the chow-diet natural old mice (Huang et al. 2019). Decreased muscle satellite cells and delayed muscle regeneration were also shown in fat infiltration animal models. After fed with high-fat diet, the mice had an estimated 12% reduction of total satellite cells (Lee et al. 2015). Obese animals needed more time for regeneration after muscle injury induced by cardiotoxin injection, indicating the resident satellite cells were blunt (Nguyen et al. 2011). Additionally, reduced grip strength and physical activities also resulted (Burchfield et al. 2018; Huang et al. 2019). This may be explained by the impediment of muscle

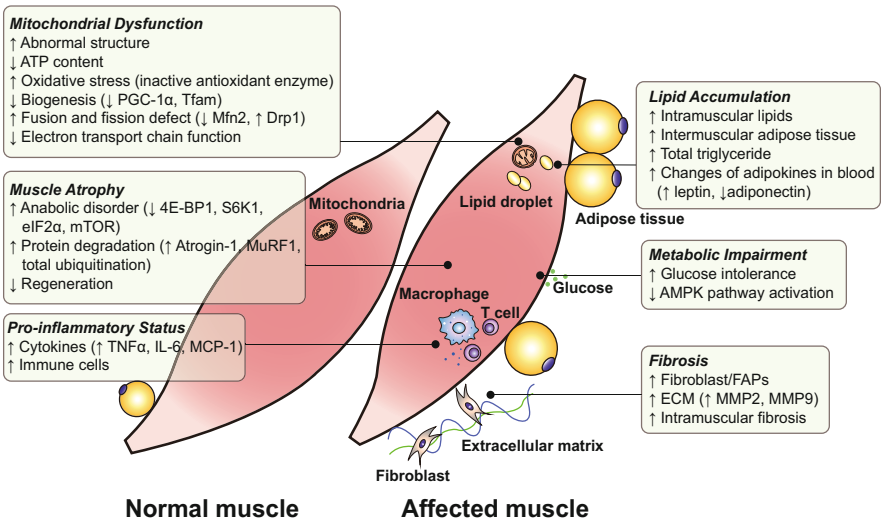


Fig. 6.1 Summary of effects and changes in adiposity-affected sarcopenic muscles. Impaired mitochondrial structure and function, accelerated muscle atrophy, increased inflammation, fibrosis, lipid accumulation, as well as metabolic dysfunction were found in muscle with excess fat deposit. 4E-BP1, eukaryotic translation initiation factor 4E eIF4E-binding protein 1; AMPK, adenosine monophosphate-activated protein kinase; ATP, adenosine triphosphate; ECM, extracellular matrix; eIF2α, eukaryotic initiation factor-2α; FAPs, fibro-adipogenic progenitors; IL-6, interleukin-6; MCP-1, monocyte chemoattractant protein-1; MMP, matrix metallopeptidase; MuRF1, muscle RING finger 1; S6K1, ribosomal protein S6 kinase beta-1; TNFα, tumour necrosis factor α

contraction caused by the increased myosteatosis and myofibrosis (Zoico et al. 2013; Bai et al. 2020).

At the molecular level, imbalanced muscle protein synthesis and degradation were detected in not only obese animal models, but also glycerin-injected and transgenic animals. To be specific, the down-regulation of mTOR, 4E-BP1, S6K1, eIF2α (known as muscle anabolic regulators) and up-regulation of ubiquitin E3 ligases genes (MuRF1 and Atrogin-1, known as muscle atrophy genes) were found (Bollheimer et al. 2012; Tardif et al. 2014; Le et al. 2014, 2017; Choi et al. 2021) (Fig. 6.1). When the protein degradation rate is higher than the synthesis rate, the muscle volume is difficult to sustain. In addition, fat accumulation was also found to associate with deterioration of mitochondrial structure and function in skeletal muscles, including distorted mitochondrial cristae, lower expression of PGC-1α, antioxidant enzyme activities, and altered electron transport pathways (Bollheimer et al. 2012; Tardif et al. 2014; Huang et al. 2019; Bai et al. 2020). Damaged mitochondrial homeostasis was unable to meet the energy demand of myocytes and would potentially activate apoptosis (Bellanti et al. 2021). Therefore, once fat deposition level increases that threatens mitochondrial homeostasis in skeletal muscle, sarcopenia may result. Glucose intolerance and pro-inflammatory status (TNFα, MCP-1, IL-6) were also observed in fat-infiltrated skeletal muscle

(Burchfield et al. 2018; Le et al. 2014, 2017; Collins et al. 2016; Laurentius et al. 2019; Bai et al. 2020; Xu et al. 2021; Choi et al. 2021). It is well known that the skeletal muscle plays essential roles in insulin sensitivity and glycemic stability (Sylow et al. 2021). Reduced lipid oxidative capacity of fat-deposited muscle may aggravate metabolic syndromes and muscle atrophy (Turcotte and Fisher 2008). Adipose tissue is a secretory organ that produces pro-inflammatory cytokines to skeletal muscle by paracrine actions. The high muscle inflammatory level was observed in mice with glycerin injection or high-fat diet. It indicated that both circulating lipid and myosteatosis contributed to the adverse muscle status in terms of inflammation (Le et al. 2014; Xu et al. 2021).

Intramuscular fat not only negatively correlated with physical ability in older people, but also related to the hospitalization, disease prognosis, and mortality (Waters 2019; Cawthon et al. 2009; Perkisas et al. 2017, 2018). In addition to muscle mass and strength, diagnosis or screening of sarcopenia is also recommended, focusing on intramuscular fat infiltration, since it is a strong predictor of functional disability and clinical outcomes (Addison et al. 2014).

Neuromuscular Junction Deterioration

The neuromuscular junction (NMJ) is a chemical synapse serving as a bridge between motor neurons and muscle fibres, which is composed of pre-synaptic active zones in motor nerve terminals, post-synaptic acetylcholine receptors (AChRs) in muscle membranes and intra-synaptic clefts (Gonzalez-Freire et al. 2014). Considering the influence of NMJ on skeletal muscles, NMJ maintenance is important for the maintenance of muscle mass through muscle contraction triggered by action potentials originated from motor neurons and transferred to the muscle cell at the NMJ (Tamaki et al. 2014). NMJ degeneration is regarded as an important cause of sarcopenia (Larsson et al. 2019). However, whether NMJ deterioration is a primary cause of sarcopenia or whether impaired NMJ structure and function is secondary to sarcopenia is still not clear.

Numerous alterations in the structure and function of NMJ have been observed during the ageing process. In human studies, ageing-related morphological changes include increased NMJ branching and peri-junctional AChRs area (Oda 1984; Wokke et al. 1990). Jitter values and the fibre density examined by single-fibre electromyography (SFEMG) were reported to increase with ageing, indicating the reduced efficacy of neuromuscular transmission (Balci et al. 2005; Juel 2012). As humans are not suitable for detailed studies of NMJ, most studies have been conducted in old animals. A recent systematic review summarized muscle fibre type dependent changes of NMJ with increasing age (Bao et al. 2020). Reduced overlapping between nerve terminals and AChRs as well as increased post-synaptic endplate fragmentation are commonly observed in different skeletal muscles of old animals (Bao et al. 2020), which are consistent with reduced compound muscle action potentials (CMAP) amplitude with ageing (Xie et al. 2018). In addition,

ageing-related denervation is more likely to be involved in fast-twitch skeletal muscles, while type I motor units tend to regain control of denervated muscle fibres by collateral reinnervation (Yuan et al. 2011). Fast-twitch skeletal muscles mainly composed of MHC IIb fibres were shown with reduced AChR number during ageing (Banker et al. 1983; Herscovich and Gershon 1987), while there was no significant difference of miniature endplate potential (MEPP) amplitude between young and old muscles (Banker et al. 1983; Willadt et al. 2016), which may be explained by increased release of acetylcholine (ACh) quanta (Banker et al. 1983; Zhao et al. 2018). The same increased release of ACh quanta is also observed in slow-twitch muscles, explaining the more severe neurotransmission failure after tetanic stimulations (Banker et al. 1983; Kulakowski et al. 2011). In contrast, AChR number and ACh quanta release present no changes during ageing in diaphragm that is mainly composed of MHC IIa fibers (Banker et al. 1983; Willadt et al. 2016). The reduced association rate constant between α-BuTx and AChRs and increased acetylcholinesterase (AChE) activity in old diaphragm explain the increased MEPP amplitude (Banker et al. 1983; Smith et al. 1990; Smith and Chapman 1987). The AChR area is another important parameter to assess morphological changes of post-synaptic endplate, which changes in AChR area are associated with muscle fibre types and the progress of muscle atrophy.

Agrin-LRP4-MuSk-Rapsyn-Dok7 is a major signalling pathway driving AChR clustering and ensuring an efficient signal transduction at NMJ. Agrin cleavage can induce endplate fragmentation, along with sarcopenia-like syndrome (Bütikofer et al. 2011). Meanwhile, the serum level of cleaved C-terminal Agrin fragments is higher in the patients with sarcopenia (Marzetti et al. 2014). Increased LRP4 mRNA level but reduced LRP4 protein expression was reported by Zhao and colleagues, which is related with increased LRP4 ubiquitination (Zhao et al. 2018). MuSk mRNA expression is also increased with ageing in both rodents and humans (Aare et al. 2016; Soendenbroe et al. 2019), while MuSk phosphorylation is reduced during ageing (Zhao et al. 2018). Other molecules like MRF4 (Apel et al. 2009), Dystrophin-Glycoprotein Complex (including Sarcospan, Sarcoglycan-α, Syntrophin and Dystrophin) (Zhao et al. 2018; Hughes et al. 2017), TrkB (Personius and Parker 2013) and mTORC1 (Ham et al. 2020) also have been reported to be associated with NMJ degeneration during ageing, but require further evidence from human studies. In summary, the NMJ undergoes both morphological and functional alterations during ageing and this degeneration may ultimately lead to ageing-related sarcopenia. Further investigating the mechanisms of NMJ degeneration with ageing is essential to provide us with new directions and targets for the diagnosis and treatment of sarcopenia.

Current Treatments

Epidemiology proposes a life course approach to tackling sarcopenia and frailty (Dodds and Sayer 2015). The life course approach comprises a maximum peak of muscle mass and strength in early life from growth and development, and the

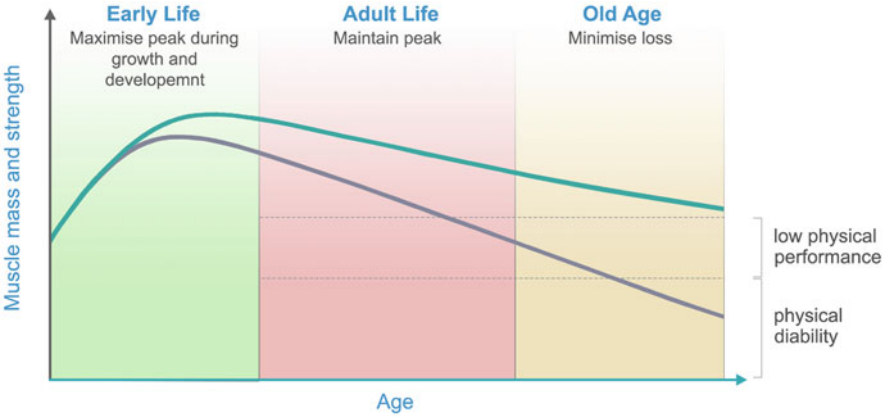


Fig. 6.2 The life course approach to maintaining physical fitness in old age to minimize physical performance limitations; green line represents the life course approach. The figure is modified from (Dodds and Sayer 2015)

subsequent age-related decline is determined by maintenance in adult life and minimizing loss in older life (Fig. 6.2). The prevention of sarcopenia and frailty need to start early to decelerate the decline associated with ageing (Azzolino et al. 2021).

Physical exercises are essential for health which include aerobic exercises, resistance exercises and combined exercises in the context of sarcopenia and the maintenance of muscle health. Combined or multimodal exercises comprise a mixture of aerobic and resistance exercise training, and sometimes together with flexibility and balance exercises. Combined exercises can complement each other and offer the benefits from different exercises (Takeshima et al. 2004). It has been shown that in combined training, aerobic exercises can even provide systemic factors that can promote muscle improvements by resistance exercises (Moberg et al. 2021; Mascher et al. 2011). Studies have demonstrated that both endurance and resistance training can stimulate morphological adaptations of the NMJ, thus counteracting NMJ degeneration with ageing (Kreko-Pierce and Eaton 2018).

Aerobic Exercises

Aerobic or endurance exercises are the performance of light-to-moderate intensity activities for extended periods of time that would increase breathing and heart rate due to increased oxygen demand. Aerobic exercises have been found to improve overall fitness, cardiovascular health, and metabolism, specifically metabolic syndrome by reducing obesity and hypertension and increasing insulin sensitivity (Marcus et al. 2010). Aerobic exercise also suppresses the expression of myostatin mRNA, reducing the inhibition on muscle growth associated with ageing (Ko et al. 2014). The benefits of aerobic exercises on prevention and treatment of major

chronic disease have been well established (Nunan et al. 2013), however, resistance exercises can provide a more immediate improvement in muscle mass, strength, power in older adults (Lovell et al. 2010).

Resistance Exercises

Resistance exercises are considered the first-line treatment for sarcopenia (Dent et al. 2018). This type of training refers to activities which produces skeletal muscle contraction using external resistance such as elastic band, training weights or even body weight. Resistance exercises have been shown to increase muscle protein synthesis and the size of muscle fibres, resulting in improvements of physical performance, muscle strength and muscle mass in older adults (Chen et al. 2021; Kim et al. 2012; Liu and Latham 2009). A meta-analysis with 1328 participants over 50 years of age showed a weighted pool estimate of mean lean body mass increase of 1.1 kg after resistance exercise training that lasted an average of 20 weeks with 2–3 sessions per week (Peterson et al. 2011). Resistance exercises can also combat the morphological changes in muscle associated with ageing by incorporating new nuclei into muscle fibres (Hikida et al. 2000), increasing mitochondrial density in muscle (White et al. 2016) and reducing intramuscular fat infiltration (Goodpaster et al. 2008), thus improving muscle quality, muscle strength (Goodpaster et al. 2001) and risk of future mobility limitations (Visser et al. 2005). Progressive increase of the intensity of resistance exercise is recommended throughout the course of a training to drive continued muscular adaptation and muscle hypertrophy (Hurst et al. 2022). Recommended resistance exercise prescription for older people with sarcopenia is summarized in Table 6.2. Resistance exercise training sessions of more than twice

Table 6.2 Table summary showing recommended resistance exercise prescription for older adults with sarcopenia. The table is modified from Hurst et al. (2022)

Training frequency	2–3 sessions per week	
Exercise selection	Upper body • Chest press • Seated row • Pull down	Lower body • Squat/leg press • Knee extension • Leg curl • Calf raise
Exercise volume	1–3 sets of 6–12 repetitions	
Exercise intensity (per set)	Repetition-continuum based • 40–60% 1RM, progressing to 70–85% 1RM Rating of perceived exertion (RPE) based • RPE 3–5 on CR10 scale, progressing to RPE 6–8	
Rest periods	Within exercise session: • 1–2 min rest between sets; 3–5 min rest between exercises Between exercise sessions: • At least 48 h	

1RM, 1 repetition maximum (maximal amount of weight that can be lifted for one complete repetition); CR10, category ratio 10 scale

per week have been shown to improve muscle strength more than a training frequency of once weekly (Kneffel et al. 2021; Borde et al. 2015). Rest periods between training sessions are also important in providing adequate time for recovery between training. The frequency of training can be increased over time to ensure appropriate amount of overload and stimulus is received (Dodds and Sayer 2015). Targeted training for specific regions to fit the needs of an individual is recommended, taking into account of a person's limitations. Correct technique should be focused, rather than simply overloading with many training exercises or high exercise volume early in the training programme, which may lead to fatigue and discomfort that discourage older adults. Resistance exercise intensity or effort is usually recommended based on the repetition maximum (RM) (Buckner et al. 2017), ratings of perceived exertion (RPE) or an effort-based approach (Buckley and Borg 2011), while resistance exercise volume is quantified by the number of sets of repetitions. A meta-analysis found that a high level of effort in resistance exercises is the most effective way for improving muscle strength (Peterson et al. 2010), while high-volume programmes are more effective for lean muscle mass gains (Peterson et al. 2011). Ageing individuals are recommended to take up resistance exercise training as early as possible and progress to high-effort exercise intensity for optimal training efficiency.

Whole body vibration is a novel treatment approach that can be offered as an alternative for older adults especially those unfit for regular exercise training (Kemmler and von Stengel 2012). It is a biophysical intervention that provides non-invasive and systemic mechanical stimulation, proven to improve muscle strength, balancing ability and fall prevention, among other benefits (Ren et al. 2020; Roelants et al. 2004). The improvement effects from vibration therapy were observed as early as 9 months after treatment commencement and were found to be retained 1 year after an 18-month treatment has ended (Cheung et al. 2016). Muscle strength and performance have been shown to significantly improve after vibration therapy (Osawa et al. 2013; Verschueren et al. 2004; Wu et al. 2020).

Nutritional Supplementation

With the increase of age and the decrease in the body absorption efficiency, malnutrition is becoming a problem for the elderly. Affected by the loss of taste and smell, chewing troubles, and damaged gut function (Murphy 2008), the food intake of older people will reduce by around 25% between 40 and 70 years of age (Nieuwenhuizen et al. 2010). Low food intake and repetitive diets may result in a risk of insufficient nutrient intakes (Wolfe et al. 2008), which aggravates muscle loss and muscle strength decrease in older people. Currently, the gold standard clinical treatment for sarcopenia is nutritional supplementation (Bauer et al. 2013; Deutz et al. 2014) combined with physical activity intervention. According to the consensus statements, to maintain sufficient protein intake, a daily protein intake of ≥1.0 g/kg body weight for healthy older adults and ≥1.2 g/kg body weight for those with

sarcopenia and frailty is recommended. For older adults in need of supplementation, high-quality protein, amino acids such as leucine and l-carnitine, or beta-hydroxy-beta-methyl butyrate (HMB) may be considered according to the specific prescribing information.

High-quality protein provides amino acids needed for the synthesis of muscle proteins, the balance between anabolism and catabolism, and preventing loss of muscle mass and strength (Wolfe et al. 2008); for some sarcopenic patients with malnutrition condition, high-protein oral nutritional supplements may be more effective. Milk fat globule membrane (MFGM) (Watanabe et al. 2020) and whey protein (Mori and Tokuda 2018; Lin et al. 2021; Nabuco et al. 2018) are mostly used in randomized controlled trials (RCT). A study by Mori and Tokuda (2018) assessed the effect of whey protein supplementation (22.3 g for 24 weeks) along with exercise on aged women (aged 65–80 years), which showed a significant increase in muscle function (grip strength increase about 1.2 kg, $P < 0.05$) and muscle mass (increase about 0.5 kg, $P < 0.01$). Other studies (Bo et al. 2019; Rondanelli et al. 2016) used whey protein with additional nutrition like vitamin D (100–702 IU) and leucine (2.5–4 g) and reported similar results. Mariangela et al. (Rondanelli et al. 2020) used a mixed formula (20 g of whey proteins, 2.8 g of leucine, 9 g of carbohydrates, 3 g of fat, 800 IU of vitamin D) for sarcopenic patients (according to EWGSOP, $n = 140$, age ≥65 years) for 4–8 weeks. They resulted in an increase of mean gait speed (0.061 m/s/month [95% CI, 0.043–0.080]) and muscle mass (0.40 [95% CI: 0.06–0.73], $P < 0.03$) compared to the placebo group. These studies support that high-quality protein can benefit the prevention or treatment of sarcopenia.

Studies demonstrated that essential amino acid leucine and its metabolite β-methyl butyric acid (HMB) had some effects in improving muscle mass and function (Cruz-Jentoft 2018; Sanz-Paris et al. 2018). HMB (Pasiakos and Carbone 2014) has been reported to have an impact on anabolic protein metabolism. Several human studies reported the benefits of HMB in both strength-power and endurance sports. The HMB dose used is 1.5–6 g/day for 4–12 weeks, of which 3 g/day is most effective (Chen et al. 2022). Combining with exercise, HMB tends to provide better effects on protecting muscles from injuries and soreness, which may improve the exercise capacity and duration (Thomson et al. 2009). By critically analyzing the existing literature on HMB supplementation before 2013, the International Society of Sports Nutrition declared that HMB enhances muscle recovery from exercise-induced muscle damage. From a variety of supplement protocols (1 day to 6 weeks; with or without exercise), age range (from 19 to 50 years old), and training status (untrained and moderately to highly resistance-trained), studies show that HMB appears to be most effective when taking 2 weeks before exercise bout (Wilson et al. 2013).

Studies (Robinson et al. 2018) about other supplements like vitamin D (Prokopidis et al. 2022), antioxidant nutrients (Cesare et al. 2020), and long-chain polyunsaturated fatty acids (Troesch et al. 2020) are observational, and the outcomes from some clinical studies are less common. These studies focused on particular groups like postmenopausal women or obese elderly, while the protocols involved a variety of outcome measures or different duration of medication. For example,

Stephen (Cornish et al. 2018) used 3.0 g/day Omega-3, a long-chain fatty acid, as a supplement for 23 older men (≥65 years old) for 12 weeks, which showed not adequate to enhance muscle mass or muscle function. While Mariasole (Da Boit et al. 2017) used a similar supplement (3 g fish oil/d) for 50 aged men and women (≥65 years old), which found muscle quality was significantly increased ($P < 0.05$). The existing evidence for nutrition supplements is not consistent (Dent et al. 2018). Large-scale trials are now undergoing to specifically address the efficacy of exercise and nutritional supplements for patients with sarcopenia, such as the European SPRINTT trial (NCT02582138) (Landi et al. 2017). Although there is insufficient evidence on the benefits of nutritional supplementation in terms of muscle health, it is believed that ongoing RCT and preclinical studies will provide more evidence to validate the efficacy of nutritional supplements on muscle health for future clinical application.

Conclusions and Future Directions

Musculoskeletal deterioration due to advancing age is a prevalent issue especially in an ageing population. Sarcopenia is defined as low appendicular skeletal muscle mass with either reduced muscle strength and/or muscle performance. Proper training and nutritional supplementation are required to achieve the maximum peak of muscle health at early age, maintained throughout adult life and into old age to minimize loss of muscle mass, strength, and function. Ageing increases the susceptibility of developing multiple comorbidities and syndromes. The traditional models of care built around single-disease treatment are unprepared for the complexity of managing geriatric health. More large-scale clinical studies are required to develop validated, ethnicity/region-based, and gender-specific cutoff points for sarcopenia; to investigate the opportunities for sarcopenia intervention across the life course; and to apply optimal sarcopenia treatments to avoid physical performance limitation, disability and the associated risks such as falls, fractures, and mortality in sarcopenic older adults.

References

Aare S, Spendiff S, Vuda M, Elkrief D, Perez A, Wu QH, Mayaki D, Hussain SNA, Hettwer S, Hepple RT (2016) Failed reinnervation in aging skeletal muscle. Skelet Muscle 6

Abellan van Kan G, Rolland Y, Andrieu S, Bauer J, Beauchet O, Bonnefoy M, Cesari M, Donini LM, Gillette Guyonnet S, Inzitari M, Nourhashemi F, Onder G, Ritz P, Salva A, Visser M, Vellas B (2009) Gait speed at usual pace as a predictor of adverse outcomes in community-dwelling older people an international academy on nutrition and aging (IANA) task force. J Nutr Health Aging 13(10):881–889. https://doi.org/10.1007/s12603-009-0246-z

Addison O, Marcus RL, Lastayo PC, Ryan AS (2014) Intermuscular fat: a review of the consequences and causes. Int J Endocrinol 2014:309570. https://doi.org/10.1155/2014/309570

Alkahtani SA (2017) A cross-sectional study on sarcopenia using different methods: reference values for healthy Saudi young men. BMC Musculoskelet Disord 18(1):119. https://doi.org/10.1186/s12891-017-1483-7

Anzell AR, Maizy R, Przyklenk K, Sanderson TH (2018) Mitochondrial quality control and disease: insights into ischemia-reperfusion injury. Mol Neurobiol 55(3):2547–2564. https://doi.org/10.1007/s12035-017-0503-9

Aoki K, Konno M, Honda K, Abe T, Nagata T, Takehara M, Sugasawa T, Takekoshi K, Ohmori H (2020) Habitual aerobic exercise diminishes the effects of sarcopenia in senescence-accelerated mice Prone8 model. Geriatrics 5(3). https://doi.org/10.3390/geriatrics5030048

Apel PJ, Alton T, Northam C, Ma J, Callahan M, Sonntag WE, Li Z (2009) How age impairs the response of the neuromuscular junction to nerve transection and repair: an experimental study in rats. J Orthop Res 27(3):385–393

Ashrafi G, Schwarz TL (2013) The pathways of mitophagy for quality control and clearance of mitochondria. Cell Death Differ 20(1):31–42. https://doi.org/10.1038/cdd.2012.81

Azzolino D, Spolidoro GCI, Saporiti E, Luchetti C, Agostoni C, Cesari M (2021) Musculoskeletal changes across the lifespan: nutrition and the life-course approach to prevention. Front Med 8:697954. https://doi.org/10.3389/fmed.2021.697954

Bai CH, Alizargar J, Peng CY, Wu JP (2020) Combination of exercise training and resveratrol attenuates obese sarcopenia in skeletal muscle atrophy. Chin J Physiol 63(3):101–112. https://doi.org/10.4103/CJP.CJP_95_19

Balci K, Turgut N, Nurlu G (2005) Normal values for single fiber EMG parameters of frontalis muscle in healthy subjects older than 70 years. Clin Neurophysiol 116(7):1555–1557. https://doi.org/10.1016/j.clinph.2005.03.001

Banker BQ, Kelly SS, Robbins N (1983) Neuromuscular transmission and correlative morphology in young and old mice. J Physiol 339:355–377. https://doi.org/10.1113/jphysiol.1983.sp014721

Bao Z, Cui C, Chow SK, Qin L, Wong RMY, Cheung WH (2020) AChRs degeneration at NMJ in aging-associated sarcopenia—a systematic review. Front Aging Neurosci 12:597811. https://doi.org/10.3389/fnagi.2020.597811

Bauer J, Biolo G, Cederholm T, Cesari M, Cruz-Jentoft AJ, Morley JE, Phillips S, Sieber C, Stehle P, Teta D, Visvanathan R, Volpi E, Boirie Y (2013) Evidence-based recommendations for optimal dietary protein intake in older people: a position paper from the PROT-AGE study group. J Am Med Dir Assoc 14(8):542–559. https://doi.org/10.1016/j.jamda.2013.05.021

Bellanti F, Lo Buglio A, Vendemiale G (2021) Mitochondrial impairment in sarcopenia. Biology (Basel) 10(1). https://doi.org/10.3390/biology10010031

Bhatti JS, Bhatti GK, Reddy PH (2017) Mitochondrial dysfunction and oxidative stress in metabolic disorders—a step towards mitochondria based therapeutic strategies. Biochim Biophys Acta Mol Basis Dis 1863(5):1066–1077. https://doi.org/10.1016/j.bbadis.2016.11.010

Bo Y, Liu C, Ji Z, Yang R, An Q, Zhang X, You J, Duan D, Sun Y, Zhu Y, Cui H, Lu Q (2019) A high whey protein, vitamin D and E supplement preserves muscle mass, strength, and quality of life in sarcopenic older adults: a double-blind randomized controlled trial. Clin Nutr 38(1):159–164. https://doi.org/10.1016/j.clnu.2017.12.020

Bohannon RW, Magasi SR, Bubela DJ, Wang YC, Gershon RC (2012) Grip and knee extension muscle strength reflect a common construct among adults. Muscle Nerve 46(4):555–558. https://doi.org/10.1002/mus.23350

Bollheimer LC, Buettner R, Pongratz G, Brunner-Ploss R, Hechtl C, Banas M, Singler K, Hamer OW, Stroszczynski C, Sieber CC, Fellner C (2012) Sarcopenia in the aging high-fat fed rat: a pilot study for modeling sarcopenic obesity in rodents. Biogerontology 13(6):609–620. https://doi.org/10.1007/s10522-012-9405-4

Borde R, Hortobagyi T, Granacher U (2015) Dose-response relationships of resistance training in healthy old adults: a systematic review and meta-analysis. Sports Med 45(12):1693–1720. https://doi.org/10.1007/s40279-015-0385-9

Buckley JP, Borg GA (2011) Borg's scales in strength training; from theory to practice in young and older adults. Appl Physiol Nutr Metab 36(5):682–692. https://doi.org/10.1139/h11-078

Buckner SL, Jessee MB, Mattocks KT, Mouser JG, Counts BR, Dankel SJ, Loenneke JP (2017) Determining strength: a case for multiple methods of measurement. Sports Med 47(2):193–195. https://doi.org/10.1007/s40279-016-0580-3

Burchfield JG, Kebede MA, Meoli CC, Stockli J, Whitworth PT, Wright AL, Hoffman NJ, Minard AY, Ma X, Krycer JR, Nelson ME, Tan SX, Yau B, Thomas KC, Wee NKY, Khor EC, Enriquez RF, Vissel B, Biden TJ, Baldock PA, Hoehn KL, Cantley J, Cooney GJ, James DE, Fazakerley DJ (2018) High dietary fat and sucrose results in an extensive and time-dependent deterioration in health of multiple physiological systems in mice. J Biol Chem 293(15): 5731–5745. https://doi.org/10.1074/jbc.RA117.000808

Bütikofer L, Zurlinden A, Bolliger MF, Kunz B, Sonderegger P (2011) Destabilization of the neuromuscular junction by proteolytic cleavage of agrin results in precocious sarcopenia. FASEB J 25(12):4378–4393. https://doi.org/10.1096/fj.11-191262

Cawthon PM, Fox KM, Gandra SR, Delmonico MJ, Chiou CF, Anthony MS, Sewall A, Goodpaster B, Satterfield S, Cummings SR, Harris TB, Health A, Body Composition S (2009) Do muscle mass, muscle density, strength, and physical function similarly influence risk of hospitalization in older adults? J Am Geriatr Soc 57(8):1411–1419. https://doi.org/10.1111/j.1532-5415.2009.02366.x

Cesare MM, Felice F, Santini V, Di Stefano R (2020) Antioxidants in sport sarcopenia. Nutrients 12(9). https://doi.org/10.3390/nu12092869

Chen LK, Arai H, Assantachai P, Akishita M, Chew STH, Dumlao LC, Duque G, Woo J (2022) Roles of nutrition in muscle health of community-dwelling older adults: evidence-based expert consensus from Asian Working Group for Sarcopenia. J Cachexia Sarcopenia Muscle. https://doi.org/10.1002/jcsm.12981

Chen LK, Liu LK, Woo J, Assantachai P, Auyeung TW, Bahyah KS, Chou MY, Chen LY, Hsu PS, Krairit O, Lee JS, Lee WJ, Lee Y, Liang CK, Limpawattana P, Lin CS, Peng LN, Satake S, Suzuki T, Won CW, Wu CH, Wu SN, Zhang T, Zeng P, Akishita M, Arai H (2014) Sarcopenia in Asia: consensus report of the Asian Working Group for Sarcopenia. J Am Med Dir Assoc 15(2):95–101. https://doi.org/10.1016/j.jamda.2013.11.025

Chen LK, Woo J, Assantachai P, Auyeung TW, Chou MY, Iijima K, Jang HC, Kang L, Kim M, Kim S, Kojima T, Kuzuya M, Lee JSW, Lee SY, Lee WJ, Lee Y, Liang CK, Lim JY, Lim WS, Peng LN, Sugimoto K, Tanaka T, Won CW, Yamada M, Zhang T, Akishita M, Arai H (2020) Asian Working Group for Sarcopenia: 2019 consensus update on sarcopenia diagnosis and treatment. J Am Med Dir Assoc 21(3):300–307 e302. https://doi.org/10.1016/j.jamda.2019.12.012

Chen N, He X, Feng Y, Ainsworth BE, Liu Y (2021) Effects of resistance training in healthy older people with sarcopenia: a systematic review and meta-analysis of randomized controlled trials. Eur Rev Aging Phys Act 18(1):23. https://doi.org/10.1186/s11556-021-00277-7

Cheng KY, Chow SK, Hung VW, Wong CH, Wong RM, Tsang CS, Kwok T, Cheung WH (2021) Diagnosis of sarcopenia by evaluating skeletal muscle mass by adjusted bioimpedance analysis validated with dual-energy X-ray absorptiometry. J Cachexia Sarcopenia Muscle 4(10):12825

Cheung WH, Li CY, Zhu TY, Leung KS (2016) Improvement in muscle performance after one-year cessation of low-magnitude high-frequency vibration in community elderly. J Musculoskelet Neuronal Interact 16(1):4–11

Choi EB, Jeong JH, Jang HM, Ahn YJ, Kim KH, An HS, Lee JY, Jeong EA, Lee J, Shin HJ, Kim KE, Roh GS (2021) Skeletal Lipocalin-2 is associated with iron-related oxidative stress in ob/ob mice with sarcopenia. Antioxidants (Basel) 10(5). https://doi.org/10.3390/antiox10050758

Collins KH, Hart DA, Reimer RA, Seerattan RA, Waters-Banker C, Sibole SC, Herzog W (2016) High-fat high-sucrose diet leads to dynamic structural and inflammatory alterations in the rat vastus lateralis muscle. J Orthop Res 34(12):2069–2078. https://doi.org/10.1002/jor.23230

Cornish SM, Myrie SB, Bugera EM, Chase JE, Turczyn D, Pinder M (2018) Omega-3 supplementation with resistance training does not improve body composition or lower biomarkers of inflammation more so than resistance training alone in older men. Nutr Res 60:87–95. https://doi.org/10.1016/j.nutres.2018.09.005

Correa-de-Araujo R, Addison O, Miljkovic I, Goodpaster BH, Bergman BC, Clark RV, Elena JW, Esser KA, Ferrucci L, Harris-Love MO, Kritchevsky SB, Lorbergs A, Shepherd JA, Shulman GI, Rosen CJ (2020) Myosteatosis in the context of skeletal muscle function deficit: an interdisciplinary workshop at the National Institute on Aging. Front Physiol 11:963. https://doi.org/10.3389/fphys.2020.00963

Cruz-Jentoft AJ (2018) Beta-hydroxy-beta-methyl butyrate (HMB): from experimental data to clinical evidence in sarcopenia. Curr Protein Pept Sci 19(7):668–672. https://doi.org/10.2174/1389203718666170529105026

Cruz-Jentoft AJ, Baeyens JP, Bauer JM, Boirie Y, Cederholm T, Landi F, Martin FC, Michel JP, Rolland Y, Schneider SM, Topinkova E, Vandewoude M, Zamboni M (2010) Sarcopenia: European consensus on definition and diagnosis: report of the European Working Group on sarcopenia in older people. Age Ageing 39(4):412–423. https://doi.org/10.1093/ageing/afq034

Cruz-Jentoft AJ, Bahat G, Bauer J, Boirie Y, Bruyere O, Cederholm T, Cooper C, Landi F, Rolland Y, Sayer AA, Schneider SM, Sieber CC, Topinkova E, Vandewoude M, Visser M, Zamboni M (2019) Sarcopenia: revised European consensus on definition and diagnosis. Age Ageing 48(1):16–31. https://doi.org/10.1093/ageing/afy169

Da Boit M, Sibson R, Sivasubramaniam S, Meakin JR, Greig CA, Aspden RM, Thies F, Jeromson S, Hamilton DL, Speakman JR, Hambly C, Mangoni AA, Preston T, Gray SR (2017) Sex differences in the effect of fish-oil supplementation on the adaptive response to resistance exercise training in older people: a randomized controlled trial. Am J Clin Nutr 105(1):151–158. https://doi.org/10.3945/ajcn.116.140780

Dent E, Morley JE, Cruz-Jentoft AJ, Arai H, Kritchevsky SB, Guralnik J, Bauer JM, Pahor M, Clark BC, Cesari M, Ruiz J, Sieber CC, Aubertin-Leheudre M, Waters DL, Visvanathan R, Landi F, Villareal DT, Fielding R, Won CW, Theou O, Martin FC, Dong B, Woo J, Flicker L, Ferrucci L, Merchant RA, Cao L, Cederholm T, Ribeiro SML, Rodriguez-Manas L, Anker SD, Lundy J, Gutierrez Robledo LM, Bautmans I, Aprahamian I, Schols J, Izquierdo M, Vellas B (2018) International clinical practice guidelines for sarcopenia (ICFSR): screening, diagnosis and management. J Nutr Health Aging 22(10):1148–1161. https://doi.org/10.1007/s12603-018-1139-9

Derbré F, Gomez-Cabrera MC, Nascimento AL, Sanchis-Gomar F, Martinez-Bello VE, Tresguerres JA, Fuentes T, Gratas-Delamarche A, Monsalve M, Viña J (2012) Age associated low mitochondrial biogenesis may be explained by lack of response of PGC-1α to exercise training. Age (Dordr) 34(3):669–679. https://doi.org/10.1007/s11357-011-9264-y

Deutz NE, Bauer JM, Barazzoni R, Biolo G, Boirie Y, Bosy-Westphal A, Cederholm T, Cruz-Jentoft A, Krznaric Z, Nair KS, Singer P, Teta D, Tipton K, Calder PC (2014) Protein intake and exercise for optimal muscle function with aging: recommendations from the ESPEN expert group. Clin Nutr 33(6):929–936. https://doi.org/10.1016/j.clnu.2014.04.007

Dodds R, Sayer AA (2015) Sarcopenia and frailty: new challenges for clinical practice. Clin Med (Lond) 15(Suppl 6):s88–s91. https://doi.org/10.7861/clinmedicine.15-6-s88

Farmer T, Naslavsky N, Caplan S (2018) Tying trafficking to fusion and fission at the mighty mitochondria. Traffic 19(8):569–577. https://doi.org/10.1111/tra.12573

Favaro G, Romanello V, Varanita T, Andrea Desbats M, Morbidoni V, Tezze C, Albiero M, Canato M, Gherardi G, De Stefani D, Mammucari C, Blaauw B, Boncompagni S, Protasi F, Reggiani C, Scorrano L, Salviati L, Sandri M (2019) DRP1-mediated mitochondrial shape controls calcium homeostasis and muscle mass. Nat Commun 10(1):2576. https://doi.org/10.1038/s41467-019-10226-9

Fielding RA, Vellas B, Evans WJ, Bhasin S, Morley JE, Newman AB, Abellan van Kan G, Andrieu S, Bauer J, Breuille D, Cederholm T, Chandler J, De Meynard C, Donini L, Harris T, Kannt A, Keime Guibert F, Onder G, Papanicolaou D, Rolland Y, Rooks D, Sieber C, Souhami E, Verlaan S, Zamboni M (2011) Sarcopenia: an undiagnosed condition in older adults. Current consensus definition: prevalence, etiology, and consequences. International working group on sarcopenia. J Am Med Dir Assoc 12(4):249–256. https://doi.org/10.1016/j.jamda.2011.01.003

Gao HE, Li FH, Xie T, Ma S, Qiao YB, Wu DS, Sun L (2021) Lifelong exercise in age rats improves skeletal muscle function and MicroRNA profile. Med Sci Sports Exerc 53(9): 1873–1882. https://doi.org/10.1249/mss.0000000000002661

Gonzalez-Freire M, de Cabo R, Studenski SA, Ferrucci L (2014) The neuromuscular junction: aging at the crossroad between nerves and muscle. Front Aging Neurosci 6:208. https://doi.org/10.3389/fnagi.2014.00208

Goodpaster BH, Carlson CL, Visser M, Kelley DE, Scherzinger A, Harris TB, Stamm E, Newman AB (2001) Attenuation of skeletal muscle and strength in the elderly: the health ABC study. J Appl Physiol 90(6):2157–2165. https://doi.org/10.1152/jappl.2001.90.6.2157

Goodpaster BH, Chomentowski P, Ward BK, Rossi A, Glynn NW, Delmonico MJ, Kritchevsky SB, Pahor M, Newman AB (2008) Effects of physical activity on strength and skeletal muscle fat infiltration in older adults: a randomized controlled trial. J Appl Physiol 105(5):1498–1503. https://doi.org/10.1152/japplphysiol.90425.2008

Gorman GS, Chinnery PF, DiMauro S, Hirano M, Koga Y, McFarland R, Suomalainen A, Thorburn DR, Zeviani M, Turnbull DM (2016) Mitochondrial diseases. Nat Rev Dis Primers 2:16080. https://doi.org/10.1038/nrdp.2016.80

Ham DJ, Borsch A, Lin S, Thurkauf M, Weihrauch M, Reinhard JR, Delezie J, Battilana F, Wang X, Kaiser MS, Guridi M, Sinnreich M, Rich MM, Mittal N, Tintignac LA, Handschin C, Zavolan M, Ruegg MA (2020) The neuromuscular junction is a focal point of mTORC1 signaling in sarcopenia. Nat Commun 11(1):4510. https://doi.org/10.1038/s41467-020-18140-1

Henríquez-Olguin C, Knudsen JR (2019) Parkin the progression of sarcopenia. J Physiol 597(9): 2333–2334. https://doi.org/10.1113/jp277770

Herscovich S, Gershon D (1987) Effects of aging and physical training on the neuromuscular junction of the mouse. Gerontology 33(1):7–13

Hikida RS, Staron RS, Hagerman FC, Walsh S, Kaiser E, Shell S, Hervey S (2000) Effects of high-intensity resistance training on untrained older men. II. Muscle fiber characteristics and nucleo-cytoplasmic relationships. J Gerontol A Biol Sci Med Sci 55(7):B347–B354. https://doi.org/10.1093/gerona/55.7.b347

Hioki M, Kanehira N, Koike T, Saito A, Shimaoka K, Sakakibara H, Oshida Y, Akima H (2020) Age-related changes in muscle volume and intramuscular fat content in quadriceps femoris and hamstrings. Exp Gerontol 132:110834. https://doi.org/10.1016/j.exger.2020.110834

Huang Y, Zhu X, Chen K, Lang H, Zhang Y, Hou P, Ran L, Zhou M, Zheng J, Yi L, Mi M, Zhang Q (2019) Resveratrol prevents sarcopenic obesity by reversing mitochondrial dysfunction and oxidative stress via the PKA/LKB1/AMPK pathway. Aging (Albany NY) 11(8):2217–2240. https://doi.org/10.18632/aging.101910

Hughes DC, Marcotte GR, Marshall AG, West DWD, Baehr LM, Wallace MA, Saleh PM, Bodine SC, Baar K (2017) Age-related differences in dystrophin: impact on force transfer proteins, membrane integrity, and neuromuscular junction stability. J Gerontol A Biol Sci Med Sci 72(5): 640–648. https://doi.org/10.1093/gerona/glw109

Hurst C, Robinson SM, Witham MD, Dodds RM, Granic A, Buckland C, De Biase S, Finnegan S, Rochester L, Skelton DA, Sayer AA (2022) Resistance exercise as a treatment for sarcopenia: prescription and delivery. Age Ageing 51(2). https://doi.org/10.1093/ageing/afac003

Janssen I, Heymsfield SB, Baumgartner RN, Ross R (2000) Estimation of skeletal muscle mass by bioelectrical impedance analysis. J Appl Physiol 89(2):465–471. https://doi.org/10.1152/jappl.2000.89.2.465

Jin SM, Lazarou M, Wang C, Kane LA, Narendra DP, Youle RJ (2010) Mitochondrial membrane potential regulates PINK1 import and proteolytic destabilization by PARL. J Cell Biol 191(5): 933–942. https://doi.org/10.1083/jcb.201008084

Juel VC (2012) Evaluation of neuromuscular junction disorders in the electromyography laboratory. Neurol Clin 30(2):621–639. https://doi.org/10.1016/j.ncl.2011.12.012

Kemmler W, von Stengel S (2012) Alternative exercise technologies to fight against sarcopenia at old age: a series of studies and review. J Aging Res 2012:109013. https://doi.org/10.1155/2012/109013

Kim HK, Suzuki T, Saito K, Yoshida H, Kobayashi H, Kato H, Katayama M (2012) Effects of exercise and amino acid supplementation on body composition and physical function in community-dwelling elderly Japanese sarcopenic women: a randomized controlled trial. J Am Geriatr Soc 60(1):16–23. https://doi.org/10.1111/j.1532-5415.2011.03776.x

Kirk B, Al Saedi A, Duque G (2019) Osteosarcopenia: a case of geroscience. Aging Med (Milton) 2(3):147–156. https://doi.org/10.1002/agm2.12080

Kleele T, Rey T, Winter J, Zaganelli S, Mahecic D, Perreten Lambert H, Ruberto FP, Nemir M, Wai T, Pedrazzini T, Manley S (2021) Distinct fission signatures predict mitochondrial degradation or biogenesis. Nature 593(7859):435–439. https://doi.org/10.1038/s41586-021-03510-6

Kneffel Z, Murlasits Z, Reed J, Krieger J (2021) A meta-regression of the effects of resistance training frequency on muscular strength and hypertrophy in adults over 60 years of age. J Sports Sci 39(3):351–358. https://doi.org/10.1080/02640414.2020.1822595

Ko IG, Jeong JW, Kim YH, Jee YS, Kim SE, Kim SH, Jin JJ, Kim CJ, Chung KJ (2014) Aerobic exercise affects myostatin expression in aged rat skeletal muscles: a possibility of antiaging effects of aerobic exercise related with pelvic floor muscle and urethral rhabdosphincter. Int Neurourol J 18(2):77–85. https://doi.org/10.5213/inj.2014.18.2.77

Kreko-Pierce T, Eaton BA (2018) Rejuvenation of the aged neuromuscular junction by exercise. Cell Stress 2(2):25–33. https://doi.org/10.15698/cst2018.02.123

Kulakowski SA, Parker SD, Personius KE (2011) Reduced TrkB expression results in precocious age-like changes in neuromuscular structure, neurotransmission, and muscle function. J Appl Physiol 111(3):844–852

Landi F, Cesari M, Calvani R, Cherubini A, Di Bari M, Bejuit R, Mshid J, Andrieu S, Sinclair AJ, Sieber CC, Vellas B, Topinkova E, Strandberg T, Rodriguez-Manas L, Lattanzio F, Pahor M, Roubenoff R, Cruz-Jentoft AJ, Bernabei R, Marzetti E, SPRINTT Consortium (2017) The "Sarcopenia and Physical fRailty IN older people: multi-component Treatment strategies" (SPRINTT) randomized controlled trial: design and methods. Aging Clin Exp Res 29(1):89–100. https://doi.org/10.1007/s40520-016-0715-2

Landi F, Cruz-Jentoft AJ, Liperoti R, Russo A, Giovannini S, Tosato M, Capoluongo E, Bernabei R, Onder G (2013) Sarcopenia and mortality risk in frail older persons aged 80 years and older: results from ilSIRENTE study. Age Ageing 42(2):203–209. https://doi.org/10.1093/ageing/afs194

Landi F, Liperoti R, Russo A, Giovannini S, Tosato M, Capoluongo E, Bernabei R, Onder G (2012) Sarcopenia as a risk factor for falls in elderly individuals: results from the ilSIRENTE study. Clin Nutr 31(5):652–658. https://doi.org/10.1016/j.clnu.2012.02.007

Larsson L, Degens H, Li M, Salviati L, Lee YI, Thompson W, Kirkland JL, Sandri M (2019) Sarcopenia: aging-related loss of muscle mass and function. Physiol Rev 99(1):427–511. https://doi.org/10.1152/physrev.00061.2017

Laurentius T, Kob R, Fellner C, Nourbakhsh M, Bertsch T, Sieber CC, Bollheimer LC (2019) Long-chain fatty acids and inflammatory markers coaccumulate in the skeletal muscle of sarcopenic old rats. Dis Markers 2019:9140789. https://doi.org/10.1155/2019/9140789

Le NH, Kim CS, Park T, Park JH, Sung MK, Lee DG, Hong SM, Choe SY, Goto T, Kawada T, Yu R (2014) Quercetin protects against obesity-induced skeletal muscle inflammation and atrophy. Mediators Inflamm 2014:834294. https://doi.org/10.1155/2014/834294

Le NH, Kim CS, Tu TH, Kim BS, Park T, Park JHY, Goto T, Kawada T, Ha TY, Yu R (2017) Absence of 4-1BB reduces obesity-induced atrophic response in skeletal muscle. J Inflamm (Lond) 14:9. https://doi.org/10.1186/s12950-017-0156-5

Lee SR, Khamoui AV, Jo E, Park BS, Zourdos MC, Panton LB, Ormsbee MJ, Kim JS (2015) Effects of chronic high-fat feeding on skeletal muscle mass and function in middle-aged mice. Aging Clin Exp Res 27(4):403–411. https://doi.org/10.1007/s40520-015-0316-5

Leong DP, Teo KK, Rangarajan S, Lopez-Jaramillo P, Avezum A Jr, Orlandini A, Seron P, Ahmed SH, Rosengren A, Kelishadi R, Rahman O, Swaminathan S, Iqbal R, Gupta R, Lear SA, Oguz A, Yusoff K, Zatonska K, Chifamba J, Igumbor E, Mohan V, Anjana RM, Gu H, Li W, Yusuf S (2015) Prognostic value of grip strength: findings from the prospective urban rural epidemiology (PURE) study. Lancet 386(9990):266–273. https://doi.org/10.1016/S0140-6736(14)62000-6

Lexell J (1995) Human aging, muscle mass, and fiber type composition. J Gerontol A Biol Sci Med Sci. 50 Spec No:11-16. https://doi.org/10.1093/gerona/50a.special_issue.11

Liang J, Zhang H, Zeng Z, Wu L, Zhang Y, Guo Y, Lv J, Wang C, Fan J, Chen N (2021) Lifelong aerobic exercise alleviates sarcopenia by activating autophagy and inhibiting protein degradation via the AMPK/PGC-1α signaling pathway. Metabolites 11(5). https://doi.org/10.3390/metabo11050323

Lin CC, Shih MH, Chen CD, Yeh SL (2021) Effects of adequate dietary protein with whey protein, leucine, and vitamin D supplementation on sarcopenia in older adults: an open-label, parallel-group study. Clin Nutr 40(3):1323–1329. https://doi.org/10.1016/j.clnu.2020.08.017

Liu CJ, Latham NK (2009) Progressive resistance strength training for improving physical function in older adults. Cochrane Database Syst Rev 3:CD002759. https://doi.org/10.1002/14651858.CD002759.pub2

Liu D, Fan YB, Tao XH, Pan WL, Wu YX, Wang XH, He YQ, Xiao WF, Li YS (2021a) Mitochondrial quality control in sarcopenia: updated overview of mechanisms and interventions. Aging Dis 12(8):2016–2030. https://doi.org/10.14336/ad.2021.0427

Liu S, Yu C, Xie L, Niu Y, Fu L (2021b) Aerobic exercise improves mitochondrial function in sarcopenia mice through Sestrin2 in an AMPKα2-dependent manner. J Gerontol A Biol Sci Med Sci 76(7):1161–1168. https://doi.org/10.1093/gerona/glab029

Lovell DI, Cuneo R, Gass GC (2010) Can aerobic training improve muscle strength and power in older men? J Aging Phys Act 18(1):14–26. https://doi.org/10.1123/japa.18.1.14

Lukjanenko L, Brachat S, Pierrel E, Lach-Trifilieff E, Feige JN (2013) Genomic profiling reveals that transient adipogenic activation is a hallmark of mouse models of skeletal muscle regeneration. PLoS One 8(8):e71084. https://doi.org/10.1371/journal.pone.0071084

Macek P, Terek-Derszniak M, Biskup M, Krol H, Smok-Kalwat J, Gozdz S, Zak M (2020) Assessment of age-induced changes in body fat percentage and BMI aided by Bayesian modelling: a cross-sectional cohort study in middle-aged and older adults. Clin Interv Aging 15:2301–2311. https://doi.org/10.2147/CIA.S277171

Marcus RL, Addison O, Kidde JP, Dibble LE, Lastayo PC (2010) Skeletal muscle fat infiltration: impact of age, inactivity, and exercise. J Nutr Health Aging 14(5):362–366. https://doi.org/10.1007/s12603-010-0081-2

Marzetti E, Calvani R, Lorenzi M, Marini F, D'Angelo E, Martone AM, Celi M, Tosato M, Bernabei R, Landi F (2014) Serum levels of C-terminal agrin fragment (CAF) are associated with sarcopenia in older hip fractured patients. Exp Gerontol 60:79–82. https://doi.org/10.1016/j.exger.2014.10.003

Mascher H, Ekblom B, Rooyackers O, Blomstrand E (2011) Enhanced rates of muscle protein synthesis and elevated mTOR signalling following endurance exercise in human subjects. Acta Physiol (Oxf) 202(2):175–184. https://doi.org/10.1111/j.1748-1716.2011.02274.x

Mayhew AJ, Amog K, Phillips S, Parise G, McNicholas PD, de Souza RJ, Thabane L, Raina P (2019) The prevalence of sarcopenia in community-dwelling older adults, an exploration of differences between studies and within definitions: a systematic review and meta-analyses. Age Ageing 48(1):48–56. https://doi.org/10.1093/ageing/afy106

Moberg M, Apro W, Cervenka I, Ekblom B, van Hall G, Holmberg HC, Ruas JL, Blomstrand E (2021) High-intensity leg cycling alters the molecular response to resistance exercise in the arm muscles. Sci Rep 11(1):6453. https://doi.org/10.1038/s41598-021-85733-1

Mori H, Tokuda Y (2018) Effect of whey protein supplementation after resistance exercise on the muscle mass and physical function of healthy older women: a randomized controlled trial. Geriatr Gerontol Int 18(9):1398–1404. https://doi.org/10.1111/ggi.13499

Muhammad MH, Allam MM (2018) Resveratrol and/or exercise training counteract aging-associated decline of physical endurance in aged mice; targeting mitochondrial biogenesis and function. J Physiol Sci 68(5):681–688. https://doi.org/10.1007/s12576-017-0582-4

Murphy C (2008) The chemical senses and nutrition in older adults. J Nutr Elder 27(3–4):247–265. https://doi.org/10.1080/01639360802261862

Nabuco HCG, Tomeleri CM, Sugihara Junior P, Fernandes RR, Cavalcante EF, Antunes M, Ribeiro AS, Teixeira DC, Silva AM, Sardinha LB, Cyrino ES (2018) Effects of whey protein supplementation pre- or post-resistance training on muscle mass, muscular strength, and functional capacity in pre-conditioned older women: a randomized clinical trial. Nutrients 10(5). https://doi.org/10.3390/nu10050563

Nguyen MH, Cheng M, Koh TJ (2011) Impaired muscle regeneration in ob/ob and db/db mice. ScientificWorldJournal 11:1525–1535. https://doi.org/10.1100/tsw.2011.137

Ni HM, Williams JA, Ding WX (2015) Mitochondrial dynamics and mitochondrial quality control. Redox Biol 4:6–13. https://doi.org/10.1016/j.redox.2014.11.006

Nieuwenhuizen WF, Weenen H, Rigby P, Hetherington MM (2010) Older adults and patients in need of nutritional support: review of current treatment options and factors influencing nutritional intake. Clin Nutr 29(2):160–169. https://doi.org/10.1016/j.clnu.2009.09.003

Novotny SA, Warren GL, Hamrick MW (2015) Aging and the muscle-bone relationship. Physiology (Bethesda) 30(1):8–16. https://doi.org/10.1152/physiol.00033.2014

Nunan D, Mahtani KR, Roberts N, Heneghan C (2013) Physical activity for the prevention and treatment of major chronic disease: an overview of systematic reviews. Syst Rev 2:56. https://doi.org/10.1186/2046-4053-2-56

Oda K (1984) Age changes of motor innervation and acetylcholine receptor distribution on human skeletal muscle fibres. J Neurol Sci 66(2–3):327–338. https://doi.org/10.1016/0022-510x(84)90021-2

Onishi M, Yamano K, Sato M, Matsuda N, Okamoto K (2021) Molecular mechanisms and physiological functions of mitophagy. EMBO J 40(3):e104705. https://doi.org/10.15252/embj.2020104705

Osawa Y, Oguma Y, Ishii N (2013) The effects of whole-body vibration on muscle strength and power: a meta-analysis. J Musculoskelet Neuronal Interact 13(3):380–390

Pacifico J, Geerlings MAJ, Reijnierse EM, Phassouliotis C, Lim WK, Maier AB (2020) Prevalence of sarcopenia as a comorbid disease: a systematic review and meta-analysis. Exp Gerontol 131:110801. https://doi.org/10.1016/j.exger.2019.110801

Pagliuso A, Cossart P, Stavru F (2018) The ever-growing complexity of the mitochondrial fission machinery. Cell Mol Life Sci 75(3):355–374. https://doi.org/10.1007/s00018-017-2603-0

Papadopoulou SK, Tsintavis P, Potsaki P, Papandreou D (2020) Differences in the prevalence of sarcopenia in community-dwelling, nursing home and hospitalized individuals. A systematic review and meta-analysis. J Nutr Health Aging 24(1):83–90. https://doi.org/10.1007/s12603-019-1267-x

Pasiakos SM, Carbone JW (2014) Assessment of skeletal muscle proteolysis and the regulatory response to nutrition and exercise. IUBMB Life 66(7):478–484. https://doi.org/10.1002/iub.1291

Peel NM, Kuys SS, Klein K (2013) Gait speed as a measure in geriatric assessment in clinical settings: a systematic review. J Gerontol A Biol Sci Med Sci 68(1):39–46. https://doi.org/10.1093/gerona/gls174

Perkisas S, De Cock AM, Verhoeven V, Vandewoude M (2017) Intramuscular adipose tissue and the functional components of sarcopenia in hospitalized geriatric patients. Geriatrics (Basel) 2(1). https://doi.org/10.3390/geriatrics2010011

Perkisas S, Lamers S, Degerickx R, Van Mieghem E, Vandewoude M, Verhoeven V, De Cock A-M (2018) The relation between mortality, intramuscular adipose tissue and sarcopenia in hospitalized geriatric patients. Eur Geriatr Med 9(6):801–807. https://doi.org/10.1007/s41999-018-0110-y

Personius KE, Parker SD (2013) TrkB expression at the neuromuscular junction is reduced during aging. Muscle Nerve 47(4):532–538. https://doi.org/10.1002/mus.23616

Petermann-Rocha F, Balntzi V, Gray SR, Lara J, Ho FK, Pell JP, Celis-Morales C (2022) Global prevalence of sarcopenia and severe sarcopenia: a systematic review and meta-analysis. J Cachexia Sarcopenia Muscle 13(1):86–99. https://doi.org/10.1002/jcsm.12783

Peterson MD, Rhea MR, Sen A, Gordon PM (2010) Resistance exercise for muscular strength in older adults: a meta-analysis. Ageing Res Rev 9(3):226–237. https://doi.org/10.1016/j.arr.2010.03.004

Peterson MD, Sen A, Gordon PM (2011) Influence of resistance exercise on lean body mass in aging adults: a meta-analysis. Med Sci Sports Exerc 43(2):249–258. https://doi.org/10.1249/MSS.0b013e3181eb6265

Pickles S, Vigié P, Youle RJ (2018) Mitophagy and quality control mechanisms in mitochondrial maintenance. Curr Biol 28(4):R170–r185. https://doi.org/10.1016/j.cub.2018.01.004

Prokopidis K, Giannos P, Katsikas Triantafyllidis K, Kechagias KS, Mesinovic J, Witard OC, Scott D (2022) Effect of vitamin D monotherapy on indices of sarcopenia in community-dwelling older adults: a systematic review and meta-analysis. J Cachexia Sarcopenia Muscle. https://doi.org/10.1002/jcsm.12976

Ren Z, Lan Q, Chen Y, Chan YWJ, Mahady GB, Lee SM (2020) Low-magnitude high-frequency vibration decreases body weight gain and increases muscle strength by enhancing the p38 and AMPK pathways in db/db mice. Diabetes Metab Syndr Obes 13:979–989. https://doi.org/10.2147/DMSO.S228674

Rivas DA, McDonald DJ, Rice NP, Haran PH, Dolnikowski GG, Fielding RA (2016) Diminished anabolic signaling response to insulin induced by intramuscular lipid accumulation is associated with inflammation in aging but not obesity. Am J Physiol Regul Integr Comp Physiol 310(7):R561–R569. https://doi.org/10.1152/ajpregu.00198.2015

Rivas DA, Morris EP, Fielding RA (2011) Lipogenic regulators are elevated with age and chronic overload in rat skeletal muscle. Acta Physiol (Oxf) 202(4):691–701. https://doi.org/10.1111/j.1748-1716.2011.02289.x

Robinson SM, Reginster JY, Rizzoli R, Shaw SC, Kanis JA, Bautmans I, Bischoff-Ferrari H, Bruyere O, Cesari M, Dawson-Hughes B, Fielding RA, Kaufman JM, Landi F, Malafarina V, Rolland Y, van Loon LJ, Vellas B, Visser M, Cooper C, ESCEO Working Group (2018) Does nutrition play a role in the prevention and management of sarcopenia? Clin Nutr 37(4):1121–1132. https://doi.org/10.1016/j.clnu.2017.08.016

Roelants M, Delecluse C, Verschueren SM (2004) Whole-body-vibration training increases knee-extension strength and speed of movement in older women. J Am Geriatr Soc 52(6):901–908. https://doi.org/10.1111/j.1532-5415.2004.52256.x

Romanello V, Sandri M (2021) The connection between the dynamic remodeling of the mitochondrial network and the regulation of muscle mass. Cell Mol Life Sci 78(4):1305–1328. https://doi.org/10.1007/s00018-020-03662-0

Rondanelli M, Cereda E, Klersy C, Faliva MA, Peroni G, Nichetti M, Gasparri C, Iannello G, Spadaccini D, Infantino V, Caccialanza R, Perna S (2020) Improving rehabilitation in sarcopenia: a randomized-controlled trial utilizing a muscle-targeted food for special medical purposes. J Cachexia Sarcopenia Muscle 11(6):1535–1547. https://doi.org/10.1002/jcsm.12532

Rondanelli M, Klersy C, Terracol G, Talluri J, Maugeri R, Guido D, Faliva MA, Solerte BS, Fioravanti M, Lukaski H, Perna S (2016) Whey protein, amino acids, and vitamin D supplementation with physical activity increases fat-free mass and strength, functionality, and quality of life and decreases inflammation in sarcopenic elderly. Am J Clin Nutr 103(3):830–840. https://doi.org/10.3945/ajcn.115.113357

Sanz-Paris A, Camprubi-Robles M, Lopez-Pedrosa JM, Pereira SL, Rueda R, Ballesteros-Pomar MD, Garcia Almeida JM, Cruz-Jentoft AJ (2018) Role of oral nutritional supplements enriched with beta-hydroxy-beta-methylbutyrate in maintaining muscle function and improving clinical outcomes in various clinical settings. J Nutr Health Aging 22(6):664–675. https://doi.org/10.1007/s12603-018-0995-7

Scuteri A, Lattanzio F, Bernabei R (2016) Life-course approach to chronic disease: the active and healthy aging perspective. J Am Geriatr Soc 64(9):e59–e61. https://doi.org/10.1111/jgs.14271

Sebastián D, Sorianello E, Segalés J, Irazoki A, Ruiz-Bonilla V, Sala D, Planet E, Berenguer-Llergo A, Muñoz JP, Sánchez-Feutrie M, Plana N, Hernández-Álvarez MI, Serrano AL, Palacín M, Zorzano A (2016) Mfn2 deficiency links age-related sarcopenia and impaired autophagy to activation of an adaptive mitophagy pathway. EMBO J 35(15):1677–1693. https://doi.org/10.15252/embj.201593084

Smith DO, Chapman MR (1987) Acetylcholine receptor binding properties at the rat neuromuscular junction during aging. J Neurochem 48(6):1834–1841

Smith DO, Williams KD, Emmerling M (1990) Changes in acetylcholine receptor distribution and binding properties at the neuromuscular junction during aging. Int J Dev Neurosci 8(6): 629–642. https://doi.org/10.1016/0736-5748(90)90058-a

Soendenbroe C, Heisterberg MF, Schjerling P, Karlsen A, Kjaer M, Andersen JL, Mackey AL (2019) Molecular indicators of denervation in aging human skeletal muscle. Muscle Nerve 60(4):453–463. https://doi.org/10.1002/mus.26638

Song J, Herrmann JM, Becker T (2021) Quality control of the mitochondrial proteome. Nat Rev Mol Cell Biol 22(1):54–70. https://doi.org/10.1038/s41580-020-00300-2

Studenski S, Perera S, Patel K, Rosano C, Faulkner K, Inzitari M, Brach J, Chandler J, Cawthon P, Connor EB, Nevitt M, Visser M, Kritchevsky S, Badinelli S, Harris T, Newman AB, Cauley J, Ferrucci L, Guralnik J (2011) Gait speed and survival in older adults. JAMA 305(1):50–58. https://doi.org/10.1001/jama.2010.1923

Studenski SA, Peters KW, Alley DE, Cawthon PM, McLean RR, Harris TB, Ferrucci L, Guralnik JM, Fragala MS, Kenny AM, Kiel DP, Kritchevsky SB, Shardell MD, Dam TT, Vassileva MT (2014) The FNIH sarcopenia project: rationale, study description, conference recommendations, and final estimates. J Gerontol A Biol Sci Med Sci 69(5):547–558. https://doi.org/10.1093/gerona/glu010

Sylow L, Tokarz VL, Richter EA, Klip A (2021) The many actions of insulin in skeletal muscle, the paramount tissue determining glycemia. Cell Metab 33(4):758–780. https://doi.org/10.1016/j.cmet.2021.03.020

Takeshima N, Rogers ME, Islam MM, Yamauchi T, Watanabe E, Okada A (2004) Effect of concurrent aerobic and resistance circuit exercise training on fitness in older adults. Eur J Appl Physiol 93(1–2):173–182. https://doi.org/10.1007/s00421-004-1193-3

Tamaki T, Hirata M, Uchiyama Y (2014) Qualitative alteration of peripheral motor system begins prior to appearance of typical sarcopenia syndrome in middle-aged rats. Front Aging Neurosci 6:296. https://doi.org/10.3389/fnagi.2014.00296

Tanimoto Y, Watanabe M, Sun W, Tanimoto K, Shishikura K, Sugiura Y, Kusabiraki T, Kono K (2013) Association of sarcopenia with functional decline in community-dwelling elderly subjects in Japan. Geriatr Gerontol Int 13(4):958–963. https://doi.org/10.1111/ggi.12037

Tardif N, Salles J, Guillet C, Tordjman J, Reggio S, Landrier JF, Giraudet C, Patrac V, Bertrand-Michel J, Migne C, Collin ML, Chardigny JM, Boirie Y, Walrand S (2014) Muscle ectopic fat deposition contributes to anabolic resistance in obese sarcopenic old rats through eIF2alpha activation. Aging Cell 13(6):1001–1011. https://doi.org/10.1111/acel.12263

Tazawa R, Uchida K, Fujimaki H, Miyagi M, Inoue G, Sekiguchi H, Murata K, Takata K, Kawakubo A, Takaso M (2019) Elevated leptin levels induce inflammation through IL-6 in skeletal muscle of aged female rats. BMC Musculoskelet Disord 20(1):199. https://doi.org/10.1186/s12891-019-2581-5

Thomson JS, Watson PE, Rowlands DS (2009) Effects of nine weeks of beta-hydroxy-beta-methylbutyrate supplementation on strength and body composition in resistance trained men. J Strength Cond Res 23(3):827–835. https://doi.org/10.1519/JSC.0b013e3181a00d47

Troesch B, Eggersdorfer M, Laviano A, Rolland Y, Smith AD, Warnke I, Weimann A, Calder PC (2020) Expert opinion on benefits of long-chain Omega-3 fatty acids (DHA and EPA) in aging and clinical nutrition. Nutrients 12(9). https://doi.org/10.3390/nu12092555

Tumasian RA, Harish A, Kundu G, Yang J-H, Ubaida-Mohien C, Gonzalez-Freire M, Kaileh M, Zukley LM, Chia CW, Lyashkov A, Wood WH, Piao Y, Coletta C, Ding J, Gorospe M, Sen R, De S, Ferrucci L (2021) Skeletal muscle transcriptome in healthy aging. Nat Commun 12(1). https://doi.org/10.1038/s41467-021-22168-2

Turcotte LP, Fisher JS (2008) Skeletal muscle insulin resistance: roles of fatty acid metabolism and exercise. Phys Ther 88(11):1279–1296. https://doi.org/10.2522/ptj.20080018

United Nations, Department of Economic and Social Affairs, Population Division (2019) World Population Ageing 2019: Highlights. (ST/ESA/SERA/430)

Vellas B, Fielding RA, Bens C, Bernabei R, Cawthon PM, Cederholm T, Cruz-Jentoft AJ, Del Signore S, Donahue S, Morley J, Pahor M, Reginster JY, Rodriguez Manas L, Rolland Y, Roubenoff R, Sinclair A, Cesari M (2018) Implications of ICD-10 for sarcopenia clinical practice and clinical trials: report by the international conference on frailty and sarcopenia research task force. J Frailty Aging 7(1):2–9. https://doi.org/10.14283/jfa.2017.30

Verschueren SM, Roelants M, Delecluse C, Swinnen S, Vanderschueren D, Boonen S (2004) Effect of 6-month whole body vibration training on hip density, muscle strength, and postural control in postmenopausal women: a randomized controlled pilot study. J Bone Miner Res Off J Am Soc Bone Miner Res 19(3):352–359. https://doi.org/10.1359/JBMR.0301245

Visser M, Goodpaster BH, Kritchevsky SB, Newman AB, Nevitt M, Rubin SM, Simonsick EM, Harris TB (2005) Muscle mass, muscle strength, and muscle fat infiltration as predictors of incident mobility limitations in well-functioning older persons. J Gerontol A Biol Sci Med Sci 60(3):324–333. https://doi.org/10.1093/gerona/60.3.324

Walston JD (2012) Sarcopenia in older adults. Curr Opin Rheumatol 24(6):623–627. https://doi.org/10.1097/BOR.0b013e328358d59b

Watanabe K, Holobar A, Tomita A, Mita Y (2020) Effect of milk fat globule membrane supplementation on motor unit adaptation following resistance training in older adults. Physiol Rep 8(12):e14491. https://doi.org/10.14814/phy2.14491

Waters DL (2019) Intermuscular adipose tissue: a brief review of etiology, association with physical function and weight loss in older adults. Ann Geriatr Med Res 23(1):3–8. https://doi.org/10.4235/agmr.19.0001

Wenz T (2009) PGC-1alpha activation as a therapeutic approach in mitochondrial disease. IUBMB Life 61(11):1051–1062. https://doi.org/10.1002/iub.261

White Z, Terrill J, White RB, McMahon C, Sheard P, Grounds MD, Shavlakadze T (2016) Voluntary resistance wheel exercise from mid-life prevents sarcopenia and increases markers of mitochondrial function and autophagy in muscles of old male and female C57BL/6J mice. Skelet Muscle 6(1):45. https://doi.org/10.1186/s13395-016-0117-3

Willadt S, Nash M, Slater CR (2016) Age-related fragmentation of the motor endplate is not associated with impaired neuromuscular transmission in the mouse diaphragm. Sci Rep 6

Wilson JM, Fitschen PJ, Campbell B, Wilson GJ, Zanchi N, Taylor L, Wilborn C, Kalman DS, Stout JR, Hoffman JR, Ziegenfuss TN, Lopez HL, Kreider RB, Smith-Ryan AE, Antonio J (2013) International Society of Sports Nutrition Position Stand: beta-hydroxy-beta-methylbutyrate (HMB). J Int Soc Sports Nutr 10(1):6. https://doi.org/10.1186/1550-2783-10-6

Wokke JH, Jennekens FG, van den Oord CJ, Veldman H, Smit LM, Leppink GJ (1990) Morphological changes in the human end plate with age. J Neurol Sci 95(3):291–310. https://doi.org/10.1016/0022-510x(90)90076-y

Wolfe RR, Miller SL, Miller KB (2008) Optimal protein intake in the elderly. Clin Nutr 27(5):675–684. https://doi.org/10.1016/j.clnu.2008.06.008

Woo J, Leung J, Morley JE (2015) Defining sarcopenia in terms of incident adverse outcomes. J Am Med Dir Assoc 16(3):247–252. https://doi.org/10.1016/j.jamda.2014.11.013

Wrighton PJ, Shwartz A, Heo JM, Quenzer ED, LaBella KA, Harper JW, Goessling W (2021) Quantitative intravital imaging in zebrafish reveals in vivo dynamics of physiological-stress-induced mitophagy. J Cell Sci 134(4). https://doi.org/10.1242/jcs.256255

Wu S, Ning HT, Xiao SM, Hu MY, Wu XY, Deng HW, Feng H (2020) Effects of vibration therapy on muscle mass, muscle strength and physical function in older adults with sarcopenia: a systematic review and meta-analysis. Eur Rev Aging Phys Act 17:14. https://doi.org/10.1186/s11556-020-00247-5

Xie F, Zhang F, Min S, Chen J, Yang J, Wang X (2018) Glial cell line-derived neurotrophic factor (GDNF) attenuates the peripheral neuromuscular dysfunction without inhibiting the activation of spinal microglia/monocyte. BMC Geriatr 18(1):110

Xie WQ, He M, Yu DJ, Wu YX, Wang XH, Lv S, Xiao WF, Li YS (2021) Mouse models of sarcopenia: classification and evaluation. J Cachexia Sarcopenia Muscle. https://doi.org/10.1002/jcsm.12709

Xu Z, You W, Chen W, Zhou Y, Nong Q, Valencak TG, Wang Y, Shan T (2021) Single-cell RNA sequencing and lipidomics reveal cell and lipid dynamics of fat infiltration in skeletal muscle. J Cachexia Sarcopenia Muscle 12(1):109–129. https://doi.org/10.1002/jcsm.12643

Yada E, Yamanouchi K, Nishihara M (2006) Adipogenic potential of satellite cells from distinct skeletal muscle origins in the rat. J Vet Med Sci 68(5):479–486. https://doi.org/10.1292/jvms.68.479

Yamada Y, Nishizawa M, Uchiyama T, Kasahara Y, Shindo M, Miyachi M, Tanaka S (2017) Developing and validating an age-independent equation using multi-frequency bioelectrical impedance analysis for estimation of appendicular skeletal muscle mass and establishing a cutoff for sarcopenia. Int J Environ Res Public Health 14(7). https://doi.org/10.3390/ijerph14070809

Yuan R, Peters LL, Paigen B (2011) Mice as a mammalian model for research on the genetics of aging. ILAR J 52(1):4–15. https://doi.org/10.1093/ilar.52.1.4

Zeng Z, Liang J, Wu L, Zhang H, Lv J, Chen N (2020) Exercise-induced autophagy suppresses sarcopenia through Akt/mTOR and Akt/FoxO3a signal pathways and AMPK-mediated mitochondrial quality control. Front Physiol 11:583478. https://doi.org/10.3389/fphys.2020.583478

Zhao K, Shen C, Li L, Wu H, Xing G, Dong Z, Jing H, Chen W, Zhang H, Tan Z, Pan J, Xiong L, Wang H, Cui W, Sun XD, Li S, Huang X, Xiong WC, Mei L (2018) Sarcoglycan alpha mitigates neuromuscular junction decline in aged mice by stabilizing LRP4. J Neurosci 38(41):8860–8873

Zhu S, Tian Z, Torigoe D, Zhao J, Xie P, Sugizaki T, Sato M, Horiguchi H, Terada K, Kadomatsu T, Miyata K, Oike Y (2019) Aging- and obesity-related peri-muscular adipose tissue accelerates muscle atrophy. PLoS One 14(8):e0221366. https://doi.org/10.1371/journal.pone.0221366

Zoico E, Corzato F, Bambace C, Rossi AP, Micciolo R, Cinti S, Harris TB, Zamboni M (2013) Myosteatosis and myofibrosis: relationship with aging, inflammation and insulin resistance. Arch Gerontol Geriatr 57(3):411–416. https://doi.org/10.1016/j.archger.2013.06.001

Chapter 7
Tendon Aging

Ana Luísa Graça, Manuel Gomez-Florit, Manuela Estima Gomes, and Denitsa Docheva

Abstract Tendons are mechanosensitive connective tissues responsible for the connection between muscles and bones by transmitting forces that allow the movement of the body, yet, with advancing age, tendons become more prone to degeneration followed by injuries. Tendon diseases are one of the main causes of incapacity worldwide, leading to changes in tendon composition, structure, and biomechanical properties, as well as a decline in regenerative potential. There is still a great lack of knowledge regarding tendon cellular and molecular biology, interplay between biochemistry and biomechanics, and the complex pathomechanisms involved in tendon diseases. Consequently, this reflects a huge need for basic and clinical research to better elucidate the nature of healthy tendon tissue and also tendon aging process and associated diseases. This chapter concisely describes the effects that the aging process has on tendons at the tissue, cellular, and molecular levels and briefly reviews potential biological predictors of tendon aging. Recent research findings that are herein reviewed and discussed might contribute to the development of precision tendon therapies targeting the elderly population.

Keywords Aging · Tendon tissue · Tendon cells · Tendinopathy · Biomarkers · Immune system

A. L. Graça · M. E. Gomes
3B's Research Group, I3Bs—Research Institute on Biomaterials, Biodegradables and Biomimetics, University of Minho, Headquarters of the European Institute of Excellence on Tissue Engineering and Regenerative Medicine, Guimarães, Portugal

ICVS/3B's–PT Government Associate Laboratory, Braga/Guimarães, Portugal
e-mail: ana.graca@i3bs.uminho.pt; megomes@i3bs.uminho.pt

M. Gomez-Florit
Health Research Institute of the Balearic Islands (IdISBa), Palma de Mallorca, Spain
e-mail: manuel.gomezflorit@ssib.es

D. Docheva (✉)
Department of Musculoskeletal Tissue Regeneration, Orthopaedic Hospital König-Ludwig-Haus, University of Würzburg, Würzburg, Germany
e-mail: d-docheva.klh@uni-wuerzburg.de

J. R. Harris, V. I. Korolchuk (eds.), *Biochemistry and Cell Biology of Ageing: Part IV, Clinical Science*, Subcellular Biochemistry 103,
https://doi.org/10.1007/978-3-031-26576-1_7

Introduction

This chapter was designed as a concise overview on the tendon aging process and prospective analysis of possible therapies. Firstly, we provide a short summary on the biology of aging, tendon tissue structure, and its cellular and biochemical composition. Then, we focus on the main age-related alterations in tendon structure, biomechanical properties, tendon cells, and changes in the tendon microenvironment. Subsequently, we discuss the relation between aging and tendon diseases. Lastly, we briefly review and discuss some recent research studies that can lead to better future therapies for the management of tendon aging/disease.

Aging Hallmarks

Aging is defined as an overall decline in the functional capacity of various organs to maintain tissue homeostasis accompanied by a diminished regeneration response (Sahin and DePinho 2010). The process of cellular and organismal aging is complex and mainly influenced by genetic factors, but as well as by external factors such as obesity, diabetes, mutagens, like alcohol or tobacco smoke, or intrinsic stresses including reactive oxygen species (ROS) and telomere erosion (Sharpless and DePinho 2007). Diverse studies have indicated that the reduced regenerative potential of adult tissues is linked to a functional decline of the stem and progenitor cell pools, which has been associated with human aging and age-associated diseases such as osteoporosis, tendon disorders, sarcopenia, anemia, dementia, cancer, or impaired wound healing (Rando 2006; Sahin and DePinho 2010). Thus, it is assumed that aging is partly driven by an age-associated decline in the number and repair capacity of tissue-specific adult stem and progenitor cells (Sharpless and DePinho 2007).

In general, cellular aging has been postulated to be linked to cellular senescence, which in turn is triggered by epigenetic alterations such as DNA methylation and histone modifications, and through DNA damage accumulation caused via ROS (Sharpless and DePinho 2007), and telomere erosion leading to the activation of the $p14^{ARF}$ and $p16^{INK4A}$ tumor suppressor pathways.

The two tumor suppressors $p14^{ARF}$ and $p16^{INK4A}$ are encoded by the cyclin-dependent kinase inhibitor 2A (CDKN2A) locus on chromosome 9p21. On the one hand, $p14^{ARF}$ sequesters the p53-specific ubiquitin ligase human double minute 2 protein (HDM2) and prevents p53 degradation. On the other hand, $p16^{INK4A}$ sequesters cyclin-dependent kinase 4 (CDK4) and 6 (CDK6), which keeps retinoblastoma protein (pRb) in a hypo-phosphorylated active state, thereby arresting the cell cycle in G_1 (Chudnovsky et al. 2005; Hirschi et al. 2010).

Telomere erosion also contributes to age-associated declines in the adult stem cell pool (Ju et al. 2007). Moreover, it is assumed that aging is not purely an intracellular process but is also linked to interactions with the cellular microenvironment.

Independently of telomere shortening, emerging evidence suggests that changes in cellular niches, which keep adult stem cells in a quiescent state, strongly contribute to the regulation of replicative senescence (Wilson et al. 2008).

In summary, distinct molecular and cellular alterations accumulate slowly during aging resulting in degeneration and atrophy, paralleled by reduction in functional properties as well as homeostatic and repair capacity of tissues and organs.

Tendon Tissues

Tendon Structure and Composition

Tendons connect muscles to bones, through the myotendinous and osteotendinous (enthesis) junctions, respectively, and thereby ensure joint movements (Sharma and Maffulli 2008). Tendons have been subclassified into energy storing tendons, which store and return energy during locomotion, such as the Achilles tendon, and positional tendons, which help in maintaining the position of certain joints. Ligaments interconnect bone to bone and exhibit a similar molecular and cellular composition to tendons. Tendons are dense connective tissues and are predominantly composed of parallel, closely packed collagen fibers and cells within well-ordered extracellular matrix (ECM). The basic tissue structure of tendons consists of collagen type I (COL I) that is arranged in hierarchical levels of complexity and constitutes 65–80% of the tendon dry mass. There are also 2–3% of other collagen types such as collagen type II (COL II) in the cartilaginous zones and collagen type X (COL X) in the mineralized fibrocartilage zone of the enthesis, collagen type III (COL III) in reticular fibers of blood vessels, collagen type IV (COL IV) in capillary membranes, collagen type V (COL V) in vascular membranes, and collagen types XII, XIV, and XV (COL XII, COL XIV, COL XV) as fibril-associated collagens (Jósza et al. 1993; Fukuta et al. 1998; Fukushige et al. 2005). The smallest collagen unit is tropocollagen, consisting of a triple-helix polypeptide containing two α1 chains and one α2 chain, which are first synthesized inside the tendon cell and then secreted into the ECM. There, the triple helix self-assembles via intermolecular enzymatic (lysyl oxidase (LOX) enzyme activity) and nonenzymatic (formation of advanced glycation end-products—AGEs) cross-links into parallel organized collagen fibrils responsible for the crimp and wave-like appearance of the tendon ECM. Fibrils in turn are bundled mainly longitudinally into collagen fibers, sub-fascicles (primary bundle), fascicles (secondary bundle), tertiary bundles, and the tendon itself. Each tendon fascicle is surrounded by a thin reticular network of connective tissue—the endotenon (or interfascicular matrix, IFM). Tertiary bundles as well as the whole tendon are covered by a fine, loose connective tissue sheath—the epitenon ensuring vascular, lymphatic, and nerve supply (Fig. 7.1a) (Hulmes 2002; James et al. 2008; Sharma and Maffulli 2008).

The ground substance of the ECM in tendons, surrounding the collagen and tendon cells, is composed of 1–5% proteoglycans and glycoproteins, 2% elastin

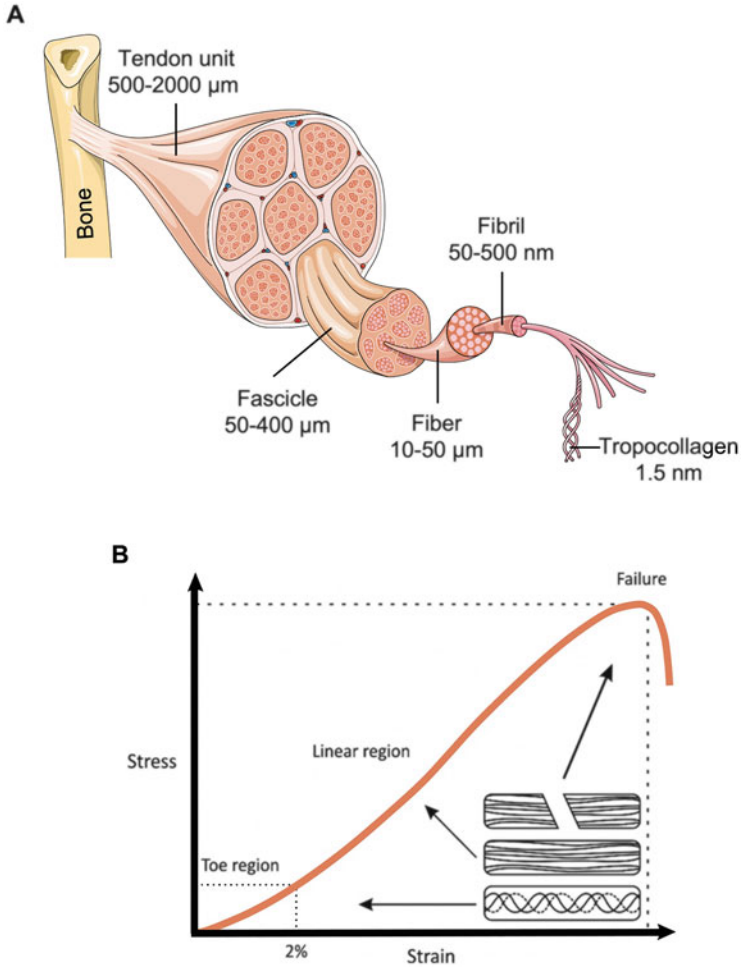


Fig. 7.1 (**a**) Hierarchical structure of tendon tissue. Tendon unit, fascicle, fiber, fibril, and tropocollagen are depicted. Adapted from Docheva et al. (2015) using mindthegraph.com software. (**b**) Tendon mechanical behavior when load is applied to the tissue. Upon increasing load, the tendon tissue experience deformation in three stages: first, toe region (crimp extension); next, linear region (microdamage); and last failure (complete rupture), from which the stress and strain can be determined. Adapted from Robi et al. (2013)

(ELN), and 0.2% inorganic molecules, including copper, manganese, and calcium (Lin et al. 2004). Via their glycosaminoglycan (GAG) side chains, proteoglycans bind to the collagen fibrils to interconnect the fibrils in a parallel alignment and to ensure gliding of collagen fibrils during locomotion. They also enable rapid diffusion of water-soluble molecules and the migration of cells. Major GAG components of the tendon are dermatan and chondroitin sulfates (Zhang et al. 2006; Sharma and Maffulli 2008). The tendon ECM contains several proteoglycans and glycoproteins

such as tenomodulin (TNMD), tenascin C (TNC), cartilage oligo matrix protein (COMP), decorin (DCN), fibromodulin (FMOD), biglycan (BGN), and lumican (LUM). Among these, TNMD, a type II transmembrane glycoprotein, which contains a highly conserved C-terminal cysteine-rich domain that after cleavage is co-localized with COL I fibrils into the tendon ECM, is a marker of mature tenocytes (Docheva et al. 2005). The glycoprotein TCN, a member of the tenascin gene family, is abundantly found in the ECM of developing vertebrate embryos, interacts with fibronectin (FN), and binds to integrins and ECM components such as collagens (Jones and Jones 2000; Pajala et al. 2009). The pentameric, non-collagenous ECM-protein COMP belongs to the thrombospondin family of extracellular calcium-binding proteins. It consists of a five-stranded coiled coil including five identical glycoprotein subunits, and its three-dimensional (3D) structure is stabilized by disulfide bonds. COMP plays a catalytic role in the assembly of the tendon ECM by binding to collagens (Paulsson and Heinegård 1981; Rock et al. 2010). DCN, FMOD, BGN, and LUM belong to the small leucine-rich proteoglycan family and consist of a protein core containing leucine repeats with varying GAG chains. DCN is known to bind directly to COL I fibrils and has been implicated in the lateral collagen fibrillogenesis (Yoon and Halper 2005). Similarly, FMOD, BGN, and LUM bind to COL I fibrils, thus participating in the lateral collagen fibrillogenesis in the tendon (Svensson et al. 2000; Rees et al. 2009).

Tendon tissue formation and homeostasis depend on a combination of transcriptions factors such as Scleraxis (SCX), Mohawk (MKX), and Early growth response protein 1 and 2 (EGR1, EGR2). SCX, a member of the basic-helix-loop-helix (bHLH) superfamily of transcription factors expressed in tendon lineage cells, is an early marker of tendon development, positively regulating COL I and TNMD expression (Bagchi and Czubryt 2011). Likewise, MKX, a member of the three amino acid loop extension (TALE) superclass of atypical homeobox genes, also regulates COL I production in tendon cells (Ito et al. 2010). Furthermore, EGR1/2, two zinc finger proteins, act as mechano-sensing molecules during tendon maturation, and are markers of mature tenocytes (Guerquin et al. 2013; Liu et al. 2014; Caceres et al. 2018).

Tendon Vasculature

The blood supply of tendons is assured by vessels originating from three different sites: the myotendinous junction, the enthesis, and the paratenon. At the myotendinous junction, the muscle provides the tendon with blood vessels running down to the proximal third of the tendon (Kvist et al. 1995). The sparse blood supply from the enthesis is restricted to the insertion site (Carr and Norris 1989). The main vascularization of the tendon is provided by the tendon sheets, where a vascular network penetrates deep into the tendon and reaches the endotenon sheets (Reynolds and Worrell 1991; Zantop et al. 2003). Nevertheless, since tendon tissues are predominantly composed of ECM showing a low metabolic rate of endogenous

cells, their vascularity and healing capability is much inferior compared to many other tissues (Jósza et al. 1993; Milz et al. 2004; Benjamin et al. 2008).

Tendon Biomechanical Properties

Tendons transmit the force generated in the muscle to the bone allowing body movements. The force transmission depends on the tendon biomechanical properties such as elasticity and tensile strength, which in turn are related to the collagen fiber diameter and orientation. Collagen fibrils and proteoglycans are differently involved in the mechanical properties: collagen fibrils allow tendons to resist tensile stress, whereas proteoglycans withstand compressive stress and enable gliding. Moreover, the crimped configuration of the collagen fibrils contributes to elasticity and compressive resistance (Ker 2002; Sharma and Maffulli 2008). The viscoelastic behavior of the tendon, generally, is characterized by stress relaxation and creep under maintained strain or stress, respectively (Fig. 7.1b). The crimped, wavy configuration of collagen fibrils disappears when the tendon is stretched up to 2%. If the strain remains between 2 and 4%, the tendon keeps its viscoelastic behavior and can return to its original length and crimp pattern after stress release. If the strain exceeds 4%, fibers are damaged on microscopic level, and a strain beyond 8–10% causes intrafibril damage leading to tendon rupture (James et al. 2008; Sharma and Maffulli 2008). The viscoelastic behavior of the tendon provides a high buffer capacity against longitudinal, transversal, horizontal, and rotational forces. Mechanical forces, generated during movements, are transmitted by focal adhesions including integrin receptors and cell–cell adherent junctions. Tendon cells transduce the physical force-induced signals into biochemical responses, a process called mechano-transduction, by inducing the mitogen-activated protein (MAP) kinase pathways such as the extracellular signal-related kinase (ERK1/2), protein kinase 38 (p38), and c-Jun-N-terminal kinase (JNK) pathways (Franchi et al. 2007a, b; James et al. 2008). In turn, kinase-dependent downstream signaling is activated resulting in mechano-adaptation of the cells.

Tendon Cell Types

Tendon cells such as tenoblasts and tenocytes constitute 90–95% of the cellular elements and are responsible for the deposition and remodeling of the tendon ECM (Fig. 7.2a). Tenoblasts are progenitor, spindle-shaped cells with high metabolic activity that are located primarily in the IFM; they are suggested to be an heterogeneous population (Thorpe and Screen 2016), which plays significant roles in tissue repair (Spiesz et al. 2015). During the tendon maturation process, the tenoblasts transform into tenocytes, which have a lower abundance (10-fold lower) as well as metabolic activity (Sharma and Maffulli 2008). Tenocytes are the terminally differentiated, specialized spindle-shaped fibroblastic cells with long membranous protrusions. They are organized in parallel rows within the longitudinally oriented

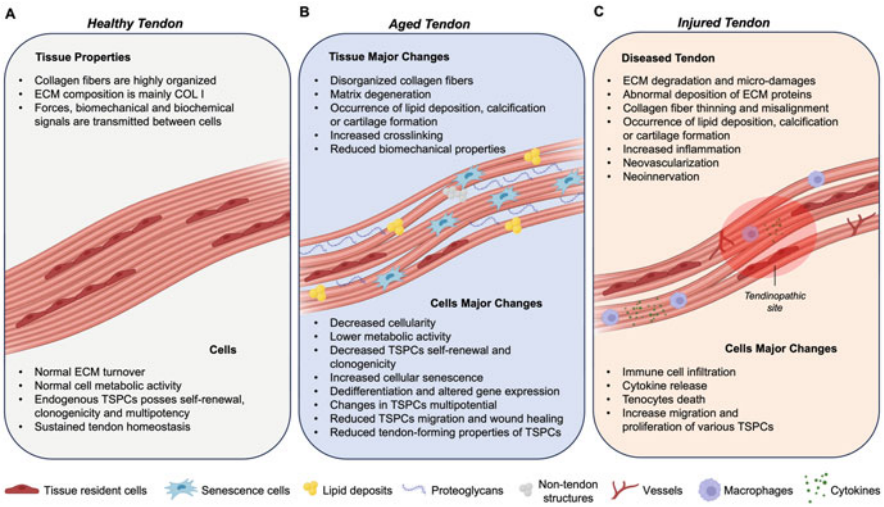


Fig. 7.2 Cartoon depicting the main biological features of (**a**) healthy, (**b**) aged, and (**c**) diseased tendon tissue. Adapted from (Kohler et al. 2013) using BioRender.com software

collagen fibers. The cell protrusions are connected via gap junctions among the tenocytes in a 3D cellular network through the tissue. The cell-to-cell communication allows for appropriate and synchronized response to mechanical loading and external and internal biochemical signals (Chuen et al. 2004; Grinstein and Galloway 2018). Both tenoblasts and tenocytes synthesize collagen as well as other ECM components and are capable of protein catabolizing. Thereby, they contribute to ECM assembly and remodeling (Jósza et al. 1993; Sharma and Maffulli 2008). The remaining 5–10% of cells include chondrocytes at the enthesis site, synovial cells in the tendon sheath (Sharma and Maffulli 2008), endothelial cells, pericytes, and immune and fat cells (Kendal et al. 2020; Akbar et al. 2021). The ratio between the different cell types seems to be dynamic, varying in healthy and diseased human tendon (Kendal et al. 2020; Akbar et al. 2021).

Bi and colleagues identified within human hamstring tendons containing a rare population of residing tendon stem/progenitor cells (TSPCs) (Bi et al. 2007). They showed that isolated TSPCs exhibit classical stem cell criteria such as self-renewal, clonogenicity, and the capability to differentiate into more specialized cell types such as adipocytes, osteoblasts, and chondrocytes (Bi et al. 2007). Since then, TSPCs have been described in various tendons from different species (Schneider and Docheva 2017). They can be found in the tendon fascicles, in the paratenon/epitenon, and in the vascularized tendon area (Walia and Huang 2019; Huang et al. 2021). The TSPCs present in these three main regions are distinguished by the different markers they express (Runesson et al. 2013). TSPCs from tendon fascicles express bone marrow stromal cell (BMSC) markers such as CD73, CD90.2, and CD105, as well as stem cell antigen 1 (SCA-1), CD44, SCX, TNC, and TNMD (Bi et al. 2007; Mienaltowski et al. 2013). On the other hand, the TSPCs in the

paratenon/epitenon area are characterized by tubulin polymerization-promotion protein family member 3 (TPP3) (Harvey et al. 2019), laminin, alpha-smooth muscle actin (αSMA), platelet-derived growth factor receptor α (PDGFRα) (Mienaltowski et al. 2014; Walia and Huang 2019), and osteocalcin (OCN) expression (Wang et al. 2017a). The TSPCs of the perivascular region express CD146, CD133, endomucin (EMCN), musashi-1 (MSI1) (Tempfer et al. 2009), and p75 neurotrophin receptor (Xu et al. 2015) and co-express αSMA (Walia and Huang 2019). In addition, Yin et al. (2016) showed that the perivascular subpopulation of TSPCs expresses nestin (NES) and high levels of tendon-related genes and exhibits elevated tenogenic potential. However, so far, there is still limited understanding of factors that regulate the tendon stem cell niche formation and maintenance. Nevertheless, the discovery of TSPCs, which fulfill the adult stem cell criteria and possess regenerative capabilities, provided new insights into tendon cell biology as well as opened new strategies for the treatment of injured or aged tendons.

Characteristics of Aged Tendon Tissue

The transition from immature (embryonic and early postnatal) to mature tendon tissue is characterized by marked changes in its structure, biomechanical properties, and the cellular and molecular composition (Figs. 7.2 and 7.3) (Lacroix et al. 2013; Magnusson and Kjaer 2019). However, the discrete changes in tendons during aging

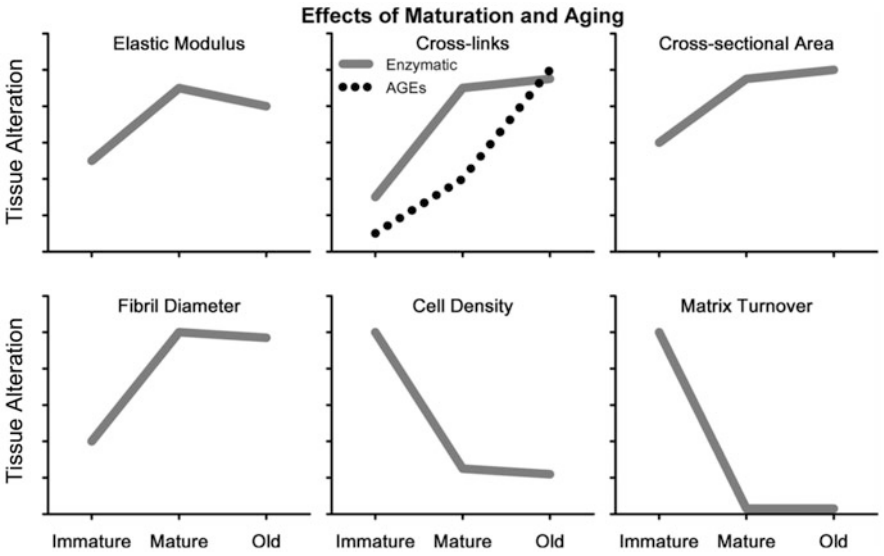


Fig. 7.3 Schematic representation of tendon alterations over time. Adapted from (Svensson et al. 2016)

are largely unknown due to the aging process complexity as well as the nonexistence of suitable age-related biomarkers for tendon tissues. There is also a lack of animal models that mimic human longevity, size to mass ratio, and the onset and progression of the multifaceted degenerative changes known to contribute to human tendon pathology. Moreover, the use of human tendon tissues is limited as they are difficult to obtain for ethical and surgical reasons. Still, human and horses share some structural and age-related degenerative characteristics, which makes the equine tendon a useful model. Tendon age-related changes have been shown to increase the susceptibility to tendon degeneration, injury, and re-injuries, as well as the risk of treatment failure and long-term rehabilitation process. In addition, with aging there is a great reduction in the regenerative capacity of the tissue (addressed in the following section). Since data from small animal models do not provide a coherent picture of the age-related changes in human tendon, in this section, we will mainly focus on data derived from human and equine studies.

Effects of Aging on Tendon Structure and Mechanical Properties

At the structural level, it is accepted that aging does not induce significant loss of tendon tissue, since only minor changes in tendon cross-sectional area (CSA) are observed in human studies (Magnusson et al. 2003; Carroll et al. 2008; Couppé et al. 2009, 2014; Stenroth et al. 2012). Regarding tendon composition, the total collagen content and collagen fibril diameter remain largely unaltered with aging in human models (Couppé et al. 2014). On the other hand, aging has been shown to induce a decrease in proteoglycans, at least in some tendons (Ippolito et al. 1980; Torricelli et al. 2013), and in elastin content and organization, in the endotenon compartment of horse energy storing tendons (Godinho et al. 2017).

The main change related to aging of human tendons is a significantly increased nonenzymatic cross-linking, resulting from the accumulation of AGEs throughout life (Couppé et al. 2009; Heinemeier et al. 2013b; Lacroix et al. 2013). In addition, Thorpe et al. (2016) analyzed aged equine tendons and reported that the rate of protein turnover is decreased especially in the IFM. AGEs also likely contribute to the loss of water with age observed in the tendon (Carroll et al. 2008), since cross-links cause dehydration of collagen. The lower proteoglycan content observed in aged horses (Ippolito et al. 1980; Torricelli et al. 2013) also contributes to a reduction in water content. In addition, different human studies reported a decrease of vascularity and associated blood flow in the elderly compared with younger subjects (Funakoshi et al. 2010). Moreover, fat and cartilage formation as well as calcification was reported in aged tendons, which compromise tendon structure and function (Zhang and Wang 2015; Gehwolf et al. 2016; Wood and Brooks 2016; Zaseck et al. 2016). Nonetheless, aged tenocytes are able to remain metabolically active and actively remodel the ECM in response to mechanical stimulus (Heinemeier et al. 2013a).

As a consequence of the above changes, tendon mechanical properties (elastic modulus and strength) in aged human tendons decline especially in energy storing tendons (Carroll et al. 2008; Couppé et al. 2009, 2014; Stenroth et al. 2012; Thorpe et al. 2017; Quinlan et al. 2018), resulting in a diminished tendon function, which is in agreement with most of the findings in animal studies. Turan et al. demonstrated that aged human Achilles tendons were stiffer (less elastic) when compared to young tendons (Turan et al. 2015). Also, Hsiao et al. showed that aged human patellar tendons had lower elastic modulus and shear wave velocity in comparison with young tendons (Hsiao et al. 2015). More recently, age-related alterations to tendon structure have been shown to decrease its ability to withstand fatigue loading, resulting from localized areas of stiffening within the endotenon, rather than a decrease of mechanical properties of the gross structure, which was probably caused by improper repair of microdamages (Thorpe et al. 2017). Nevertheless, some in vivo human studies have reported unchanged mechanical properties (modulus) with aging (Carroll et al. 2008; Couppé et al. 2009). Some of these discrepancies might be related to the decreased strain applied to tendon as a consequence of a lower force placed on the tissue due to age-related loss of muscle mass and strength (sarcopenia) (Doherty 2003). Hence, it is of great importance to match old and young specimens for activity levels when evaluating tendon mechanical performance. Furthermore, research evidence has suggested that tendon aging also causes loss of distal myelinated sensory fibers and decrease in mechanoreceptor numbers accompanied with morphology changes (Aydoğ et al. 2006). Table 7.1 summarizes the effects of the aging process on human and animal tendon tissue characteristics.

Age-Related Changes in Tendon Cells

Studies evaluating changes in tendon cells, their numbers, and functions in humans are limited. Instead, animal studies have shown that aging induces a decline in tendon cell density in rabbits and rats, whereas in horses there is no clear decline in cell density with age (Zs-Nagy et al. 1969; Nakagawa et al. 1996; Thorpe et al. 2015).

A cell type that has been largely studied due to its easier accessibility are mesenchymal stromal cells (MSCs) isolated from bone marrow (BM). These cells exhibit age-dependent changes in clonogenicity, proliferative capacity, and differentiation potential, an observation consistent among different species (Baxter et al. 2004; Stolzing et al. 2008; Kasper et al. 2009; Zhou et al. 2010; Yu et al. 2011). Stolzing et al. demonstrated that BM-MSCs derived from human young individuals expanded more rapidly and had a longer lifespan in vitro compared to human MSCs from elderly patients (Stolzing et al. 2008). MSCs isolated from BM of the young, middle, and older rhesus monkeys exhibited a decreased proliferation and two-lineage differentiation capacity with aging (Yu et al. 2011). Interestingly, Zhou and colleagues compared cells isolated from tendon tissue of young and aged rats and also revealed a decline in clonogenicity, proliferation rate, and

Table 7.1 Changes in tendon tissue over the aging process

Structural changes	Species	Biomechanical changes	References
Change in gross appearance Yellowish color Disrupted collagen fiber structure Accumulation of proteoglycan/glycosaminoglycan Mucoid and lipoid accumulation	Human	Not assessed	(Adams et al. 1974; Kannus and Józsa 1991)
Decrease in water and mucopolysaccharide content	Rabbit	Change in stiffness and reduction of tendon gliding properties	(Ippolito et al. 1980; Tuite et al. 1997)
Reduction of collagen turnover and synthesis Increased presence of COL III Delayed repair	Equine Human	Impaired adaptive response to mechanical loading	(Birch et al. 1999; Kjaer et al. 2009; Peffers et al. 2014)
Decrease of ELN and proteoglycans in tendon ECM	Rat	Reduced tissue elasticity Increased tendon stiffness	(Kostrominova and Brooks 2013)
Increased collagen cross-linking	Rabbit Human Rat	Loss of mechanical competence Decrease in ultimate strain Changes in ultimate load modulus and elasticity Increased stiffness	(Carlstedt 1987; Thermann et al. 1995; Lewis and Shaw 1997; Pardes et al. 2017)
Thickening of collagen fibril diameter	Mice Equine	Increased stiffening and elastic modulus	(Gehwolf et al. 2016; Ribitsch et al. 2020)
Increased cross-sectional tendon area	Human	Variation in tendon stiffness and Young's modulus	(Stenroth et al. 2012)
Loss of waviness Uncrimped collagen fibrils Disintegrated and frayed collagen bundles	Equine Mice	Impaired energy storage and release Loss of tissue integrity and diminished biomechanical properties	(Thorpe et al. 2014; Zuskov et al. 2020)
Enlarging of COL II-containing region from the enthesis to the tendon mid-portion and accumulation of calcium deposits	Mice Human	Detriment of mechanical and viscoelastic properties	(Rooney et al. 1993; Kawashiri et al. 2021)

chondrogenic potential; however, osteogenic and adipogenic differentiation capacities were unaffected (Zhou et al. 2010). In a recent study, using the self-assembly cell sheet model for tendon differentiation, it has been demonstrated that aged TSPCs are in fact less competent in forming tendon organoids, which were characterized by higher failure rate, smaller diameters, poorer ECM composition and quality, and reduced cell density due to increased cell death (Yan et al. 2020). In

vitro studies of aged human tendon-derived cells and TSPCs also reported significantly decreased proliferative, metabolic, and migratory capacity (Klatte-Schulz et al. 2012; Kohler et al. 2013). Consequently, the early entry into the growth plateau phase, the flattened cell morphology, and the reduced self-renewability of aged tendon cells and TSPCs suggested that these cells experience an early onset of cellular senescence, a phenomenon validated by increased activity of β-galactosidase and higher expression levels of the cell cycle regulator gene p16, both being hallmarks of senescence (Collado et al. 2007; Hoare et al. 2010; Kohler et al. 2013). Hence, the decrease of cell proliferation capacity and the increase in cellular senescence are among the best features to describe the aging process in tendons (Fig. 7.2b) (Tsai et al. 2011; Kohler et al. 2013; Hu et al. 2017).

Senescence is the phenomenon when cells lose their ability to divide and enter a growth arrest state. This also occurs in vitro after approximately 55 cell divisions, which is also known as Hayflick's limit or replicative senescence (Hermann et al. 2004). Cellular senescence is linked to organismal and tissue aging, and the underlying complex process is triggered by several partially known mechanisms. A number of studies have reported increased levels of senescence-associated genes such as $p14^{ARF}$, $p16^{INK4A}$, $p21^{WAF1}$, p53, and pRb in MSCs derived from aged BM aspirates (Shibata et al. 2007; Stolzing et al. 2008; Wagner et al. 2010). Janzen et al. created a $p16^{INK4A}$ knockout mice model and showed that hematopoietic cells (HSCs) of aged mutant mice exhibited diminished proliferation and apoptosis rates as well as improved stress tolerance (Janzen et al. 2006). Therefore, on the one hand, senescence prevents the proliferation of damaged cells, acting as a tumor suppressor mechanism. However, on the other hand, the $p14^{ARF}$ and $p16^{INK4A}$ pathways possess pro-aging effects which are linked to a reduced regeneration potential of adult stem cells accompanied by an increasing accumulation of senescent cells in the tissue over time (Sharpless and DePinho 2007). Senescent cells have been shown to exert side effects on neighboring cells and contribute to an inflammatory profile, which originated the term "inflammageing" (Franceschi et al. 2000).

Tendon healing involves the migration of tendon cells to the repair or damaged site. Subsequently, the cells begin to proliferate and synthesize collagen, proteoglycans, and other proteins, as well as they remodel the ECM ensuring tissue continuity at the injury site. However, the repaired tissue usually does not regain the characteristics of normal tendons, especially in aged people whose healing rates are retarded (James et al. 2008). Aged TSPCs have been reported to display reduced migratory activity along with increased content of actin stress fibers (Ross et al. 2011; Wei et al. 2011; Kohler et al. 2013). A lower migration potential of different cell types due to aging was stated in numerous studies (Sandeman et al. 2000; Xia et al. 2001; Mogford et al. 2002; Ruiz-Torres et al. 2003; Mishima and Lotz 2008; Sopko et al. 2010; Ross et al. 2011). Cell migration is a complex process that involves cell–matrix interaction and profound actin cytoskeleton reorganization. The actin cytoskeleton is composed of actin filaments which in turn are attached to the ECM proteins via focal adhesions. Actin stress fibers are composed of linear polymers of actin subunits that are elongated at one end and simultaneously shrunk at the other end. This dynamical process of polymerization and depolymerization of

actin stress fibers along with the assembly and disassembly of focal adhesions enables the cell to migrate (Wei et al. 2011). So far, literature indicates that cells derived from aged donors have a lower migratory capacity than cells derived from young donors which are probably induced by an altered turnover of actin fibers. Indeed, Kohler et al. (2013) demonstrated that human aged TSPCs not only have larger size filled with robust stress fibers, but also that the acting turnover is significantly lower. Actin dynamics are critical to cell migration but also to cell division and differentiation processes. Thus, actin-related alterations might be a central problem in tendon aging.

Beyond accumulation of senescence and decline in migratory properties, the expression of stemness cell markers, octamer-binding transcription factor 4 (OCT-4), nucleostemin (NS), SCA-1, and stage-specific embryonic antigen-1 (SSEA-1), in TSPCs reduces with aging and compromises the TSPC multipotential (Zhang and Wang 2015). By implementing microarray technology, Kohler et al. (2013) investigated the transcriptomal shift in human TSPCs with aging, revealing the differential expression of multiple genes. Interestingly, gene ontology and literature annotation aproaches showed that genes with a significant change in their mRNA expression levels relate to cell–cell and cell–ECM communication, cytoskeleton and actin dynamics, and motility and migration. Similarly, Peffers and colleagues have shown that the transcriptome of macroscopically normal human Achilles tendon is dynamically altered with age (Peffers et al. 2015). It was found that tendon aging does not result in affected expression of ECM-encoding genes. However, the authors identified differentially expressed gene sets with aging related to a dysregulation of cellular function and maintenance, cellular growth, and cellular development. Therefore, the above studies suggest that the cellular component of tendon tissues may lose the ability to respond appropriately to mechanical and chemical signals.

In Table 7.2, the main characteristics that tendon cells present during the progress of the aging process are described.

Aging and Tendon Diseases

Although aging and tendon diseases share common mechanisms, there are some molecular and cellular differences that distinguish these processes. Tendon disease can occur at earlier age, while aging is not a disease but a significant risk factor for development of many major chronic diseases (Hopkins et al. 2016; Riel et al. 2019).

In contrast to aging, tendon diseases, representing 30–50% of musculoskeletal-related clinical visits (Vos et al. 2017), are medical conditions including inflammation and degeneration as well as ruptures and overuse injuries. Tendinopathy is the broader term encompassing tendinitis that is tendon inflammation and pain, and tendinosis that is inter-tendinous degeneration without an evidence of robust inflammatory processes (Fig. 7.2c) (Mazzone and McCue 2002; Zafar et al. 2009). Tendon diseases cause patient disability and reduced work productivity, representing a significant economic burden (Sleeswijk Visser et al. 2021). Tendinopathy and

Table 7.2 Most common features of aged tendon cells

Cellular changes	Species	Gene changes	References
Decreased cell proliferation capacity Increased cellular senescence	Rat Human	Low CITED2 mRNA and protein levels	(Zhou et al. 2010; Hu et al. 2017; Yan et al. 2020)
Lower proliferation rates Increased number of cells in cycle arrest Higher fraction of cells in G2/M phase Impaired multipotency Reduced tenocyte but increased adipocyte differentiation	Rat	Lower expression levels of CD90.1 and higher levels of CD44	(Zhou et al. 2010)
Decreased population doubling rate and response to growth factors Lower colony numbers Increased senescence and β-galactosidase activity Enlarged cell area and robust actin stress fibers Slower actin turnover Reduced migratory ability Retained multipotential	Human	Upregulation of $p16^{INKA}$ Altered expression of multiple genes Augmented ROCK protein levels and activity	(Kohler et al. 2013)
Altered cell morphology Cells with few protrusions Increased cell volume Change in actin cytoskeleton organization Increased cellular stiffness	Rat Human	Not assessed	(Wu et al. 2015; Kiderlen et al. 2019)
Reduction of self-renewal and proliferation G1/S phase cell cycle arrest Increased senescence Lesser migration	Rat	Downregulation of Forkhead box P1 (FOXP1), E2F1, cyclin D1, and pRb protein levels	(Xu and Liu 2018)
Increased senescence	Human	Increased expression of Pin1	(Chen et al. 2015)

rupture can happen in all age groups, in physically active youths and adults, and among the sedentary population with moderate physical activity. Next to sports, several intrinsic factors including body weight, nutrition, and age may be responsible for tendon damage. For detailed information, readers are addressed to a comprehensive review on tendinopathy by Millar et al. (2021).

At present, common conservative treatments for tendinopathies include immobilization, physiotherapy, shockwave-, ultrasound-, and magnetic-based therapies, the administration of steroidal and nonsteroidal anti-inflammatory drugs, and injection of platelet-rich plasma (Leadbetter 2005; Mishra et al. 2009; Lomas et al. 2015;

Zhou and Wang 2016; Aicale et al. 2020). Among these options, platelet-rich therapies are being used increasingly in the treatment of injuries of musculoskeletal soft tissues, including tendon (Costa-Almeida et al. 2020). Theoretically, platelet-rich content in growth factors and other scaffolding proteins should support healing and regeneration of tendon. However, systematic reviews of clinical trials have not confirmed the significant efficacy of platelet-rich plasma in the management of tendinopathies (De Vos et al. 2014; Liddle and Rodríguez-Merchán 2015; Scott et al. 2019). In severe cases, when the conservative approaches fail, surgery is also a therapeutic option, although a recent systematic review of clinical trials found that it was as good as exercise-based therapies in mid- and long-term pain reduction and in quality of life in patients (Challoumas et al. 2019). However, surgery can prevail conservative therapy in terms of time for return to function and can reduce the risk of chronic rupture. Tendon treatment often requires long periods of rehabilitation, while the original biological properties and mechanical strengths are rarely restored, and often result in chronic pain (Millar et al. 2021). Despite the great incidence of tendinopathies in the worldwide population, the currently available clinical strategies do not tackle the etiology of the disease which increases the susceptibility to acute tendon rupture.

Beyond the different above-mentioned pathophysiological features that lead to the tendon aging progression, the unbalance of immune cell populations, the cell-to-cell communication, and the cell–matrix interactions have also crucial roles in tendon diseases and rupture (Kuhn and Tuan 2010). Tendon pain, edema, and inflammation represent the immediate response to tendon micro- and macrodamage (Bianchi et al. 2021). The initial inflammatory stage begins with the formation of a hematoma. Inflammatory cells, such as neutrophils and macrophages, are attracted to the tendon injury site by pro-inflammatory cytokines. It has been suggested that resident tenocytes have the capacity to trigger the inflammatory response, potentially through the recognition of damage-associated molecular patterns (DAMPs) (Millar et al. 2017). A high macrophage number accompanied by hypervascularization as well as erroneous ECM deposition was identified in the early phase of tendon repair (Lin et al. 2017). Imbalanced and activated immune cells, primed by both endothelial and tendon cells, promote a cycle of inflammation and aberrant tissue repair that drives disease chronicity rather than regeneration (Akbar et al. 2021). Due to this failed regenerative response, the second hallmark of tendinopathies is fibrosis, which is characterized by the exacerbated accumulation of ECM in a highly disorganized matter (Wynn and Ramalingam 2012).

Another characteristic of diseased tendon microenvironments is hypervascularization of tendon ECM, in comparison to the poorly vascularized healthy tendons (Tempfer and Traweger 2015). The subsequent increase in oxygen and nutrients supply has been shown to promote degenerative ECM remodeling within the tissue stroma (Wunderli et al. 2020). Nevertheless, newly formed blood vessels harbor high numbers of perivascular cells, which hold a subpopulation of TSPCs (Tempfer et al. 2009). The neovascularization process is mainly governed by vascular endothelial growth factor (VEGF) signaling, the negligible levels of which found in healthy tendon show a significant increase during disease (Liu et al. 2021).

VEGF signaling is critical for blood and lymph vessel formation, although it can also elicit responses in other cells (Liu et al. 2021). Among these, tendon cells express VEGF receptors in response to inflammatory stimulation and injury, and binding with its ligand promotes tendon degeneration-associated events (Tempfer et al. 2022). Moreover, TNMD has also been suggested to play a role in vasculature-related processes in tendon. In healthy conditions, TNMD expressed by tendon-resident cells exhibits antiangiogenic properties, when released in a secreted form, indicating a crucial role in maintaining a low vascularized state of tendon tissue (Oshima et al. 2004). Further, TNMD has recently been demonstrated to limit the formation of a fibrovascular scar during early events in tendon healing (Lin et al. 2017) and ameliorate in-tendinous heterotopic ossification at the later repair stages (Delgado Caceres et al. 2021). Even though neovascularization is indispensable during the regeneration of the majority of tissues, including tendon, it may also exert a negative role in tendinopathy (Tempfer and Traweger 2015; Korntner et al. 2019).

A number of molecular studies have advanced the understanding that underlines tendon degenerative process, which often goes hand in hand with aging (Jones and Jones 2000). The major molecular changes include a shift to a higher COL III abundance in relation to COL I in the ECM, as well as higher levels of FN, TNC, GAGs, aggrecan (ACAN), and BGN (Riley 2008). There are also changes in the activity of various metalloproteinases (MMPs) and tissue inhibitors of metalloproteinases (TIMPs), contributing to a weaker tendon ECM (Riley 2008; Sharma and Maffulli 2008). In tendon ruptures, it has been reported that levels of metalloproteinase-1, -9, -19, and -25 (MMP1, MMP9, MMP19, MMP25) and tissue inhibitor factor-1 (TIMP1) were increased, while the expression of metalloproteinase-3 and -7 (MMP3, MMP7) and tissue inhibitor-2, -3, and -4 (TIMP2, TIMP3, TIMP4) were decreased (Jones and Jones 2000; Riley 2008). Tendinopathy also involves an increase of inflammatory mediators such as prostaglandin E2 (PGE2) and interleukin -1 (IL-1), an enhanced expression of cyclooxygenase 2 (COX2), different growth factors (GF), including transforming growth factor beta (TGF-β), platelet-derived growth factor (PDGF), and insulin-like growth factor-1 (IGF-1), and neurotransmitters, such as glutamate and substance B (Riley 2008; Sharma and Maffulli 2008). It is important to state that the above data are based largely on experimental studies, and despite all the advancements in tendon cell and molecular biology research, there is still a complete lack of diagnostic biomarkers for tendon diseases, including age-related tendinopathies.

Future Perspectives

Tendons composition, hierarchical design, and anisotropy make it a unique structure with exceptional mechanical properties. The aging process results in a failure to sustain tendon homeostasis, which may result in functional changes and/or disease. Furthermore, the reduced ability of tendons to self-repair and the limits of the

currently available therapeutics have increased the interest in developing therapeutic options that promote the tendon's endogenous reparative ability. In this sense, the field of tissue engineering and regenerative medicine can bring promising strategies to promote in situ tissue regeneration or even tissue rejuvenation, and slow or prevent aging and age-associated diseases. The field typically relies in a combination of cells, materials, and biochemical and biomechanical stimulation.

Cell-based strategies, obtained either from tendons or from non-tendon sources, like bone marrow- or adipose tissue-derived cells, have shown promising results to enhance tendon regeneration and improve the function of impacted tendons (Costa-Almeida et al. 2019; Migliorini et al. 2020). Nonetheless, the best cell source, isolation, expansion, and differentiation methods, characterization of the tenogenic differentiation pathways, and clarifications of tendon-specific molecular markers have not been consensual. Moreover, the exact mechanisms underlying the cellular mode of action are yet to be fully elucidated. Recent studies have proposed that extracellular vesicles secreted by the cells can exert regenerative effects (Marote et al. 2016; Riazifar et al. 2017; Witwer et al. 2019; Graça et al. 2022). Thus, it will be of great interest to investigate whether cell- and nanovesicle-based strategies can not only support regeneration but also combat aging and associated diseases.

Cell secretome and cellular crosstalk have a critical function in tendon aging (Gomez-Florit et al. 2022). Thus, the successful identification of specific biomolecules will benefit the development of tendon therapeutics and open new perspectives for translational medicine both in diagnostics and in therapy. In the last decade, a number of studies for enhancing tendon regeneration have focused on applying growth factors, singly or in combination (reviewed in Docheva et al. 2015; Schneider et al. 2018). However, the clinical translation of growth factor-based therapeutics has numerous limitations due to safety and cost-effectiveness issues. Aspects such as short half-life, low protein stability, and rapid deactivation of their specialized properties by enzymes at body temperature represent a major constraint for their extended use in clinical applications (Wang et al. 2017b; Caballero Aguilar et al. 2019). An interesting class of biomolecules that hold promising potential are senomorphics and senolytics, which specifically target senescent cells and help their clearing in the tissue, thus reducing the detrimental side effects of senescence and at the same time mitigate the aging process progression (Lagoumtzi and Chondrogianni 2021).

The use of biomaterials and bioengineering tools to promote tendon regeneration has also attracted considerable interest among the research community. Different techniques, including cell sheets (Yan et al. 2018; Vinhas et al. 2021), collagen structures (Puetzer et al. 2021), fiber-based approaches (Laranjeira et al. 2017; Almeida et al. 2019), and bioprinting (Merceron et al. 2015), have been proposed. Among these, fibrous materials fabricated using different techniques, such as electrospinning, wet spinning, or melt electrowriting (No et al. 2020; Rinoldi et al. 2021), have a high aptitude for mimicking fibrous tissue architecture and anisotropy.

Another trend in tendon regenerative biology is magnetically assisted therapies. These therapies take advantage of the magnetic field alone or in combination with magnetically responsive biomaterials. The magnetic stimulation can modulate

tendon cell adhesion, migration, proliferation, and differentiation acting as guides for promoting tissue regeneration (Pesqueira et al. 2018). However, it has to be considered that such approaches will most likely be adequate to treat aged-related tendinosis or subsequent rupture rather than the aging process itself.

Concluding Remarks

A variety of advanced research studies have revealed that tendon aging results from a combination of multiple changes occurring at all levels of the tissue—molecular, cellular, structural, and biomechanical. Central players are endogenous tissue cells, which accumulate pathological changes such as gene expression shift, reduced self-renewability, increased senescence, altered differentiation, hampered migratory, and overall regenerative potential. Thus, the tissues experience disbalance over time that can further progress in degenerative processes and even tendon rupture. Future research is still much needed to clarify the exact molecular and cellular mechanisms and to identify biomarkers and early diagnostic tools. Moreover, the development of efficient and multifunctional approaches that encounter the needs for tendon regeneration in young and elderly patients should also be pursued from the scientific community.

Acknowledgements The authors acknowledge Dr. Julia Kohler. Parts of this book chapter were grounded in her doctoral thesis "Phenotypic and transcriptional alterations in tendon stem/progenitor cells during the process of ageing," 2012. The authors also thank the H2020-WIDESPREAD-05-2017-Twinning Grant ACHILLES No. 810850. D.D. acknowledges EU MSCA-COFUND-DP Grant OSTASKILLS No. 101034412 and BMBF Grant CellWiTaL No. 13N15874. A.L.G. and M.E.G. acknowledge Fundação para a Ciência e a Tecnologia (FCT) for PhD grants PD/59/2013, PD/BD/135255/2017, and COVID/BD/152017/2021. M.G.F. acknowledges Carlos III Health Institute and the EU through the European Social Fund Plus for the Miguel Servet contract CP21/00136.

References

Adams CWM, Bayliss OB, Baker RWR et al (1974) Lipid deposits in ageing human arteries, tendons and fascia. Atherosclerosis 19:429–440. https://doi.org/10.1016/S0021-9150(74)80007-9

Aicale R, Bisaccia RD, Oliviero A et al (2020) Current pharmacological approaches to the treatment of tendinopathy. Expert Opin Pharmacother 21:1467–1477. https://doi.org/10.1080/14656566.2020.1763306

Akbar M, MacDonald L, Crowe LAN et al (2021) Single cell and spatial transcriptomics in human tendon disease indicate dysregulated immune homeostasis. Ann Rheum Dis 80:1494–1497. https://doi.org/10.1136/annrheumdis-2021-220256

Almeida H, Domingues RMA, Mithieux SM et al (2019) Tropoelastin-coated tendon biomimetic scaffolds promote stem cell tenogenic commitment and deposition of elastin-rich matrix. ACS Appl Mater Interfaces 11:19830–19840. https://doi.org/10.1021/acsami.9b04616

Aydoğ ST, Korkusuz P, Doral MN et al (2006) Decrease in the numbers of mechanoreceptors in rabbit ACL: the effects of ageing. Knee Surg Sports Traumatol Arthrosc 14:325–329. https://doi.org/10.1007/s00167-005-0673-2

Bagchi RA, Czubryt MP (2011) Scleraxis: a new regulator of extracellular matrix formation. In: Genes and cardiovascular function, pp 57–65

Baxter MA, Wynn RF, Jowitt SN et al (2004) Study of telomere length reveals rapid ageing of human marrow stromal cells following in vitro expansion. Stem Cells 22:675–682. https://doi.org/10.1634/stemcells.22-5-675

Benjamin M, Kaiser E, Milz S (2008) Structure-function relationships in tendons: a review. J Anat 212:211–228. https://doi.org/10.1111/j.1469-7580.2008.00864.x

Bi Y, Ehirchiou D, Kilts TM et al (2007) Identification of tendon stem/progenitor cells and the role of the extracellular matrix in their niche. Nat Med 13:1219–1227. https://doi.org/10.1038/nm1630

Bianchi E, Ruggeri M, Rossi S et al (2021) Innovative strategies in tendon tissue engineering. Pharmaceutics 13:89. https://doi.org/10.3390/pharmaceutics13010089

Birch HL, Bailey JVB, Bailey AJ, Goodship AE (1999) Age-related changes to the molecular and cellular components of equine flexor tendons. Equine Vet J 31:391–396. https://doi.org/10.1111/j.2042-3306.1999.tb03838.x

Caballero Aguilar LM, Silva SM, Moulton SE (2019) Growth factor delivery: defining the next generation platforms for tissue engineering. J Control Release 306:40–58. https://doi.org/10.1016/j.jconrel.2019.05.028

Caceres MD, Pfeifer CG, Docheva D (2018) Understanding tendons: lessons from transgenic mouse models. Stem Cells Dev 27:1161–1174. https://doi.org/10.1089/scd.2018.0121

Carlstedt CA (1987) Mechanical and chemical factors in tendon healing: effects of indomethacin and surgery in the rabbit. Acta Orthop 58:1–75. https://doi.org/10.3109/17453678709154163

Carr AJ, Norris SH (1989) The blood supply of the calcaneal tendon. J Bone Joint Surg Br 71:100–101. https://doi.org/10.1302/0301-620x.71b1.2914976

Carroll CC, Dickinson JM, Haus JM et al (2008) Influence of ageing on the in vivo properties of human patellar tendon. J Appl Physiol 105:1907–1915. https://doi.org/10.1152/japplphysiol.00059.2008

Challoumas D, Clifford C, Kirwan P, Millar NL (2019) How does surgery compare to sham surgery or physiotherapy as a treatment for tendinopathy? A systematic review of randomised trials. BMJ Open Sport Exerc Med 5:1–15. https://doi.org/10.1136/bmjsem-2019-000528

Chen L, Liu J, Tao X et al (2015) The role of Pin1 protein in ageing of human tendon stem/progenitor cells. Biochem Biophys Res Commun 464:487–492. https://doi.org/10.1016/j.bbrc.2015.06.163

Chudnovsky Y, Khavari PA, Adams AE (2005) Melanoma genetics and the development of rational therapeutics. J Clin Invest 115:813–824. https://doi.org/10.1172/jci24808

Chuen FS, Chuk CY, Ping WY et al (2004) Immunohistochemical characterization of cells in adult human patellar tendons. J Histochem Cytochem 52:1151–1157. https://doi.org/10.1369/jhc.3A6232.2004

Collado M, Blasco MA, Serrano M (2007) Cellular senescence in cancer and ageing. Cell 130:223–233. https://doi.org/10.1016/j.cell.2007.07.003

Costa-Almeida R, Babo PS, Reis RL, Gomes ME (2020) Platelet-rich blood derivatives for tendon regeneration. J Am Acad Orthop Surg 28:e202–e205. https://doi.org/10.5435/JAAOS-D-18-00638

Costa-Almeida R, Calejo I, Gomes ME (2019) Mesenchymal stem cells empowering tendon regenerative therapies. Int J Mol Sci 20. https://doi.org/10.3390/ijms20123002

Couppé C, Hansen P, Kongsgaard M et al (2009) Mechanical properties and collagen cross-linking of the patellar tendon in old and young men. J Appl Physiol 107:880–886. https://doi.org/10.1152/japplphysiol.00291.2009

Couppé C, Svensson RB, Grosset JF et al (2014) Life-long endurance running is associated with reduced glycation and mechanical stress in connective tissue. Age (Omaha) 36. https://doi.org/10.1007/s11357-014-9665-9

De Vos RJ, Windt J, Weir A (2014) Strong evidence against platelet-rich plasma injections for chronic lateral epicondylar tendinopathy: a systematic review. Br J Sports Med 48:952–956. https://doi.org/10.1136/bjsports-2013-093281

Delgado Caceres M, Angerpointner K, Galler M et al (2021) Tenomodulin knockout mice exhibit worse late healing outcomes with augmented trauma-induced heterotopic ossification of Achilles tendon. Cell Death Dis 12. https://doi.org/10.1038/s41419-021-04298-z

Docheva D, Hunziker EB, Fassler R, Brandau O (2005) Tenomodulin is necessary for tenocyte proliferation and tendon maturation. Mol Cell Biol 25:699–705. https://doi.org/10.1128/mcb.25.2.699-705.2005

Docheva D, Müller SA, Majewski M, Evans CH (2015) Biologics of tendon repair. Adv Drug Deliv Rev 84:222–239. https://doi.org/10.1016/j.addr.2014.11.015.Biologics

Doherty TJ (2003) Invited review: ageing and sarcopenia. J Appl Physiol 95:1717–1727. https://doi.org/10.1152/japplphysiol.00347.2003

Franceschi C, Bonafè M, Valensin S et al (2000) Inflamm-ageing. An evolutionary perspective on immunosenescence. Ann N Y Acad Sci 908:244–254. https://doi.org/10.1111/j.1749-6632.2000.tb06651.x

Franchi M, Fini M, Quaranta M et al (2007a) Crimp morphology in relaxed and stretched rat Achilles tendon. J Anat 210:1–7. https://doi.org/10.1111/j.1469-7580.2006.00666.x

Franchi M, Quaranta M, de Pasquale V et al (2007b) Tendon crimps and peritendinous tissues responding to tensional forces. Eur J Histochem 51(Suppl 1):9–14

Fukushige T, Kanekura T, Ohuchi E et al (2005) Immunohistochemical studies comparing the localization of type XV collagen in normal human skin and skin tumors with that of type IV collagen. J Dermatol 32:74–83

Fukuta S, Oyama M, Kavalkovich K et al (1998) Identification of types II, IX and X collagens at the insertion site of the bovine Achilles tendon. Matrix Biol 17:65–73. https://doi.org/10.1016/s0945-053x(98)90125-1

Funakoshi T, Iwasaki N, Kamishima T et al (2010) In vivo visualization of vascular patterns of rotator cuff tears using contrast-enhanced ultrasound. Am J Sports Med 38:2464–2471. https://doi.org/10.1177/0363546510375536

Gehwolf R, Wagner A, Lehner C et al (2016) Pleiotropic roles of the matricellular protein Sparc in tendon maturation and ageing. Sci Rep 6. https://doi.org/10.1038/srep32635

Godinho MSC, Thorpe CT, Greenwald SE, Screen HRC (2017) Elastin is localised to the interfascicular matrix of energy storing tendons and becomes increasingly disorganised with ageing. Sci Rep 7:1–11. https://doi.org/10.1038/s41598-017-09995-4

Gomez-Florit M, Labrador-Rached CJ, Domingues RMA, Gomes ME (2022) The tendon microenvironment: engineered in vitro models to study cellular crosstalk. Adv Drug Deliv Rev 185:114299. https://doi.org/10.1016/j.addr.2022.114299

Graça AL, Domingues RMA, Calejo I et al (2022) Therapeutic effects of platelet-derived extracellular vesicles in a bioengineered tendon disease model. Int J Mol Sci 23. https://doi.org/10.3390/ijms23062948

Grinstein M, Galloway JL (2018) Developmental biology in tendon tissue engineering. Elsevier

Guerquin MJ, Charvet B, Nourissat G et al (2013) Transcription factor EGR1 directs tendon differentiation and promotes tendon repair. J Clin Invest 123:3564–3576. https://doi.org/10.1172/JCI67521

Harvey T, Flamenco S, Fan C-M (2019) A Tppp3+Pdgfra+ tendon stem cell population contributes to regeneration and reveals a shared role for PDGF signalling in regeneration and fibrosis. Nat Cell Biol 21:1490–1503. https://doi.org/10.1038/s41556-019-0417-z

Heinemeier KM, Bjerrum SS, Schjerling P, Kjaer M (2013a) Expression of extracellular matrix components and related growth factors in human tendon and muscle after acute exercise. Scand J Med Sci Sports 23:1–12. https://doi.org/10.1111/j.1600-0838.2011.01414.x

Heinemeier KM, Schjerling P, Heinemeier J et al (2013b) Lack of tissue renewal in human adult Achilles tendon is revealed by nuclear bomb 14 C. FASEB J 27:2074–2079. https://doi.org/10.1096/fj.12-225599

Hermann A, Gastl R, Liebau S et al (2004) Efficient generation of neural stem cell-like cells from adult human bone marrow stromal cells. J Cell Sci 117:4411–4422. https://doi.org/10.1242/jcs.01307

Hirschi A, Cecchini M, Steinhardt R et al (2010) An overlapping kinase and phosphatase docking site regulates activity of the retinoblastoma protein. Biophys J 98:248a. https://doi.org/10.1016/j.bpj.2009.12.1349

Hoare M, Das T, Alexander G (2010) Ageing, telomeres, senescence, and liver injury. J Hepatol 53:950–961. https://doi.org/10.1016/j.jhep.2010.06.009

Hopkins C, Fu SC, Chua E et al (2016) Critical review on the socio-economic impact of tendinopathy. Asia-Pacific J Sport Med Arthrosc Rehabil Technol 4:9–20. https://doi.org/10.1016/j.asmart.2016.01.002

Hsiao MY, Chen YC, Lin CY et al (2015) Reduced patellar tendon elasticity with ageing: in vivo assessment by shear wave elastography. Ultrasound Med Biol 41:2899–2905. https://doi.org/10.1016/j.ultrasmedbio.2015.07.008

Hu C, Zhang Y, Tang K et al (2017) Downregulation of CITED2 contributes to TGFβ-mediated senescence of tendon-derived stem cells. Cell Tissue Res 368:93–104. https://doi.org/10.1007/s00441-016-2552-1

Huang Z, Yin Z, Xu J et al (2021) Tendon stem/progenitor cell subpopulations and their implications in tendon biology. Front Cell Dev Biol 9. https://doi.org/10.3389/fcell.2021.631272

Hulmes DJS (2002) Building collagen molecules, fibrils, and suprafibrillar structures. J Struct Biol 137:2–10. https://doi.org/10.1006/jsbi.2002.4450

Ippolito E, Natali PG, Postacchini F et al (1980) Morphological, immunochemical, and biochemical study of rabbit achilles tendon at various ages. J Bone Joint Surg 62:583–598

Ito Y, Toriuchi N, Yoshitaka T et al (2010) The Mohawk homeobox gene is a critical regulator of tendon differentiation. Proc Natl Acad Sci U S A 107:10538–10542. https://doi.org/10.1073/pnas.1000525107

James R, Kesturu G, Balian G, Chhabra AB (2008) Tendon: biology, biomechanics, repair, growth factors, and evolving treatment options. J Hand Surg Am 33:102–112. https://doi.org/10.1016/j.jhsa.2007.09.007

Janzen V, Forkert R, Fleming HE et al (2006) Stem-cell ageing modified by the cyclin-dependent kinase inhibitor p16INK4a. Nature 443:421–426. https://doi.org/10.1038/nature05159

Jones FS, Jones PL (2000) The tenascin family of ECM glycoproteins: structure, function, and regulation during embryonic development and tissue remodeling. Dev Dyn 218:235–259. https://doi.org/10.1002/(sici)1097-0177(200006)218:2<235::Aid-dvdy2>3.0.Co;2-g

Jósza L, Lehto MU, Järvinen M et al (1993) A comparative study of methods for demonstration and quantification of capillaries in skeletal muscle. Acta Histochem 94:89–96. https://doi.org/10.1016/s0065-1281(11)80344-2

Ju Z, Jiang H, Jaworski M et al (2007) Telomere dysfunction induces environmental alterations limiting hematopoietic stem cell function and engraftment. Nat Med 13:742–747. https://doi.org/10.1038/nm1578

Kannus P, Józsa L (1991) Histopathological changes preceding spontaneous rupture of a tendon. A controlled study of 891 patients. J Bone Joint Surg 73:1507–1525

Kasper G, Mao L, Geissler S et al (2009) Insights into mesenchymal stem cell ageing: involvement of antioxidant defense and actin cytoskeleton. Stem Cells 27:1288–1297. https://doi.org/10.1002/stem.49

Kawashiri M-A, Okada H, Tada H (2021) Severe calcification of the Achilles' tendon. Eur Heart J 42:2217. https://doi.org/10.1093/eurheartj/ehab017

Kendal AR, Layton T, Al-Mossawi H et al (2020) Multi-omic single cell analysis resolves novel stromal cell populations in healthy and diseased human tendon. Sci Rep 10. https://doi.org/10.1038/s41598-020-70786-5

Ker RF (2002) The implications of the adaptable fatigue quality of tendons for their construction, repair and function. Comp Biochem Physiol A Mol Integr Physiol 133:987–1000. https://doi.org/10.1016/s1095-6433(02)00171-x

Kiderlen S, Polzer C, Rädler JO et al (2019) Age related changes in cell stiffness of tendon stem/progenitor cells and a rejuvenating effect of ROCK-inhibition. Biochem Biophys Res Commun 509:839–844. https://doi.org/10.1016/j.bbrc.2019.01.027

Kjaer M, Langberg H, Heinemeier K et al (2009) From mechanical loading to collagen synthesis, structural changes and function in human tendon. Scand J Med Sci Sports 19:500–510. https://doi.org/10.1111/j.1600-0838.2009.00986.x

Klatte-Schulz F, Pauly S, Scheibel M et al (2012) Influence of age on the cell biological characteristics and the stimulation potential of male human tenocyte-like cells. Eur Cell Mater 24:74–89. https://doi.org/10.22203/eCM.v024a06

Kohler J, Popov C, Klotz B et al (2013) Uncovering the cellular and molecular changes in tendon stem/progenitor cells attributed to tendon ageing and degeneration. Ageing Cell 12:988–999. https://doi.org/10.1111/acel.12124

Korntner S, Lehner C, Gehwolf R et al (2019) Limiting angiogenesis to modulate scar formation. Adv Drug Deliv Rev 146:170–189. https://doi.org/10.1016/j.addr.2018.02.010

Kostrominova TY, Brooks SV (2013) Age-related changes in structure and extracellular matrix protein expression levels in rat tendons. Age (Omaha) 35:2203–2214. https://doi.org/10.1007/s11357-013-9514-2

Kuhn NZ, Tuan RS (2010) Regulation of stemness and stem cell niche of mesenchymal stem cells: implications in tumorigenesis and metastasis. J Cell Physiol 222:268–277. https://doi.org/10.1002/jcp.21940

Kvist M, Hurme T, Kannus P et al (1995) Vascular density at the myotendinous junction of the rat gastrocnemius muscle after immobilization and remobilization. Am J Sports Med 23:359–364. https://doi.org/10.1177/036354659502300320

Lacroix AS, Duenwald-Kuehl SE, Brickson S et al (2013) Effect of age and exercise on the viscoelastic properties of rat tail tendon. Ann Biomed Eng 41:1120–1128. https://doi.org/10.1007/s10439-013-0796-4

Lagoumtzi SM, Chondrogianni N (2021) Senolytics and senomorphics: natural and synthetic therapeutics in the treatment of ageing and chronic diseases. Free Radic Biol Med 171:169–190. https://doi.org/10.1016/j.freeradbiomed.2021.05.003

Laranjeira M, Domingues RMA, Costa-Almeida R et al (2017) 3D mimicry of native-tissue-fiber architecture guides tendon-derived cells and adipose stem cells into artificial tendon constructs. Small 13:1–13. https://doi.org/10.1002/smll.201700689

Leadbetter WB (2005) Anti-inflammatory therapy in tendinopathy: the role of nonsteroidal drugs and corticosteroid injections. Tendon Inj Basic Sci Clin Med 211–232. https://doi.org/10.1007/1-84628-050-8_22

Lewis G, Shaw KM (1997) Tensile properties of human tendo Achillis: effect of donor age and strain rate. J Foot Ankle Surg 36:435–445. https://doi.org/10.1016/s1067-2516(97)80096-8

Liddle AD, Rodríguez-Merchán EC (2015) Platelet-rich plasma in the treatment of patellar tendinopathy: a systematic review. Am J Sports Med 43:2583–2590. https://doi.org/10.1177/0363546514560726

Lin D, Alberton P, Caceres MD et al (2017) Tenomodulin is essential for prevention of adipocyte accumulation and fibrovascular scar formation during early tendon healing. Cell Death Dis 8:e3116–e3116. https://doi.org/10.1038/cddis.2017.510

Lin TWTW, Cardenas L, Soslowsky LJLJ (2004) Biomechanics of tendon injury and repair. J Biomech 37:865–877. https://doi.org/10.1016/j.jbiomech.2003.11.005

Liu H, Zhu S, Zhang C et al (2014) Crucial transcription factors in tendon development and differentiation: their potential for tendon regeneration. Cell Tissue Res 356:287–298. https://doi.org/10.1007/s00441-014-1834-8

Liu X, Zhu B, Li Y et al (2021) The role of vascular endothelial growth factor in tendon healing. Front Physiol 12. https://doi.org/10.3389/fphys.2021.766080

Lomas AJ, Ryan CNM, Sorushanova A et al (2015) The past, present and future in scaffold-based tendon treatments. Adv Drug Deliv Rev 84:257–277. https://doi.org/10.1016/j.addr.2014.11.022

Magnusson SP, Beyer N, Abrahamsen H et al (2003) Increased cross-sectional area and reduced tensile stress of the Achilles tendon in elderly compared with young women. J Gerontol Ser A Biol Sci Med Sci 58:123–127. https://doi.org/10.1093/gerona/58.2.b123

Magnusson SP, Kjaer M (2019) The impact of loading, unloading, ageing and injury on the human tendon. J Physiol 597:1283–1298. https://doi.org/10.1113/JP275450

Marote A, Teixeira FG, Mendes-Pinheiro B, Salgado AJ (2016) MSCs-derived exosomes: cell-secreted nanovesicles with regenerative potential. Front Pharmacol 7:1–8. https://doi.org/10.3389/fphar.2016.00231

Mazzone MF, McCue T (2002) Common conditions of the Achilles tendon. Am Fam Physician 65:1805–1810

Merceron TK, Burt M, Seol YJ et al (2015) A 3D bioprinted complex structure for engineering the muscle-tendon unit. Biofabrication 7:35003. https://doi.org/10.1088/1758-5090/7/3/035003

Mienaltowski MJ, Adams SM, Birk DE (2014) Tendon proper- and peritenon-derived progenitor cells have unique tenogenic properties. Stem Cell Res Ther 5:1–15. https://doi.org/10.1186/scrt475

Mienaltowski MJ, Adams SM, Birk DE (2013) Regional differences in stem cell/progenitor cell populations from the mouse Achilles tendon. Tissue Eng Part A 19:199–210. https://doi.org/10.1089/ten.tea.2012.0182

Migliorini F, Tingart M, Maffulli N (2020) Progress with stem cell therapies for tendon tissue regeneration. Expert Opin Biol Ther 20:1373–1379. https://doi.org/10.1080/14712598.2020.1786532

Millar NL, Murrell GAC, McInnes IB (2017) Inflammatory mechanisms in tendinopathy—towards translation. Nat Rev Rheumatol 13:110–122. https://doi.org/10.1038/nrrheum.2016.213

Millar NL, Silbernagel KG, Thorborg K et al (2021) Tendinopathy. Nat Rev Dis Primers 7:1–21. https://doi.org/10.1038/s41572-020-00234-1

Milz S, Tischer T, Buettner A et al (2004) Molecular composition and pathology of entheses on the medial and lateral epicondyles of the humerus: a structural basis for epicondylitis. Ann Rheum Dis 63:1015–1021. https://doi.org/10.1136/ard.2003.016378

Mishima Y, Lotz M (2008) Chemotaxis of human articular chondrocytes and mesenchymal stem cells. J Orthop Res 26:1407–1412. https://doi.org/10.1002/jor.20668

Mishra A, Woodall J, Vieira A (2009) Treatment of tendon and muscle using platelet-rich plasma. Clin Sports Med 28:113–125

Mogford JE, Tawil N, Chen A et al (2002) Effect of age and hypoxia on TGFbeta1 receptor expression and signal transduction in human dermal fibroblasts: impact on cell migration. J Cell Physiol 190:259–265. https://doi.org/10.1002/jcp.10060

Nakagawa Y, Hayashi K, Yamamoto N, Nagashima K (1996) Age-related changes in biomechanical properties of the Achilles tendon in rabbits. Eur J Appl Physiol Occup Physiol 73:7–10. https://doi.org/10.1007/BF00262803

No YJ, Castilho M, Ramaswamy Y, Zreiqat H (2020) Role of biomaterials and controlled architecture on tendon/ligament repair and regeneration. Adv Mater 32:1–16. https://doi.org/10.1002/adma.201904511

Oshima Y, Sato K, Tashiro F et al (2004) Anti-angiogenic action of the C-terminal domain of tenomodulin that shares homology with chondromodulin-I. J Cell Sci 117:2731–2744. https://doi.org/10.1242/jcs.01112

Pajala A, Melkko J, Leppilahti J et al (2009) Tenascin-C and type I and III collagen expression in total Achilles tendon rupture. An immunohistochemical study. Histol Histopathol 24:1207–1211. https://doi.org/10.14670/hh-24.1207

Pardes AM, Beach ZM, Raja H et al (2017) Ageing leads to inferior Achilles tendon mechanics and altered ankle function in rodents. J Biomech 60:30–38. https://doi.org/10.1016/j.jbiomech.2017.06.008

Paulsson M, Heinegård D (1981) Purification and structural characterization of a cartilage matrix protein. Biochem J 197:367–375. https://doi.org/10.1042/bj1970367
Peffers M, Fang Y, Cheung K et al (2015) Transcriptome analysis of ageing in uninjured human Achilles tendon. Arthritis Res Ther 17:33. https://doi.org/10.1186/s13075-015-0544-2
Peffers MJ, Thorpe CT, Collins JA et al (2014) Proteomic analysis reveals age-related changes in tendon matrix composition, with age- and injury-specific matrix fragmentation. J Biol Chem 289:25867–25878. https://doi.org/10.1074/jbc.M114.566554
Pesqueira T, Costa-Almeida R, Gomes ME (2018) Magnetotherapy: the quest for tendon regeneration. J Cell Physiol 233:6395–6405. https://doi.org/10.1002/jcp.26637
Puetzer JL, Ma T, Sallent I et al (2021) Driving hierarchical collagen fiber formation for functional tendon, ligament, and meniscus replacement. Biomaterials 269:120527. https://doi.org/10.1016/j.biomaterials.2020.120527
Quinlan JI, Maganaris CN, Franchi MV et al (2018) Muscle and tendon contributions to reduced rate of torque development in healthy older males. J Gerontol Ser A Biol Sci Med Sci 73:539–545. https://doi.org/10.1093/gerona/glx149
Rando TA (2006) Stem cells, ageing and the quest for immortality. Nature 441:1080–1086. https://doi.org/10.1038/nature04958
Rees SG, Dent CM, Caterson B (2009) Metabolism of proteoglycans in tendon. Scand J Med Sci Sports 19:470–478. https://doi.org/10.1111/j.1600-0838.2009.00938.x
Reynolds NL, Worrell TW (1991) Chronic achilles peritendinitis: etiology, pathophysiology, and treatment. J Orthop Sports Phys Ther 13:171–176. https://doi.org/10.2519/jospt.1991.13.4.171
Riazifar M, Pone EJ, Lötvall J, Zhao W (2017) Stem cell extracellular vesicles: extended messages of regeneration. Annu Rev Pharmacol Toxicol 57:125–154. https://doi.org/10.1016/S0002-9440(10)63912-0
Ribitsch I, Gueltekin S, Keith MF et al (2020) Age-related changes of tendon fibril micromorphology and gene expression. J Anat 236:688–700. https://doi.org/10.1111/joa.13125
Riel H, Lindstrøm CF, Rathleff MS et al (2019) Prevalence and incidence rate of lower-extremity tendinopathies in a Danish general practice: a registry-based study. BMC Musculoskelet Disord 20:4–9. https://doi.org/10.1186/s12891-019-2629-6
Riley G (2008) Tendinopathy - from basic science to treatment. Nat Clin Pract Rheumatol 4:82–89. https://doi.org/10.1038/ncprheum0700
Rinoldi C, Kijeńska-Gawrońska E, Khademhosseini A et al (2021) Fibrous systems as potential solutions for tendon and ligament repair, healing, and regeneration. Adv Healthc Mater 10. https://doi.org/10.1002/adhm.202001305
Robi K, Jakob N, Matevz K, Matjaz V (2013) The physiology of sports injuries and repair processes. In: Current issues in sports and exercise medicine, pp 43–86
Rock MJ, Holden P, Horton WA, Cohn DH (2010) Cartilage oligomeric matrix protein promotes cell attachment via two independent mechanisms involving CD47 and alphaVbeta3 integrin. Mol Cell Biochem 338:215–224. https://doi.org/10.1007/s11010-009-0355-3
Rooney P, Walker D, Grant ME, McClure J (1993) Cartilage and bone formation in repairing Achilles tendons within diffusion chambers: evidence for tendon-cartilage and cartilage-bone conversion in vivo. J Phatol 169:375–381. https://doi.org/10.1002/path.1711690315
Ross C, Alston M, Bickenbach JR, Aykin-Burns N (2011) Oxygen tension changes the rate of migration of human skin keratinocytes in an age-related manner. Exp Dermatol 20:58–63. https://doi.org/10.1111/j.1600-0625.2010.01190.x
Ruiz-Torres A, Lozano R, Melón J, Carraro R (2003) Age-dependent decline of in vitro migration (basal and stimulated by IGF-1 or insulin) of human vascular smooth muscle cells. J Gerontol A Biol Sci Med Sci 58:B1074–B1077. https://doi.org/10.1093/gerona/58.12.b1074
Runesson E, Ackermann P, Brisby H et al (2013) Detection of slow-cycling and stem/progenitor cells in different regions of rat Achilles tendon: response to treadmill exercise. Knee Surg Sports Traumatol Arthrosc 21:1694–1703. https://doi.org/10.1007/s00167-013-2446-7
Sahin E, DePinho RA (2010) Linking functional decline of telomeres, mitochondria and stem cells during ageing. Nature 464:520–528. https://doi.org/10.1038/nature08982

Sandeman SR, Allen MC, Liu C et al (2000) Human keratocyte migration into collagen gels declines with in vitro ageing. Mech Ageing Dev 119:149–157. https://doi.org/10.1016/s0047-6374(00)00177-9

Schneider M, Angele P, Järvinen TAH, Docheva D (2018) Rescue plan for Achilles: therapeutics steering the fate and functions of stem cells in tendon wound healing. Adv Drug Deliv Rev 129: 352–375. https://doi.org/10.1016/j.addr.2017.12.016

Schneider M, Docheva D (2017) Mysteries behind the cellular content of tendon tissues. J Am Acad Orthop Surg 25:e289–ee290. https://doi.org/10.5435/JAAOS-D-17-00520

Scott A, LaPrade RF, Harmon KG et al (2019) Platelet-rich plasma for patellar tendinopathy: a randomized controlled trial of leukocyte-rich PRP or leukocyte-poor PRP versus saline. Am J Sports Med 47:1654–1661. https://doi.org/10.1177/0363546519837954

Sharma P, Maffulli N (2008) Tendinopathy and tendon injury: the future. Disabil Rehabil 30:1733–1745. https://doi.org/10.1080/09638280701788274

Sharpless NE, DePinho RA (2007) How stem cells age and why this makes us grow old. Nat Rev Mol Cell Biol 8:703–713. https://doi.org/10.1038/nrm2241

Shibata KR, Aoyama T, Shima Y et al (2007) Expression of the p16INK4A gene is associated closely with senescence of human mesenchymal stem cells and is potentially silenced by DNA methylation during in vitro expansion. Stem Cells 25:2371–2382. https://doi.org/10.1634/stemcells.2007-0225

Sleeswijk Visser TSO, Van Der Vlist AC, Van Oosterom RF et al (2021) Impact of chronic Achilles tendinopathy on health-related quality of life, work performance, healthcare utilisation and costs. BMJ Open Sport Exerc Med 7:1–7. https://doi.org/10.1136/bmjsem-2020-001023

Sopko NA, Turturice BA, Becker ME et al (2010) Bone marrow support of the heart in pressure overload is lost with ageing. PLoS One 5:e15187. https://doi.org/10.1371/journal.pone.0015187

Spiesz EM, Thorpe CT, Chaudhry S et al (2015) Tendon extracellular matrix damage, degradation and inflammation in response to in vitro overload exercise. J Orthop Res 33:889–897. https://doi.org/10.1002/jor.22879

Stenroth L, Peltonen J, Cronin NJ et al (2012) Age-related differences in Achilles tendon properties and triceps surae muscle architecture in vivo. J Appl Physiol 113:1537–1544. https://doi.org/10.1152/japplphysiol.00782.2012

Stolzing A, Jones E, McGonagle D, Scutt A (2008) Age-related changes in human bone marrow-derived mesenchymal stem cells: consequences for cell therapies. Mech Ageing Dev 129:163–173. https://doi.org/10.1016/j.mad.2007.12.002

Svensson L, Närlid I, Oldberg A (2000) Fibromodulin and lumican bind to the same region on collagen type I fibrils. FEBS Lett 470:178–182. https://doi.org/10.1016/s0014-5793(00)01314-4

Svensson RB, Heinemeier KM, Couppé C et al (2016) Effect of ageing and exercise on the tendon. J Appl Physiol 121:1353–1362. https://doi.org/10.1152/japplphysiol.00328.2016

Tempfer H, Spitzer G, Lehner C et al (2022) VEGF-D-mediated signaling in tendon cells is involved in degenerative processes. FASEB J 36. https://doi.org/10.1096/fj.202100773RRR

Tempfer H, Traweger A (2015) Tendon vasculature in health and disease. Front Physiol 6. https://doi.org/10.3389/fphys.2015.00330

Tempfer H, Wagner A, Gehwolf R et al (2009) Perivascular cells of the supraspinatus tendon express both tendon- and stem cell-related markers. Histochem Cell Biol 131:733–741. https://doi.org/10.1007/s00418-009-0581-5

Thermann H, Frerichs O, Biewener A et al (1995) Biomechanical studies of human Achilles tendon rupture. Unfallchirurgie 98:570–575

Thorpe CT, Chaudhry S, Lei II et al (2015) Tendon overload results in alterations in cell shape and increased markers of inflammation and matrix degradation. Scand J Med Sci Sports 25:e381–e391. https://doi.org/10.1111/sms.12333

Thorpe CT, Peffers MJ, Simpson D et al (2016) Anatomical heterogeneity of tendon: fascicular and interfascicular tendon compartments have distinct proteomic composition. Sci Rep 6. https://doi.org/10.1038/srep20455

Thorpe CT, Riley GP, Birch HL et al (2017) Fascicles and the interfascicular matrix show decreased fatigue life with ageing in energy storing tendons. Acta Biomater 56:58–64. https://doi.org/10.1016/j.actbio.2017.03.024

Thorpe CT, Riley GP, Birch HL et al (2014) Fascicles from energy-storing tendons show an age-specific response to cyclic fatigue loading. J R Soc Interface 11. https://doi.org/10.1098/rsif.2013.1058

Thorpe CT, Screen HRC (2016) Tendon structure and composition. In: Advances in experimental medicine and biology, pp 3–10

Torricelli P, Veronesi F, Pagani S et al (2013) In vitro tenocyte metabolism in ageing and oestrogen deficiency. Age (Omaha) 35:2125–2136. https://doi.org/10.1007/s11357-012-9500-0

Tsai W-C, Chang H-N, Yu T-Y et al (2011) Decreased proliferation of ageing tenocytes is associated with down-regulation of cellular senescence-inhibited gene and up-regulation of p27. J Orthop Res 29:1598–1603. https://doi.org/10.1002/jor.21418

Tuite DJ, Renström PAFH, O'Brien M (1997) The ageing tendon. Scand J Med Sci Sports 7:72–77. https://doi.org/10.1111/j.1600-0838.1997.tb00122.x

Turan A, Teber MA, Yakut ZI et al (2015) Sonoelastographic assessment of the age-related changes of the Achilles tendon. Med Ultrason 17:58–61. https://doi.org/10.11152/mu.2013.2066.171.ayt

Vinhas A, Gonçalves AI, Rodrigues MT, Gomes ME (2021) Human tendon-derived cell sheets created by magnetic force-based tissue engineering hold tenogenic and immunomodulatory potential. Acta Biomater 131:236–247. https://doi.org/10.1016/j.actbio.2021.06.036

Vos T, Abajobir AA, Abate KH et al (2017) Global, regional, and national incidence, prevalence, and years lived with disability for 328 diseases and injuries for 195 countries, 1990–2016: a systematic analysis for the global burden of disease study 2016. Lancet 390:1211–1259. https://doi.org/10.1016/S0140-6736(17)32154-2

Wagner W, Ho AD, Zenke M (2010) Different facets of ageing in human mesenchymal stem cells. Tissue Eng Part B Rev 16:445–453. https://doi.org/10.1089/ten.TEB.2009.0825

Walia B, Huang AH (2019) Tendon stem progenitor cells: understanding the biology to inform therapeutic strategies for tendon repair. J Orthop Res 37:1270–1280. https://doi.org/10.1002/jor.24156

Wang Y, Zhang X, Huang H et al (2017a) Osteocalcin expressing cells from tendon sheaths in mice contribute to tendon repair by activating hedgehog signaling. eLife 6. https://doi.org/10.7554/eLife.30474

Wang Z, Wang Z, Lu WW et al (2017b) Novel biomaterial strategies for controlled growth factor delivery for biomedical applications. NPG Asia Mater 9:e435–e417. https://doi.org/10.1038/am.2017.171

Wei S, Gao X, Du J et al (2011) Angiogenin enhances cell migration by regulating stress fiber assembly and focal adhesion dynamics. PLoS One 6:e28797. https://doi.org/10.1371/journal.pone.0028797

Wilson A, Laurenti E, Oser G et al (2008) Hematopoietic stem cells reversibly switch from dormancy to self-renewal during homeostasis and repair. Cell 135:1118–1129. https://doi.org/10.1016/j.cell.2008.10.048

Witwer KW, Van Balkom BWM, Bruno S et al (2019) Defining mesenchymal stromal cell (MSC)-derived small extracellular vesicles for therapeutic applications. J Extracell Vesicles 8:1–12. https://doi.org/10.1080/20013078.2019.1609206

Wood LK, Brooks SV (2016) Ten weeks of treadmill running decreases stiffness and increases collagen turnover in tendons of old mice. J Orthop Res 34:346–353. https://doi.org/10.1002/jor.22824

Wu H, Zhao G, Zu H et al (2015) Ageing-related viscoelasticity variation of tendon stem cells (TSCs) characterized by quartz thickness shear mode (TSM) resonators. Sens Actuators B 210:369–380. https://doi.org/10.1016/j.snb.2014.12.117

Wunderli SL, Blache U, Beretta Piccoli A et al (2020) Tendon response to matrix unloading is determined by the patho-physiological niche. Matrix Biol 89:11–26. https://doi.org/10.1016/j.matbio.2019.12.003

Wynn TA, Ramalingam TR (2012) Mechanisms of fibrosis: therapeutic translation for fibrotic disease. Nat Med 18:1028–1040. https://doi.org/10.1038/nm.2807

Xia YP, Zhao Y, Tyrone JW et al (2001) Differential activation of migration by hypoxia in keratinocytes isolated from donors of increasing age: implication for chronic wounds in the elderly. J Invest Dermatol 116:50–56. https://doi.org/10.1046/j.1523-1747.2001.00209.x

Xu H, Liu F (2018) Downregulation of FOXP1 correlates with tendon stem/progenitor cells ageing. Biochem Biophys Res Commun 504:96–102. https://doi.org/10.1016/j.bbrc.2018.08.136

Xu W, Sun Y, Zhang J et al (2015) Perivascular-derived stem cells with neural crest characteristics are involved in tendon repair. Stem Cells Dev 24:857–868. https://doi.org/10.1089/scd.2014.0036

Yan Z, Yin H, Brochhausen C et al (2020) Aged tendon stem/progenitor cells are less competent to form 3D tendon organoids due to cell autonomous and matrix production deficits. Front Bioeng Biotechnol 8. https://doi.org/10.3389/fbioe.2020.00406

Yan Z, Yin H, Nerlich M et al (2018) Boosting tendon repair: interplay of cells, growth factors and scaffold-free and gel-based carriers. J Exp Orthop 5:1–13. https://doi.org/10.1186/s40634-017-0117-1

Yin Z, Hu JJ, Yang L et al (2016) Single-cell analysis reveals a nestin+ tendon stem/progenitor cell population with strong tenogenic potentiality. Sci Adv 2:1–14. https://doi.org/10.1126/sciadv.1600874

Yoon JH, Halper J (2005) Tendon proteoglycans: biochemistry and function. J Musculoskelet Neuronal Interact 5:22–34

Yu JM, Wu X, Gimble JM et al (2011) Age-related changes in mesenchymal stem cells derived from rhesus macaque bone marrow. Ageing Cell 10:66–79. https://doi.org/10.1111/j.1474-9726.2010.00646.x

Zafar MS, Mahmood A, Maffulli N (2009) Basic science and clinical aspects of Achilles tendinopathy. Sports Med Arthrosc 17:190–197. https://doi.org/10.1097/JSA.0b013e3181b37eb7

Zantop T, Tillmann B, Petersen W (2003) Quantitative assessment of blood vessels of the human Achilles tendon: an immunohistochemical cadaver study. Arch Orthop Trauma Surg 123:501–504. https://doi.org/10.1007/s00402-003-0491-2

Zaseck LW, Miller RA, Brooks SV (2016) Rapamycin attenuates age-associated changes in tibialis anterior tendon viscoelastic properties. J Gerontol Ser A Biol Sci Med Sci 71:858–865. https://doi.org/10.1093/gerona/glv307

Zhang G, Ezura Y, Chervoneva I et al (2006) Decorin regulates assembly of collagen fibrils and acquisition of biomechanical properties during tendon development. J Cell Biochem 98:1436–1449. https://doi.org/10.1002/jcb.20776

Zhang J, Wang JHC (2015) Moderate exercise mitigates the detrimental effects of ageing on tendon stem cells. PLoS One 10:1–20. https://doi.org/10.1371/journal.pone.0130454

Zhou Y, Wang JHC (2016) PRP treatment efficacy for tendinopathy: a review of basic science studies. Biomed Res Int

Zhou Z, Akinbiyi T, Xu L et al (2010) Tendon-derived stem/progenitor cell ageing: defective self-renewal and altered fate. Ageing Cell 9:911–915. https://doi.org/10.1111/j.1474-9726.2010.00598.x

Zs-Nagy I, von Hahn HP, Verzar F (1969) Age-related alterations in the cell nuclei and the DNA content of rat tail tendon. Gerontologia 15:258–264

Zuskov A, Freedman BR, Gordon JA et al (2020) Tendon biomechanics and crimp properties following fatigue loading are influenced by tendon type and age in mice. J Orthop Res 38:36–42. https://doi.org/10.1002/jor.24407

Chapter 8
Virus Infections in Older People

Roy L. Soiza, Chiara Scicluna, and Sana Bilal

Abstract Older people are more prone to viral infections, and often have worse outcomes. This was well demonstrated during the COVID-19 pandemic, where a disproportionate number of deaths occurred in the oldest and frailest people. The assessment of the older person with a viral infection is complicated by the high prevalence of multiple comorbidities and sensory or cognitive impairment. They often present with common geriatric syndromes such as falls or delirium, rather than the more typical features of a viral illness in younger people. Comprehensive geriatric assessment by a specialist multidisciplinary team is the gold standard of management, as viral illness is unlikely to present in isolation of other healthcare needs. We discuss the presentation, diagnosis, prevention, and management of common viral infections—respiratory syncytial virus, coronavirus, norovirus, influenza, hepatitis, herpes, and dengue viruses—with special consideration of infections in the older patient.

Keywords Ageing · Coronavirus · COVID-19 · Diagnosis · Hepatitis · Infection · Influenza · Norovirus · Senescence · Treatment · Virology

Introduction

Viruses are an integral part of the human microbiome, probably numbering in the tens of trillions in every human body (Liang and Bushman 2021). Of these, over 250 viruses capable of causing clinical infections leading to illness in humans have been identified (Carroll et al. 2018). However, this number increases steadily each year with an estimated 1.67 million viruses in the animal kingdom remaining unidentified and unclassified. The best estimates are that between 631,000 and 827,000 of them will have the potential to infect humans, though luckily few will

R. L. Soiza (✉) · C. Scicluna · S. Bilal
Ageing Clinical and Experimental Research Group, University of Aberdeen, Aberdeen, UK
e-mail: r.l.soiza@abdn.ac.uk; chiara.sciculuna@nhs.scot; sana.bilal@nhs.scot

J. R. Harris, V. I. Korolchuk (eds.), *Biochemistry and Cell Biology of Ageing: Part IV, Clinical Science*, Subcellular Biochemistry 103,
https://doi.org/10.1007/978-3-031-26576-1_8

probably cause illness. Nevertheless, the global pandemic of COVID-19 has served as a reminder of the susceptibility of any species to succumb to new viruses. Immunosenescence, age-related changes in our immune system, render older people at higher risk of both viral infections and worse outcomes when infection occurs. The clinical manifestation of infection is further influenced by comorbidities and physical and cognitive function, such that viral infections may commonly present atypically with physical and/or cognitive decline, falls, or delirium. The differential diagnosis can therefore be wide, and most clinical presentations in older people have multifactorial aetiology in any case. In this chapter, we present a recommended holistic approach to the assessment of the older person with suspected viral infection and an overview of the more common or serious viral infections in old age.

Comprehensive Geriatric Assessment

The diagnosis and management of the older patient is usually more challenging and more prone to errors than that of younger patients (Wenger et al. 2003). This may be due to a multitude of reasons, including a lack of specific geriatric medicine education in medical school and beyond. Conventional medical wisdom that encourages clinicians to come up with a single unifying diagnosis to explain all symptoms and clinical findings (sometimes referred to as 'Ockam's razor') is usually unsuccessful in the older patient because the diagnosis is more likely to be multifactorial. Comprehensive geriatric assessment (CGA) is considered to be the gold standard for effective management of health problems in old age, and suspected viral infections are no different. It is usually delivered by a multidisciplinary team that incudes medical, nursing, and therapist staff. The role of the doctor includes taking a careful history, conducting a relevant physical examination, and organising a series of relevant tests to aid diagnoses and management (see later on in the chapter for specific viral illnesses). Diagnoses would usually include a list of contributory and causative factors that explain all symptoms, though a full diagnostic list may only be possible once the whole multidisciplinary team has assessed the patient. A comprehensive management plan is then discussed and agreed with the patient and the whole team.

Functional Status

The presentation of any viral infection is likely to be hugely influenced by the patient's functional status, with people who are less resilient more likely to present atypically with diminished mobility, falls, or other common geriatric syndromes. Functional status covers basic self-care tasks or basic activities of daily living (ADLs) such as bathing, dressing, toileting, tasks that are required to live

independently (such as shopping, doing laundry and housework), and in some instances tasks related to technology (such as internet and cell phone use).

A number of scales exist to measure functional status at basic and independent level, including the Vulnerable Elders Scale-13 (VES-13) (Saliba et al. 2001), Clinical Frailty Scale (Rockwood et al. 2005), and the Katz index for ADLs (Wallace et al. 2007).

Mobility

Virus infections in older people may present with difficulties with mobility, and a specialist physiotherapist assessment and management programme is recommended where there is significant immobility or falls. Gait speed is a useful measure for predicting functional decline in the elderly. In a systematic review of 49 studies by Binotto et al. (2018), reduced gait speed was associated with disability, frailty, muscle atrophy, stress, reduced quality of life, and mortality. Low energy use associated with reduced gait speed was also associated with increased risk of cardiovascular disease. The demographic most likely to experience reduced gait speed were people over 75 years of age, low physical activity levels, history of stroke, urinary incontinence, and diabetes.

Loss of gait speed is also associated with increased risk of falls. The older adult is more susceptible to injury from falls due to physiological changes of old age (such as reduced reflexes) combined with other disease such as osteoporosis. Recovery also tends to be delayed which in turn results in deconditioning and post-fall anxiety, which causes further deconditioning (Rubenstein 2006).

Falls are particularly serious in the older adult age group due to an increased susceptibility to injury. Accidental injury is also the cause of one-fifth of deaths in the elderly, and two-thirds of these deaths are due to falls. Half of elderly patients discharged from hospital after a fall do not survive the year and repeated falls are also a significant reason for nursing home admission.

Cognition

Viral infections of any type can cause cognitive decline, even where the central nervous system is not directly affected by viral infection. Cognitive decline is usually acute, leading to delirium, but can also worsen any pre-existing mental health problem. A viral infection can therefore highlight an undiagnosed case of dementia, and it is important all clinicians are familiar with this condition, as well as delirium. For non-specialists, the use of screening tools for delirium and dementia is recommended early on in the consultation, and a recent guideline highlighted the 4As Test may be particularly suitable due to its ease of use and high sensitivity and

specificity (Soiza and Myint 2019). A positive result should prompt consideration of a diagnosis of delirium and/or dementia, and a specialist referral if necessary.

Several screening tools exist for the diagnosis of dementia. A systematic review by Abd Razak et al. (2019) indicated that the Addenbrooke's Cognitive Examination-III (ACE-III) was the overall best screening tool, with 100% sensitivity and 96% specificity (Hsieh et al. 2013). In primary care, the best tool for detecting mild cognitive impairment is the Montreal Cognitive Assessment (MoCA) (Nasreddine et al. 2005) with a sensitivity of 83–97%.

Mood Disorders

An evaluation of mood is an integral part of CGA. When depression is not diagnosed and treated, it brings with it disability and suffering to both patients and their family members. Depression is more common in patients who suffer from other comorbidities, and can occur both as early onset (in patients who have had depression in their younger years) or as late onset (the first episode occurs over the age of 65) (Alexopoulos et al. 2002). Comorbidities can worsen due to depression, and overall, older adults with depression have poorer functioning than with other diseases such as lung disease or diabetes (World Health Organization 2017a).

A useful screening tool for depression in the elderly is the Geriatric Depression Scale (Yesevage et al. 1982–1983). Originally containing 30 items, shorter versions have been developed and used. A study on 147 elderly persons in the community found the GDS to be an accurate tool (Dias et al. 2017).

Polypharmacy

Polypharmacy is defined as a situation where multiple medications are prescribed to one person, often by different prescribers. The exact number that defines polypharmacy varies between studies, but is typically five or more. A systematic review by Davies et al. (2020) found that polypharmacy is associated with frailty, malnutrition, impaired balance, anxiety, reduced perceived health status, and various system diseases. Polypharmacy is also associated with hospitalisation and unplanned admissions.

Medications that may be particularly dangerous in older people include anticholinergics (due to delirium, blurry vision, increased falls risk) sedatives, psycholeptics (due to confusion, falls, and dependency), cardiovascular drugs (due to hypotension and sedation), sulfonylureas (cause hypoglycaemia), nitrofurantoin (cause liver and lung toxicity), and NSAIDs (cause renal failure and gastrointestinal bleeding).

Some strategies to reduce polypharmacy include judicious prescribing and involvement of the patient and/or caregiver before starting any new medications, with a discussion of the benefits and risks (Caslake et al. 2013). Appropriate

deprescribing is also important. Medications should be revisited at every opportunity, and their effects and safety followed up (Dahal and Bista 2022).

Social Factors

Social isolation and loneliness are more prevalent in the older adult population due to a shrinking social network, decline in mobility, and children moving away. A review done by Yang and Victor (2011) revealed that 20–34% of older people suffered from loneliness in 25 European countries. A study by Gardiner et al. (2020) indicated that nursing home residents experienced significant amounts of moderate to severe loneliness. Homebound status and social isolation are also shown to be linked to all-cause mortality, especially the former, although this is not specific to the geriatric population (Sakurai et al. 2019).

The three-item loneliness scale is a tool that can be used to determine if patients are affected by loneliness (Hughes et al. 2004). The scale has been adapted in other countries and languages. In English, it focuses on self-reported feelings of isolation, lack of companionship, and generally feeling 'left out'.

An advocacy brief issued by the WHO in 2021 acknowledges the complexity of the issue and that solutions are not straightforward. Activity-based social interaction, more education, and digital interventions are successful in some cases but not all. Higher quality evidence and more research in lower socio-economic areas in the world are required to establish better solutions to tackle loneliness.

Nutritional Status

Malnutrition can be defined as a state of either deficiency or excess of calories and micronutrients that impacts the individual in a negative way (Stratton et al. 2003).

A 2021 meta-analysis of studies in Europe showed that in the older adult populations, 28% of those hospitalised, 17.5% of those in residential care, and 8.5% of those in the community were at high malnutrition risk (Leij-Halfwerk et al. 2019).

Malnutrition causes disease, can worsen existing comorbidities, and compounds frailty, thereby increasing risk of viral infections and poor outcomes. A study by Neumann et al. (2005) of 133 older adults in rehabilitation was done and the outcomes assessed based on starting nutritional status. Subjects who were malnourished had poorer function on admission as well as at 90 days, had longer lengths of stay, and were more likely to require higher level care.

Screening for malnutrition can be done in several ways. The Nutritional Risk Index (NRI) and Geriatric Nutritional Risk Index (GNRI) focus on albuminaemia and current and ideal weight (Wolinsky et al. 1990; Cereda and Pedrolli 2009). The Malnutrition Universal Screening Tool (MUST) score is used in clinical practice and

considers BMI, degree of unintentional weight loss, acute illness, and/or decreased intake (Malnutrition Advisory Group 2003). The Nutritional Risk Screen (NRS-2002) is also for hospital use and considers BMI, severity of weight loss and reduced intake, and severity of surgery/condition that the patient has been admitted for; for example, head injuries and ICU admission score at a higher risk than hip fractures and diabetes (Kondrup et al. 2003).

Sensory Impairment

Sensory impairment includes hearing loss only, visual impairment only, and dual sensory impairment. It can make assessment of the older person with viral infection more challenging, but it is important clinicians have the skill set and facilities to overcome any difficulties. In the USA, almost 29 million people over the age of 60 suffer from some degree of hearing loss (Goman and Lin 2016).

Sensory impairment has been associated with a reduced quality of life (Tseng et al. 2018), mental health decline such as increased anxiety and depression (Simning et al. 2019), decline in ADLs (Bouscaren et al. 2019), increased perceived discrimination (Shakarchi et al. 2020), and all-cause mortality (Fisher et al. 2014). Outcomes also tend to be worse with dual sensory impairment as opposed to only one sensory impairment.

A simple screening test for hearing loss is to ask patients 'Do you have any difficulty with your hearing?' A study conducted in 2020 on 14,877 people over the age of 55 revealed that 93% required appropriate referrals for further testing (Zazove et al. 2020).

A Cochrane review 'Community screening for visual impairment in older people' in 2018 determined that visual screening in the community was not likely to be useful in general due to poor uptake of interventions. Therefore any screening offered should be followed by assessing the desire for intervention by the patient. Formal visual acuity testing (Snellen chart) was the method with most sensitivity and specificity, although this does not encompass early detection of macular degeneration or cataract (Clarke et al. 2018; Seematter-Bagnoud and Büla 2018).

Social Factors

Social isolation and loneliness are more prevalent in the older adult population due to a shrinking social network, decline in mobility, and children moving away. A review by Yang and Victor (2011) revealed that 20–34% of older people suffered from loneliness in 25 European countries. A study by Gardiner et al. (2020) indicated that nursing home residents experienced significant amounts of moderate to severe loneliness. Homebound status and social isolation are also shown to be linked to

all-cause mortality, especially the former, although this is not specific to the geriatric population (Sakurai et al. 2019).

The three-item loneliness scale is a tool that can be used to determine if patients are affected by loneliness (Hughes et al. 2004). The scale has been adapted in other countries and languages. In English, it focuses on self-reported feelings of isolation, lack of companionship, and generally feeling 'left out'.

An advocacy brief issued by the WHO in 2021 acknowledges the complexity of the issue and that solutions are not straightforward. Activity-based social interaction, more education, and digital interventions are successful in some cases but not all. Higher quality evidence and more research in lower socio-economic areas in the world are required to establish better solutions to tackle loneliness.

Specific Viral Infections

Having undertaken a holistic and multidisciplinary assessment, the possible diagnosis of a viral infection contributing to the presenting illness can then be confirmed by use of blood or swab tests. It is rarely possible to make a confident diagnosis purely on clinical grounds, though index of suspicion can be high where there are recognised outbreaks and known infectious contacts. Non-specific presentations with lethargy, malaise, and fever may be the only symptoms. Respiratory illness, with breathlessness and cough, and gastrointestinal illness, with diarrhoea and vomiting, are especially common, but all these symptoms have a wide differential in the older patient, including non-infective exacerbations of chronic lung disease and heart failure, overflow diarrhoea, and adverse reactions to medications. Routine investigations, such as a full blood count, may show characteristic lymphocytosis or lymphopenia, rather than the neutrophilia seen in bacterial infections, but will not be diagnostic. Some of the more common or dangerous global viral infections in older people are highlighted in the remainder of the chapter.

Respiratory Syncytial Virus (RSV)

Respiratory syncytial virus (RSV) was discovered in 1956 and has since been recognised as one of the most common causes of childhood illness. However, it causes annual outbreaks of respiratory illnesses in all age groups including older people.

It belongs to the recently defined Pneumoviridae family, Orthopneumovirus genus. It is a negative sense, single-stranded RNA virus that results in epidemics of respiratory infections that typically peak in the winter in temperate climates and during the rainy season in tropical climates.

Transmission

RSV can spread when

- An infected person coughs or sneezes.
- A person can get virus droplets from a cough or sneeze in the eyes, nose, or mouth.
- Someone touches a surface that has the virus on it, like a doorknob, and then touching the face before washing hands.
- Someone has direct contact with the virus, like kissing the face of a child with RSV.

People infected with RSV are usually contagious for 3–8 days. However, some infants, and people with weakened immune systems, can continue to spread the virus even after they stop showing symptoms, for as long as 4 weeks. Grandchildren are often exposed to and infected with RSV outside the home, such as in school or child-care centres. They can then transmit the virus to other members of the family.

RSV can survive for many hours on hard surfaces such as tables and crib rails. It typically lives on soft surfaces such as tissues and hands for shorter amounts of time.

People of any age can get RSV infection, but infections in younger adult life are generally less severe. People at highest risk for severe disease include:

- Premature infants
- Young children with congenital (from birth) heart or chronic lung disease
- Young children with compromised (weakened) immune systems due to a medical condition or medical treatment
- Adults with compromised immune systems
- Older adults, especially those with underlying heart or lung disease

RSV infections can be dangerous for certain adults. Adults at highest risk for severe RSV infection include

- Older adults, especially those 65 years and older
- Adults with chronic heart or lung disease
- Adults with weakened immune systems

Symptoms

Adults who get infected with RSV usually have mild or no symptoms. Symptoms are usually consistent with an upper respiratory tract infection. People infected with RSV usually show symptoms within 4–6 days of getting infected. Symptoms of RSV infection usually include:

- Runny nose
- Decrease in appetite

- Coughing
- Sneezing
- Fever
- Wheezing
- Headache
- Fatigue

These symptoms usually appear in stages and not all at once.

Severe RSV Infection

Most people who get an RSV infection will have mild illness and will recover in a week or two. Some people, however, are more likely to develop severe RSV infection and may need to be hospitalised. RSV can also make chronic health problems worse. For example, people with asthma may experience asthma attacks as a result of RSV infection, and people with congestive heart failure may experience more severe symptoms triggered by RSV. The following serious complications can occur with RSV:

- Pneumonia (infection of the lungs)
- More severe symptoms for people with asthma
- More severe symptoms for people with chronic obstructive pulmonary disease (COPD)
- Congestive heart failure (when the heart cannot pump blood and oxygen to the body's tissues)

Older adults who get very sick from RSV may need to be hospitalised. Some may even die. Older adults are at greater risk than young adults for serious complications from RSV because of weaker immune systems and frailty.

RSV Prevention

There are steps one can take to help prevent the spread of RSV, and these apply to other respiratory viral infections too. Specifically, if someone has cold-like symptoms they should:

- Cover their coughs and sneezes with a tissue or upper shirt sleeve, not with hands.
- Wash hands often with soap and water for at least 20 s.
- Avoid close contact, such as kissing, shaking hands, and sharing cups and eating utensils, with others.
- Clean frequently touched surfaces such as doorknobs and mobile devices.

Ideally, people with cold-like symptoms should not interact with children at high risk for severe RSV disease, including premature infants, children younger than 2 years of age with chronic lung or heart conditions, and children with weakened immune systems. If this is not possible, they should carefully follow the prevention steps mentioned above and wash their hands before interacting with such children. They should also refrain from kissing high-risk children whilst they have cold-like symptoms.

Diagnosis

Because mild RSV symptoms are similar to the common cold, testing often is not undertaken to diagnose the infection. However, doctors may suspect RSV based on medical history, time of year, and a physical exam. In this case, they may want to run laboratory tests to confirm the diagnosis. The most common is a mouth swab or a blood test to check white blood cell counts and look for viruses using polymerase chain reaction (PCR) tests.

In severe RSV cases that require hospitalisation, additional testing may be needed. Imaging tests, such as a chest X-ray or computerised topography (CT) scan, can check for lung complications.

Treatment

For mild symptoms, prescription treatment is usually not needed. RSV goes away on its own in 1–2 weeks. Antibiotics are not used to treat viral infections, including those caused by RSV. Antibiotics may be prescribed, however, if testing shows bacterial pneumonia or other infection.

In older adults, especially if they have a weakened immune system, they may need to be hospitalised if the RSV is severe. Whilst in the hospital, they may receive oxygen or be put on a ventilator to help them breathe or receive IV fluids to help with dehydration. In an older adult or an adult with a weakened immune system or long-term heart or lung disease, RSV infection may be more serious if there is superinfection, often with bacterial pneumonia.

No vaccine against RSV has yet been approved by any licensing authority, but a number of promising vaccine candidates are in advanced stages of development and testing (Vekemans et al. 2019). Pavlivimab is a drug approved to prevent severe RSV in certain infants and children at high risk for severe disease. The drug does not cure RSV, is not used to treat children who already have severe RSV, and cannot prevent RSV infection. It is given as monthly injections during the RSV season. In older people, its use is not recommended on grounds of cost and because risks may outweigh benefits.

COVID-19

Introduction

Coronavirus disease-19 (COVID-19) is a viral disease first discovered in December 2019 in Wuhan, China, initially spreading zoonotically and then from human to human (Li et al. 2020). The virus is transmitted via aerosol droplets and through fomites (van Doremalen et al. 2020).

The World Health Organization (WHO) declared a COVID-19 pandemic on the 11th of March 2020 after the rapid spread of the virus to 114 countries. By the middle of 2022, there had been 529 million reported cases worldwide and six million deaths attributed to the infection (Anonymous 2022), mostly in older people.

Background

Coronaviridae are a family of viruses that infect animals and humans, and are characterised by a fringe or 'corona' appearance of their envelope. Until the 2002–2004 SARS outbreak, Coronaviridae were thought to only cause mild respiratory illness. The family is subdivided into four genera, of which Betacoronavirus is the most important in terms of human infection and this genus contains the severe acute respiratory virus (SARS-CoV-1), Middle Eastern respiratory syndrome coronavirus (MERS), and the newly emerged SARS-CoV-2 (Burrell et al. 2017; Hsieh et al. 2004) that causes COVID-19.

Pathophysiology

SARS-CoV-2 is made up of four structural proteins: the spike (S), membrane (M), envelop (E), and nucleocapsid (N) (Jiang et al. 2020).

The spike protein is the main component responsible for attaching and entering host cells. Its subunit S1 binds to the host cell receptor, whereas S2 functions to fuse the virus to the cell membrane (Bosch et al. 2003). The host cell receptor is ACE-2, which is commonly found on the pulmonary epithelial cells (Wang et al. 2008; Shirbhate et al. 2021).

Within the cell the virus replicates to transcribe negative-strand RNA from the original positive-strand RNA, after which more positive-strand RNA is produced and is translated into protein (Lai and Cavanagh 1997). At this stage, the virus spreads within the nasal ciliated cells and individuals are typically asymptomatic, highly infectious, and likely to test positive on nasal swab testing (Sims et al. 2005).

The virus can then migrate to the upper respiratory tract and produce systemic symptoms like fever and malaise, where chemokines and interferons are released

from the infected cells (Mason 2020). In 81% of patients, the infection does not progress beyond this point and self-resolves within 10–14 days (Wu and McGoogan 2020).

However, some patients develop severe symptoms following the initial infection. The virus proceeds to invade type 2 alveolar epithelial cells via ACE-2 receptors, and triggers a release of cytokines causing a cytokine storm. This subsequently attracts immune cells whose function is to clear the virus, but also has the unfortunate effect of lung inflammation and injury. This is followed by pneumocyte loss and culminates in acute respiratory distress syndrome [ARDS] (Xu et al. 2020).

Since the start of the pandemic the virus has shown high mutagenic potential and five major variants of concern have been identified (Cascella et al. 2022) by July 2022:

- Alpha (B.1.1.7): first variant of concern described in the United Kingdom (UK) in late December 2020
- Beta (B.1.351): first reported in South Africa in December 2020
- Gamma (P.1): first reported in Brazil in early January 2021
- Delta (B.1.617.2): first reported in India in December 2020
- Omicron (B.1.1.529): first reported in South Africa in November 2021

Symptoms

COVID-19 presents with a range of symptoms, the most common being fever, shortness of breath, cough, diarrhoea, sore throat, and fatigue. Other symptoms reported are a runny nose, headache, loss of smell or taste, muscle aches, and chills (Struyf et al. 2020).

Diagnosis and Testing

The gold standard is a PCR test for viral RNA on a nasopharyngeal swab. Inexpensive lateral flow tests have been developed that have high specificity, such that a positive result is extremely likely to mean infection has occurred. The latter are particularly helpful to identify asymptomatic cases and take pre-emptive steps to prevent outbreaks, particularly in places such as care homes where outbreaks can be deadly.

Imaging tests, such as a chest X-ray, can be helpful as COVID-19 can sometimes show bilateral infiltrates that are uncommon in other conditions. A CT scan can check for lung complications and pulmonary embolism is common and may require a CT pulmonary angiogram if suspected.

Treatment

A number of therapies are currently used in COVID-19 illness, including antivirals such as molnupiravir, anti-SARS-CoV-2 neutralising antibody products such as casirivimab and imdevimab, immunomodulatory agents such as dexamethasone and tocilizumab, and oxygen therapy through high-flow nasal cannula, non-invasive positive-pressure ventilation, and in severe cases, intubation (Cascella et al. 2022).

Vaccination

Numerous novel vaccines against COVID-19 have been developed since the start of the pandemic. As of the June 2022, 11.9 billion doses of COVID-19 vaccine had been administered, covering 61% of the world's population. 25% of whom have also received at least 1 booster dose (WHO Coronavirus [COVID-19] Dashboard 2022). Vaccines have a good safety and efficacy profile, and are recommended even in the very elderly and frail (Soiza et al. 2021).

The vaccines currently available on the market are produced by Bharat Biotech, Gamaleya Research Institute, Sinovac, Pfizer, Moderna, Janssen, Oxford/AstraZeneca, Novavax, and Medicago.

Specific Considerations in Older People

Early statistical data in 2019 revealed a higher case fatality rate in the over-60-year-olds, the highest being in the over-80 s group (Huang et al. 2020). In another study by Wang et al. (2020), 70% of the elderly patients suffered from severe or critical pneumonia due to COVID-19, and the case fatality rate was 19%. The onset of ARDS was noted to be a very poor prognostic factor, whilst an increased lymphocyte count was protective. Other poor prognostic factors included coagulation disorders, myocardial injury, reduced renal function, and bacterial infections.

The COVID pandemic caused societal impacts in addition to its immediate health effects. Due to the high rate of spread, lockdown measures were implemented in most countries in an attempt to mitigate the infection rate and avoid overwhelming the medical services.

Lockdown measures had several effects on the elderly, both those living at home or those in long-term care facilities. Lockdown caused a drastic decrease in the ability of the individuals to partake in social activities and physical exercise, causing boredom, a sedentary lifestyle, and loneliness. This was especially exacerbated for residents of facilities suffering from dementia who could not understand why they were confined alone and to their rooms (Bouillon-Minois et al. 2020).

Influenza

Influenza ('flu') is an acute respiratory illness due to infection with the influenza virus. Uncomplicated influenza is defined as influenza presenting with fever, coryza, generalised symptoms (headache, malaise, myalgia, arthralgia), and sometimes gastrointestinal symptoms, but without any features of complicated influenza. Complicated influenza is defined as influenza requiring hospital admission and/or with symptoms and signs of lower respiratory tract infection (hypoxaemia, dyspnoea, lung infiltrate), central nervous system involvement, and/or a significant exacerbation of an underlying medical condition.

Influenza in Older People

The risk of flu-related complications and hospitalisation is particularly high in adults aged 65 years and older.

Immune systems decline as adults age. Older adults are at higher risk of serious flu and flu-related complications including pneumonia and hospitalisation. There are also other risks that may not be as obvious; flu increases the risk of heart attack by 3–5 times and stroke by 2–3 times in the first 2 weeks of infection for those 65+. The risk remains elevated for several months. This all adds up to a six-times higher risk of dying from flu and related complications if you are aged 65 years or older.

Pathogenesis

There are three serotypes—A, B, and C. Influenza A and B viruses cause most clinical disease:

- A is the more frequent and the cause of major influenza outbreaks.
- B tends to circulate with type A in yearly outbreaks and causes less severe illness.
- C tends to cause a mild or asymptomatic illness akin to the common cold.

Influenza A serotypes are further categorised by their surface antigens:

- H: haemagglutinin—facilitates entry of the virus into the host respiratory cell.
- N: neuraminidase—facilitates release of virions from the infected host cells.

There are 15 H and 9 N subtypes of the A virus in aquatic birds, which together with pigs (often termed the 'mixing vessel' for scrambling human and avian virus genetic material) are the natural reservoir of the virus. Many of the newer types of influenza are thought to have arisen in China because of the often-close co-habitation there of pigs, fowl, and humans. Swine flu is an influenza A virus most frequently of subtype H1N1, usually found in pigs but able to be transferred to humans.

The influenza virus undergoes minor mutations to one or both of its surface antigens—antigenic drift. This causes seasonal epidemics where people have only partial immunity from previous infection.

Risk Factors

- Closed environments—such as: residential homes, schools, and prisons
- Advanced age (65 and older)
- Pre-existing cardiac or respiratory illness

Presentation

Transmission is either by:

- Droplet due to coughing/sneezing
- Direct nasal or eye contact with hands carrying the virus

After an incubation period of 1–3 days, the patient commonly presents with rapid onset of:

- Anorexia
- Malaise
- Headache (retro-orbital)
- Fever
- Myalgia
- Non-productive cough and sore throat

Nasal discharge/obstruction and sneezing can occur but are not usually prominent features of the illness. Fever may not be seen in older patients. Gastrointestinal symptoms are not usual but may occur in a minority of patients.

Swine flu is like the usual human seasonal influenza infection, with most cases in adults and children being mild. Clinicians are encouraged to diagnose swine flu based on symptoms if there is a pyrexia ($\geq$38 °C), fever, or history of fever and flu-like illness (two or more of the following symptoms: cough, sore throat, rhinorrhoea, widespread muscle and joint aches, headache). There may also be any of the following: fatigue, loss of appetite and sometimes diarrhoea, nausea, vomiting, otitis media, and (rarely) cerebral irritability $\pm$ seizures.

Most symptoms typically last for 3–5 days but cough, tiredness, and malaise may last for 1–2 weeks. Infectivity continues for 5 days from onset, although children can remain infectious for 2 weeks, and the severely immunocompromised can shed virus for weeks.

Differential Diagnosis

The most important infectious causes are listed below:

- Common cold/upper respiratory tract infection
- Pharyngitis
- Meningitis
- Bacterial or viral lower respiratory tract infection, including pneumonia
- Malaria or dengue fever in returning travellers
- Infectious mononucleosis
- Cytomegalovirus

Investigations

The diagnosis is a clinical one, so investigations are usually reserved for community surveillance purposes. Available tests include:

- Direct viral culture of nasopharyngeal swabs/aspirates
- Immunofluorescence of nasopharyngeal swabs/aspirates
- Acute and convalescent sera, 10–14 days apart
- Polymerase chain reaction
- Rapid bedside antigen tests. These currently have low positive predictive values

Management

General Measures

In otherwise healthy individuals with uncomplicated illness, self-management is recommended, including resting at home, increased fluid intake, analgesics, and antipyretics.

Pharmacological

Antiviral drugs now form one important part of plans to prevent and contain epidemics of influenza infection, but it should be selective and appropriate.

Routine prescription of antiviral drugs for people with influenza who are otherwise healthy is not recommended. The patient should be reassured that the worst symptoms of uncomplicated influenza resolve after about 1 week, although other symptoms (such as cough, headache, insomnia, weakness, and loss of appetite) may take longer than 2 weeks to resolve.

Oseltamivir and zanamivir reduce replication of influenza A and B viruses by inhibiting viral neuraminidase. They are most effective if started within a few hours of the onset of symptoms. In otherwise healthy individuals they reduce the duration of symptoms by about 1–1.5 days.

There is evidence that some strains of influenza A virus have reduced susceptibility to oseltamivir but may retain susceptibility to zanamivir. Resistance to oseltamivir may be greater in severely immunocompromised patients.

National Institute for Health and Care Excellence (NICE) 2020 guidance for the treatment of influenza includes the following:

- The guidance does not cover treatment in a pandemic, an impending pandemic, or a widespread epidemic of a new strain of influenza to which there is little or no immunity in the community.
- Amantadine is not recommended for treatment of influenza.
- When influenza is circulating in the community, either oseltamivir or zanamivir is recommended for the treatment of influenza in at-risk patients who can start treatment within 48 h of the onset of symptoms.
- During local outbreaks of influenza-like illness, when there is a high level of certainty that influenza is present, either oseltamivir or zanamivir may be used for treatment in at-risk patients living in long-term residential or nursing homes.
- At-risk patients include those aged over 65 years or those who have one or more of the following conditions:
  - Chronic respiratory disease, including asthma and chronic obstructive pulmonary disease (COPD)
  - Chronic heart disease
  - Chronic kidney disease
  - Chronic liver disease
  - Chronic neurological disease
  - Immunosuppression
  - Diabetes mellitus
  - A pregnancy

Complications

Respiratory complications include:

- Acute bronchitis (about 20% of cases, with increased risk in the elderly and those with chronic disease)
- Secondary bacterial pneumonia (especially *Staphylococcus aureus*)
- Primary viral pneumonia
- Exacerbations of asthma and COPD
- Empyema
- Pulmonary aspergillosis
- Sinusitis

Non-respiratory complications include:

- Febrile convulsions
- Otitis media
- Toxic shock syndrome
- Myositis and myoglobinaemia
- Heart failure
- Myocarditis
- Reye's syndrome
- Guillain-Barré syndrome
- Transverse myelitis
- Encephalitis

Risks of complications with hospitalisation and death are higher amongst:

- Those aged >65 years
- Very young children
- Those with at-risk factors

Residents of nursing homes are particularly at risk of serious complications because of their age, high rate of chronic disease, and living in a closed community.

Norovirus

Introduction

Norovirus was first identified as an outbreak of gastroenteritis in Norwalk, Ohio, in 1968 and eponymously named Norwalk virus. Norovirus belongs to the family of Caliciviridae viruses and is a positive-sense, single-stranded RNA virus. Amongst the virus genogroups, GI, GII, and GIV infect humans, with the norovirus strain GII.4 being the most widespread overall. GII.4 variants emerge with mutations, with at least 8 variants identified in the last 22 years (Chhabra et al. 2019).

Norovirus is a significant cause of gastroenteritis in both developing and developed nations, typically causing nausea, vomiting, and diarrhoea, leading to severe dehydration if untreated. According to the World Health Organisation (2019), out of an average of 23 million cases of food poisoning in Europe each year, 15 million are attributed to norovirus.

Epidemiology

A systematic review of 175 studies conducted by Ahmed et al. (2014) extracted data from 48 countries. Norovirus was associated with 18% of all cases of acute gastroenteritis and overall was found to be the more common cause of mild gastroenteritis

The norovirus was also prevalent in a lower proportion of gastroenteritis cases in high mortality countries, and was attributed to a larger variety of pathogens potentially causing gastroenteritis, which may be controlled for by better hygiene and safety standards in lower mortality countries. This would appear to result in a lower apparent burden of norovirus cases, but this is simply diluted by other pathogens causing similar illness. Unfortunately, studies specific to norovirus infection in the elderly were few, and thus, it was not possible to stratify for this age group in this chapter.

Norovirus in Older Adults

The elderly are a high-risk group for norovirus infection. Over-85-year-olds recover at a slower rate than in the younger population, with 50% remaining symptomatic after 4 days. Diarrhoea can last from 3 to 9 days. Older adults who are hospitalised due to norovirus infection are more likely to be admitted to intensive care, and older adults hospitalised for other reasons are more likely to contract nosocomial norovirus infection. It is unclear whether this is because of longer hospital stays causing more likelihood for exposure rather than immunosenescence or comorbidities (Cardemil et al. 2017). Harris et al. (2008) looked at death rates associated with norovirus in England and Wales. In the over-65-year-olds, the researchers found an average of 80 deaths a year resulting from norovirus as the primary cause of death.

Long-Term Care Facilities and Infection Risk

Facilities for long-term care of the elderly are a likely breeding ground for norovirus, due to the close proximity of residents and high degree of contact with staff. An analysis conducted in 566 gastroenteritis outbreaks in care homes from 2016 to 2018 in England revealed norovirus to be the sole pathogen in 64% of cases (Inns et al. 2020). A smaller study by Bruggink et al. (2015) focused on norovirus outbreaks of people living in residential aged-care facilities in Victoria Australia in 2013. Out of the 206 cases of acute gastroenteritis, 65% were found positive for norovirus on faecal PCR testing. The average age of the patients was 84.9, with an age range of 61–98 years.

Prevention

Norovirus spreads via the faecal-oral route, either from direct hand contamination or through food contaminated with faecal matter and is highly infectious due to a

number of reasons. The virus is stable across a broad range of temperatures (0–60 °C) and persists in the environment for days. Only 18–1000 viral particles are required to cause infection; infected individuals shed viral particles prior to becoming symptomatic and keep shedding for an extended period following resolution. Finally, there is lack of cross-protection from exposure to other viruses, and norovirus mutates to produce new strains periodically.

Public health advice to prevent spread includes isolating infected individuals for up to 2 days after symptoms resolve, as well as frequent handwashing with soap and water (alcohol-based hand rubs have not been found to be suitable to eliminate viral particles). High-risk workers like healthcare staff and those in contact with food should also stay home for up to 2 days after symptoms resolve. Chlorine bleach solutions of 1000–5000 ppm should be used to sanitise surfaces to limit the environmental spread (Bányai et al. 2018).

Vaccine

Norovirus is broadly classified into 7 genogroups, which then subdivide into genotypes. Genogroups I and II are the most relevant in human infection, and they are subdivided into 9 and 22 genotypes, respectively (Kroneman et al. 2013; Green 2007). GII.4 causes the largest burden of disease in humans (Vega et al. 2013).

Immunity to norovirus is not well understood. A study in 1977 of 12 male persons in North America demonstrated that half had natural immunity to an ingested norovirus inoculum when challenged twice 27–42 months apart, but 4 of the 6 who were unwell the first time became unwell again the second time, with 1 becoming ill a third time. This suggested that some degree of natural immunity exists, as well as short-term immunity following first-time exposure, although this did not develop in everyone (Parrino et al. 1977).

Norovirus vaccine development faces some challenges. Due to lack of small animal models or cell culture systems, it is difficult to assess immunity to norovirus post-vaccination as it is not possible to conduct virus neutralisation assays (Melhem 2016). These are immunoassays which measure antibodies that stop norovirus from replicating (Payne 2017).

Due to these issues, vaccines that utilise live attenuated or killed virus are currently not possible (Melhem 2016). Current vaccine research is mainly focused on developing virus-like particles (VLPs). VLPs are nanostructures without genetic material which emulate authentic viruses. Their surface is densely coated with multiple copies of viral surface proteins which are also functional, meaning they can enter cells (Chroboczek et al. 2014).

Research has also been looking into P particles as vaccines. P particles are protrusions occurring on the virus shell, first described by Prasad et al. (1999). When isolated, these dimerise in vitro and can bind to histo-blood group antigen receptors (HBGAs), which are implicated in immunity to norovirus. P particles are

therefore promising in the development of a norovirus vaccine in the future (Tan 2021).

Research carried out in paediatric norovirus infection appears to show that natural immunity is genotype-specific, with only modest cross-protection in the same genogroup (Parra and Green 2014; Saito et al. 2014). Therefore, vaccines which protect against multiple genogroups are the sensible approach.

There are 5 vaccines that have reached human trials, three of which are in the preclinical stage: UMN Pharma Inc., Ology Bioservices, and Medicago, and two in the clinical phase: Vaxart Inc. and Takeda Vaccines, Inc. (Kim et al. 2018; Heinimäki et al. 2018; Nooraei et al. 2021; Ball et al. 2017; Atmar et al. 2011). Takeda is at the most advanced stage, in phase 2b which is shown to result in a 47% reduction in norovirus-associated gastroenteritis, 35% reduction of severe disease, and a 26% reduction of norovirus infection following challenge with virus (Atmar et al. 2011).

Cost-effectiveness is a further challenge for a norovirus vaccine, and depends on its efficacy and duration of cover relative to the cost of the vaccine itself. For the over 65 s, a vaccine would save money per case with efficacy of at least 50%, covering 24 months, costing around $25, and this is negated if the cost is higher. For all age groups, the efficacy would need to be at least 75%, protection lasting 48 months and costing USD$25. According to Bartsch et al. (2012) who conducted this analysis, the group most benefitting from a vaccine in terms of cost-effectiveness would be the paediatric group, followed by the over 65 age group.

In low- to middle-income countries, the cost per illness is much lower than in high-income countries ($45 and $247 per illness, respectively), due to differences in the cost of healthcare. Therefore, in lower income countries, a cost-saving vaccine would need to be 70% effective and cost $17 (Svennson 2016).

Norovirus is also poorly understood by the public. In a survey conducted in 2018 asking 806 adults who ate shellfish, 73% claimed to have at least heard of norovirus (Farkas et al. 2021). Another older study reported a larger percentage of people who have heard of the 'cruise ship virus'. 60% knew transmission was possible through food prepared by an infected person, but not that infection was possible from contact with surfaces touched by an infected person, infected faeces or vomitus, or even by close contact with an infected person. Another 76% were not aware that spread can occur up to 2 weeks even after an infected persons feel better. There was also lack of awareness of the type of foods associated with transmission, such as leafy vegetables, and decontamination methods such as undiluted bleach to disinfect surfaces (Cates et al. 2015).

Hepatitis

Introduction

Hepatitis is a burdensome disease in public health terms. According to the WHO 2017 Global Hepatitis Report, in 2015 there were 1.34 million deaths, a number

comparable to deaths due to tuberculosis and exceeding those solely due to HIV. The latter two diseases both have declining death rates, whereas deaths due to viral hepatitis were noted to be increasing. The main causes of death are chronic liver disease and primary liver cancer, respectively (World Health Organisation 2017b).

Hepatitis B virus [HBV] belongs to the family *Hepadnaviridae*, which infects the liver, is mostly species-specific, causing both acute and chronic infection, and results in the presence of the virus and antigen in the bloodstream. Hepatitis C virus [HCV] belongs to *Flaviviridae*, which are all pathogenic and infect the liver. Both viruses are enveloped; HBV is a partially double-stranded DNA virus, whilst HCV is an RNA virus (Glebe and Bremer 2013; Li and Lo 2015).

HBV and HCV are acquired through mucosal or percutaneous exposure to infected bodily fluids. The bulk of HBV infections are acquired perinatally and are most likely to progress to chronic infection. The main modes of transmission for HCV are horizontal, including through sexual contact and sharing of infected injecting devices, and nosocomial, such as transfusion of infected blood products. The WHO reported that in 2015, 7.3% of people infected with HIV had concurrent HBV infection, and 7.3% had concurrent HCV infection. In this group, liver disease causes significant morbidity and mortality (World Health Organisation 2017b).

Acute infection with HBV or HCV causes symptoms in some but not all people. A small proportion develop acute hepatitis, but most go unnoticed. Some of these new infections can self-resolve; other persons develop chronic infection (World Health Organisation 2017b). Chronic HBV infection is determined through the presence of the HBsAg marker in blood.

Of the 1% of the world's population, 3.5% live with chronic HBV and HCV, respectively. 68% of HBV sufferers are from the African and Western Pacific regions, whilst HCV is more common in Europe and Eastern Europe (World Health Organization 2017b). According to the Centre for Disease Control [CDC] 2004–2019 data, the rate of hepatitis B amongst people aged 60 and over in the USA was in decline until the year 2014, seeing a rise thereafter (CDC 2019).

Differences in Older People

The liver suffers age-related changes such as genomic instability, telomere attrition, epigenetic alterations, mitochondrial dysfunction, and cellular senescence (Hunt et al. 2019). These changes impact the rate of progression from acute to chronic hepatitis and may cause low clearance of HBV (Floreani 2009). A small study in 1993 followed an HBV outbreak in a nursing facility where 59% of the residents older than 65 years progressed to chronic infection (Kondo et al. 1993). Similarly higher rates of progression to chronicity were noted in a Northern Ireland study where 33% of persons aged over 50 years progressed, in comparison to 7% of the under 50 years. This study also revealed a lower clearance of the virus in the older age group (64% in comparison to 84% in the younger group) (McKeating et al. 2018).

Vaccinations

Vaccination of infants is an effective preventer of new chronic HBV. Current sufferers of HBV were largely born before the widespread availability of the vaccine and infant vaccination programmes (World Health Organisation 2017a, b). A number of hepatitis B vaccines are commercially available, including single-antigen vaccines, combination hepatitis A and B vaccines, and other combinations with diphtheria, tetanus, pertussis, poliovirus, and haemophilus influenzae type b (Davis 2005).

A hepatitis C vaccine is not currently available as there are difficulties in development of such a vaccine due to several factors. HCV exhibits a high genetic diversity and mutation rate, it evades the host's adaptive immune response, and there are no small animal or in vitro models. Potential vaccine prospects include recombinant subunit vaccines, VLPs (virus-like particles), and virus-vector vaccines (Duncan et al. 2020).

Management

Acute HBV infection has no significant treatment avenues. Chronic HBV treatment focuses on reducing viral replication, which is correlated to reduction in chances of cirrhosis and cancer. Interferon therapy can cause virological cure in up to 30% of cases; however, it has significant side effects which are less tolerated in the older population (Kemp et al. 2019). Non-immunological therapy includes nucleoside analogues such as entecavir and tenofovir. According to the European Association for the Study of the Liver (2017) guidelines, the latter non-immunological therapies are likely to be safer in older adults particularly if they have comorbidities related to osteopenia and declining renal function.

Hepatitis C is managed with direct-acting antivirals, most of which are tolerated equally by adults and older adults (Jhaveri et al. 2018).

Varicella Zoster

Introduction

Varicella zoster virus is a double-stranded DNA alphaherpes virus that commonly occurs in childhood leading to the illness known as 'chicken pox' with the typical blistering rash of herpes virus infections (John and Canaday 2017). It is usually self-limiting, though it can be very dangerous in newborn children who contract it from their mother. Primary infection in old age is unusual, and a vaccine has been available for over 25 years. However, herpes zoster is a significant problem in

older age due to reactivation of latent varicella zoster that lies dormant for years in nerve ganglia after the primary infection. It classically presents as 'shingles' when the characteristic blistering skin lesions recur in the distribution of a single nerve root, though it can cause several other clinical presentations and has numerous complications.

Epidemiology

The incidence of varicella zoster has been increasing for decades (Kawaii et al. 2017). The lifetime incidence amongst people in the USA aged 85 or more is around 50%. So far, the immunisation programme against varicella zoster started in the mid-1990 s has had little impact on the growing incidence of varicella zoster in old age (John and Cannaday 2017).

Clinical Presentation

Primary infection is characterised by a widespread itchy blistering rash, commonly referred to as chicken pox. The blisters are highly contagious but dry over and heal with a scab that can leave a scar if picked. Infection usually lasts only a few days, but the virus persists within the nervous system, where it is usually kept in check by the body's immune system. Reactivation can occur many years later, usually due to immunosuppression from medications, diseases such as leukaemia, or the immunosenescence of old age. However, even immunocompetent individuals can present with varicella zoster reactivation. The typical presentation is with 'shingles', the recurrence of unilateral blistering lesions along the distribution of a cutaneous nerve root that classically do not cross the midline. This may occur anywhere in the body and the lesions may be asymptomatic or itchy or painful. In elderly individuals, the rash may be atypical and limited to a small patch within the dermatome or have a maculopapular appearance with no blisters. Of special concern is ophthalmic shingles, also known as herpes zoster ophthalmicus, where involvement of the ophthalmic branch of the trigeminal branch can lead to serious eye problems. Involvement of other cranial nerves can also result in presentations with cranial nerve palsies, such as the Ramsay Hunt syndrome where unilateral facial nerve palsy is associated with lesions within the external ear and in the mouth. Rarely, varicella zoster can present with meningitis, encephalitis, or myelitis. Immunocompromised individuals can present with widespread disseminated infection. Diagnosis can usually be made on clinical grounds alone, but viral PCR of skin lesions would confirm the diagnosis.

Complications

The commonest complication is post-herpetic neuralgia. This is an extremely painful condition that can persist for many months after the shingles rash disappears. It can be challenging to treat and highly debilitating in frail older people. Bacterial superinfection of the vesicular lesions is another common complication. Nerve palsies and infections of the central nervous system can also occur, whilst ophthalmic involvement can result in blindness. Epidemiological studies show the risk of stroke and heart attacks is elevated for a year after zoster infection.

Treatment

The acute infection can be treated with antiviral agents such as acyclovir, and is recommended in all older adults (Dworkin et al. 2007). Steroids are sometimes used in more serious infections such as ophthalmic shingles, though the evidence they help is weak and their use needs to be weighed against the potential for serious side effects. Simple analgesics such as paracetamol or nonsteroidal anti-inflammatory drugs are often required during the acute infection or for post-herpetic neuralgia. Drugs such as gabapentin specifically targeting neuralgia are often needed for the latter condition and topically administered anaesthetic agents such as lidocaine patches are also helpful. Ophthalmic shingles should always be referred to an eye specialist.

Herpes Simplex

Herpes simplex viruses (HSV) are members of the herpesviridae family and only infect humans (Whitley and Roizman 2001). Types 1 and 2 are the most serious human pathogens. Together they are more prevalent than the related zoster virus and share some features with it, causing blistering skin lesions and remaining latent within neurons after primary infection. Type 1 is classically associated with oro-facial lesions commonly referred to as ‘cold sores’. Type 2 classically affects the genitals and is usually transmitted through sex. Reactivation of virus is common even in immunocompetent individuals and usually results in lesions recurring at or near the original site of entry. Despite being so common that most of the population shows seroconversion to either or both viruses by adulthood, the illness they cause tends to be relatively mild and not life-threatening. Rarely, HSV-1 can cause encephalitis in people of any age. It can be especially difficult to diagnose in older people as it usually presents with delirium, which has many other causes and is common in old age. The presence on imaging of the brain of oedema of the right temporal lobe is suggestive of HSV encephalitis. Diagnosis can be confirmed by

PCR testing of cerebrospinal fluid. Treatment involves at least 2 weeks of intravenous acyclovir. Neurological complications and disability are common and the condition can be fatal.

Dengue

Dengue fever is the most common mosquito-borne viral infection transmitted by female mosquitoes mainly of the species *Aedes aegypti* and to a lesser extent *Aedes albopictus*. The incubation period is 3–14 days (average 4–7 days) (Kuluratne 2015).

Pathogenesis

Dengue is a single-stranded RNA virus of Flaviviridae family. It is subdivided into primary and secondary infections. The primary infection is usually benign; however, secondary infection can be further classified as either dengue haemorrhagic fever or dengue shock syndrome, depending on the clinical features.

The virus infects and replicates inside the Langerhans cells which release interferons. The infected Langerhans cells go to the lymphatic system to activate the host immune mechanism, and eventually release into the circulation, resulting in viremia. The activation of immune response causes an increase in the number of lymphocytes and a decrease in neutrophils and white blood cells resulting in early symptoms of dengue such as fever, rashes, and joint and musculoskeletal pain.

If the disease is left untreated, the production of pro-inflammatory cytokines and proliferation of memory T cells could cause vascular endothelial cell dysfunction, which results in plasma leakage causing dengue shock syndrome and dengue haemorrhagic fever (Kularatne 2015).

Clinical Features

The clinical features of dengue vary with the age of the patient and can be classified by clinical presentations (Hadinegor 2012):

Non-specific febrile illness: Mild fever is the common presentation in children and young adults.

Classic dengue: More common in older children, adolescents, and adults. It is also known as ‘break bone fever’ and characterised by acute onset of high fever associated with frontal headache, retro-orbital pain, myalgias, arthralgia, haemorrhagic manifestations, and rash (macular or maculopapular).

Dengue haemorrhagic fever: It is primarily a disease of children under 15 years of age in hyperendemic areas. Some patients with dengue fever go on to develop

dengue haemorrhagic fever (DHF) a severe and sometimes fatal form of the disease. DHF is currently defined by the following four World Health Organization (WHO) criteria:

– Fever or recent history of fever lasting 2–7 days
– Any haemorrhagic manifestations
– Thrombocytopenia (platelet count of $<100{,}000/mm^3$)
– Evidence of increased vascular permeability causing plasma leakage

Dengue shock syndrome (DSS): Dengue shock syndrome is defined as any case that meets the four criteria for DHF and has evidence of circulatory failure manifested by (1) rapid, weak pulse and narrow pulse pressure (≤20 mmHg [2.7 kPa]) or (2) hypotension for age, restlessness, and cold, clammy skin. Patients with dengue can rapidly progress into DSS, which, if not treated correctly, can lead to severe complications and death. Warning signs include severe abdominal pain, persistent vomiting, marked change in temperature (from fever to hypothermia), and change in mental status (irritability, confusion, or somnolence).

Diagnostic Testing

Nucleic acid amplification tests (NAATs) such as PCR are the preferred method of laboratory diagnosis. IgM antibody testing can identify additional infections and is an important diagnostic tool. However, interpreting the results is complicated by cross-reactivity with other flaviviruses, like Zika, and determining the specific timing of infection can be difficult. If infection is likely to have occurred in a place where other potentially cross-reactive flaviviruses circulate, both molecular and serologic diagnostic testing for dengue and other flaviviruses should be carried out. People infected with or vaccinated against other flaviviruses may produce cross-reactive flavivirus antibodies, resulting in false-positive test results (CDC 2022).

Investigations

- Full blood count testing may show high packed cell volume with low platelets. There may be lymphocytosis with more than 15% of circulating white cells, but overall picture is of leukopenia.
- Clotting studies can show rise in fibrin degradation products along with prolongation of activated partial thromboplastin time (APTT) and prothrombin time (PT).
- Electrolyte disturbance is common. Liver function tests might be elevated—especially AST.
- Acidosis might result in decreased bicarbonate levels.
- Infection may be confirmed by isolation of virus in serum and detection of IgM and IgG antibodies by ELISA, monoclonal antibody, or haemagglutination.

- X-rays may be useful to exclude other sources of sepsis/assess complications. Chest X-ray may show abnormalities, such as pleural effusion, in the first week.
- Blood cultures and repeated malaria films should be checked in the traveller returning with a high fever (Ray Junhao et al. 2017).

Management

There is no specific treatment for dengue; however, early recognition and symptomatic management can reduce the mortality rate. Majority of the patients could recover without hospital admission, whereas others may quickly become severely unwell and close monitoring of clinical signs and laboratory measurements is required. Management principles include:

- Recognising the febrile phase and controlling the fever with paracetamol. NSAIDs (nonsteroidal anti-inflammatory drugs), such as ibuprofen and aspirin, should be avoided.
- Intravenous fluid resuscitation with close monitoring, observing for increased capillary permeability. Monitor CVP and urine output, electrolytes, packed cell volume, platelets, and LFTs.
- Secondary bacterial infections may occur and require treatment.
- Haemorrhage, shock, and severe organ impairment require early and prompt management.
- Those with severe dengue are likely to require intensive care.

Prognosis

Dengue is typically a self-limiting flu-like disease. For severe dengue, medical care by experienced team could decrease the mortality rates to less than 1% in majority of the countries.

Prevention

In late 2015, the first dengue vaccine, Dengvaxia® (CYD-TDV) by Sanofi Pasteur, was registered in several countries for use in individuals aged 9–45 years living in endemic areas. Dengue prevention and control also depends on effective vector control measures. These include anti-mosquito public health measures, such as reducing breeding sites, good sewage management, removing artificial man-made habitats that can hold water, and use of insecticides. Using of personal household protection measures, such as window screens, are also helpful. These measures must be observed during the day both inside and outside of the home because the primary

mosquito vectors bite throughout the day. Wearing clothing that minimises skin exposure to mosquitoes is recommended. Repellents may reduce the risk by reducing the overall number of bites, especially those containing N, N-diethyl-3-methylbenzamide (DEET).

Dengue in Older People

During the time of COVID-19 pandemic, differentiating between the symptoms of dengue and the common flu and cold that are common in the winters is difficult. Older people with comorbidities like COVID-19 are more likely to develop severe symptoms, but otherwise the management is unchanged.

References

Abd Razak MA, Ahmad NA, Chan YY et al (2019) Validity of screening tools for dementia and mild cognitive impairment among the elderly in primary health care: a systematic review. Public Health 169:84–92. https://doi.org/10.1016/j.puhe.2019.01.001

Ahmed SM, Hall AJ, Robinson AE, Verhoef L, Premkumar P, Parashar UD, Koopmans M, Lopman BA (2014) Global prevalence of norovirus in cases of gastroenteritis: a systematic review and meta-analysis. Lancet Infect Dis 14(8):725–730. S1473309914707674. https://doi.org/10.1016/S1473-3099(14)70767-4

Alexopoulos GS, Buckwalter K, Olin J et al (2002) Comorbidity of late life depression: an opportunity for research on mechanisms and treatment. Biol Psychiatry 52(6):543–558. https://doi.org/10.1016/S0006-3223(02)01468-3

Anonymous (2016) Takeda Initiates world's first norovirus vaccine field trial. https://www.takeda.com/newsroom/newsreleases/2016/takeda-initiates-worlds-first-norovirus-vaccine-field-trial/. Accessed 26 July 2021

Anonymous (2020) Prime Minister's statement on coronavirus (COVID-19): 23 March 2020. https://www.gov.uk/government/speeches/pm-address-to-the-nation-on-coronavirus-23-march-2020. Accessed 30 June 2022

Anonymous (2022) Worldometer. https://www.worldometers.info/coronavirus/. Accessed 26 June

Atmar RL, Bernstein DI, Harro CD et al (2011) Norovirus vaccine against experimental human Norwalk virus illness. N Engl J Med 365(23):2178–2187. https://doi.org/10.1056/NEJMoa1101245

Ball JP, Springer MJ, Ni Y, Finger-Baker I, Martinez J, Hahn J, Suber JF, DiMarco AV, Talton JD, Cobb RR, Xing Z (2017) Intranasal delivery of a bivalent norovirus vaccine formulated in an in situ gelling dry powder. PLoS One 12(5):e0177310. https://doi.org/10.1371/journal.pone.017731010.1371/journal.pone.0177310

Bartsch SM, Lopman BA, Hall AJ et al (2012) The potential economic value of a human norovirus vaccine for the United States. Vaccine 30(49):7097–7104. https://doi.org/10.1016/j.vaccine.2012.09.040

Bányai K, Estes MK, Martella V, Parashar UD (2018) Viral gastroenteritis. Lancet 392 (10142):175–186. S0140673618311280. https://doi.org/10.1016/S0140-6736(18)31128-0

Binotto MA, Lenardt MH, Rodríguez-Martínez MDC (2018) Physical frailty and gait speed in community elderly: a systematic review. Rev Esc Enferm USP 52:e03392-220X2017028703392. S0080-62342018000100810 [pii]

Bosch BJ, van der Zee R, de Haan CA et al (2003) The coronavirus spike protein is a class I virus fusion protein: structural and functional characterization of the fusion core complex. J Virol 77(16):8801–8811. 0053 [pii]

Bouillon-Minois JB, Lahaye C, Dutheil F (2020) Coronavirus and quarantine: will we sacrifice our elderly to protect them? Arch Gerontol Geriatr 90:104118. S0167-4943(20)30112-6 [pii]

Bouscaren N, Yildiz H, Dartois L et al (2019) Decline in instrumental activities of daily living over 4-year: the association with hearing, visual and dual sensory impairments among non-institutionalized women. J Nutr Health Aging 23(8):687–693. https://doi.org/10.1007/s12603-019-1231-9

Bruggink LD, Dunbar NL, Marshall JA (2015) Norovirus genotype diversity in community-based sporadic gastroenteritis incidents: a five-year study. J Med Virol 87(6):961–969. https://doi.org/10.1002/jmv.24154

Burrell CJ, Howard CR, Murphy FA (2017) Chapter 31—coronaviruses. In: Burrell CJ, Howard CR, Murphy FA (eds) Fenner and White's medical virology, 5th edn. Academic Press, London, pp 437–446

Cardemil CV, Parashar UD, Hall AJ (2017) Norovirus infection in older adults. Infect Dis Clin N Am 31(4):839–870. S0891552017300661. https://doi.org/10.1016/j.idc.2017.07.012

Carroll D, Daszak P, Wolfe ND et al (2018) The global virome project. Science 359(6378):872–874. https://doi.org/10.1126/science.aap7463

Cascella M, Rajnik M, Aleem A et al (2022) Features, evaluation, and treatment of coronavirus (COVID-19). In: Anonymous StatPearls StatPearls Publishing LLC, Treasure Island, FL

Caslake R, Soiza RL, Mangoni AA (2013) Practical advice for prescribing in old age. Medicine 41(1):9–12

Cates SC, Kosa KM, Brophy JE et al (2015) Consumer education needed on norovirus prevention and control: findings from a nationally representative survey of US. Adults J Food Prot 78(3):484–490. https://doi.org/10.4315/0362-028X.JFP-14-313

Centre for Disease Control (2022). https://www.cdc.gov/dengue/healthcare-providers/diagnosis.html. Accessed 11 May 2022

Centre for Disease Control (2004–2019) National notifiable diseases surveillance system figure 2.4. Rates of reported acute hepatitis B virus infection, by age group—United States. https://www.cdc.gov/hepatitis/statistics/2019surveillance/Figure2.4.htm. Accessed 04/05 2022

Cereda E, Pedrolli C (2009) The geriatric nutritional risk index. Curr Opin Clin Nutr Metab Care 12(1):1–7. https://doi.org/10.1097/MCO.0b013e3283186f59

Chroboczek J, Szurgot I, Szolajska E (2014) Virus-like particles as vaccine. Acta Biochim Pol 61(3):531–539. 2014_863 [pii]

Chhabra P, de Graaf M, Parra GI, Chan MC-W, Green K, Martella V, Wang Q, White PA, Katayama K, Vennema H, Koopmans MPG, Vinjé J (2019) Updated classification of norovirus genogroups and genotypes. J Gen Virol 100(10):1393–1406. https://doi.org/10.1099/jgv.0.001318

Clarke EL, Evans JR, Smeeth L (2018) Community screening for visual impairment in older people. Cochrane Database Syst Rev 2(2):CD001054. https://doi.org/10.1002/14651858.CD001054.pub3

Collins CL, Pollard AJ (2002) Respiratory syncytial virus in children and adults. J Infect 45(1):10–17

Dahal R, Bista S (2022) Strategies to reduce polypharmacy in the elderly [updated 2022 Feb 22]. In: Anonymous StatPearls [internet] StatPearls Publishing

Davies LE, Spiers G, Kingston A et al (2020) Adverse outcomes of polypharmacy in older people: systematic review of reviews. J Am Med Dir Assoc 21(2):181–187. https://doi.org/10.1016/j.jamda.2019.10.022

Davis JP (2005) Experience with hepatitis A and B vaccines. Am J Med 118(Suppl 10A):7S–15S. S0002-9343(05)00581-4 [pii]

Dias FLDC, Teixeira AL, Guimarães HC et al (2017) Accuracy of the 15-item geriatric depression scale (GDS-15) in a community-dwelling oldest-old sample: the Pietà study. Trends Psychiatry Psychother 39(4):276–279. S2237-60892017000400276 [pii]

Duncan JD, Urbanowicz RA, Tarr AW et al (2020) Hepatitis C virus vaccine: challenges and prospects. Vaccine 8(1)

Dworkin RHJR, Breuer J, Gnann JW et al (2007) Recommendations for the management of herpes zoster. Clin Infect Dis 1(44 Suppl 1):S1–S26

European Association for the Study of the Liver (2017) EASL 2017 clinical practice guidelines on the management of hepatitis B virus infection. J Hepatol 67(2):370–398. Electronic address: easloffice@easloffice.eu. S0168-8278(17)30185-X [pii]

Farkas K, Green E, Rigby D et al (2021) Investigating awareness, fear and control associated with norovirus and other pathogens and pollutants using best-worst scaling. Sci Rep 11(1): 11194–11194. https://doi.org/10.1038/s41598-021-90704-7

Fisher D, Li CM, Chiu MS et al (2014) Impairments in hearing and vision impact on mortality in older people: the AGES-Reykjavik study. Age Ageing 43(1):69–76. https://doi.org/10.1093/ageing/aft122

Floreani A (2009) Liver disorders in the elderly. Best Pract Res Clin Gastroenterol 23(6):909–917. Management of GI Diseases in the Elderly. https://doi.org/10.1016/j.bpg.2009.10.005

Gardiner C, Laud P, Heaton T et al (2020) What is the prevalence of loneliness amongst older people living in residential and nursing care homes? A systematic review and meta-analysis. Age Ageing 49(5):748–757. https://doi.org/10.1093/ageing/afaa049

World Health Organization (2021) Social isolation and loneliness among older people, World Health Organization, Geneva

Glebe D, Bremer CM (2013) The molecular virology of hepatitis B virus. Semin Liver Dis 33(2): 103–112. https://doi.org/10.1055/s-0033-1345717

Goman AM, Lin FR (2016) Prevalence of hearing loss by severity in the United States. Am J Public Health 106(10):1820–1822. https://doi.org/10.2105/AJPH.2016.303299

Green K (2007) The noroviruses. In: Knipe D, Howley P (eds) Fields virology, 5th edn. Lippincott Williams and Wilkins, Philadelphia, pp 949–980

Hadinegoro SRS (2012) The revised WHO dengue case classification: does the system need to be modified? Paediatr Int Child Health 32(Suppl 1):33–38. https://doi.org/10.1179/2046904712Z.00000000052

Harris JP, Edmunds WJ, Pebody R, Brown DW, Lopman BA (2008) Deaths from Norovirus among the Elderly England and Wales. Emerg Infect Dis 14(10):1546–1552. https://doi.org/10.3201/eid1410.080188

Heinimäki S, Malm M, Vesikari T, Blazevic V (2018) Parenterally administered Norovirus GII.4 virus-like particle vaccine formulated with aluminum hydroxide or monophosphoryl lipid A adjuvants induces systemic but not mucosal immune responses in mice. J Immunol Res 2018:1–8. https://doi.org/10.1155/2018/3487095

Hsieh S, Schubert S, Hoon C et al (2013) Validation of the Addenbrooke's cognitive examination III in frontotemporal dementia and Alzheimer's disease. Dement Geriatr Cogn Disord 36(3–4): 242–250. https://doi.org/10.1159/000351671

Hsieh YH, Chen CW, Hsu SB (2004) SARS outbreak, Taiwan, 2003. Emerg Infect Dis 10(2): 201–206. 03-0515 [pii]

Huang C, Wang Y, Li X et al (2020) Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet 395(10223):497–506. https://doi.org/10.1016/S0140-6736(20)30183-5

Hughes ME, Waite LJ, Hawkley LC et al (2004) A short scale for measuring loneliness in large surveys: results from two population-based studies. Res Aging 26(6):655–672. https://doi.org/10.1177/0164027504268574

Hunt NJ, Kang SWS, Lockwood GP et al (2019) Hallmarks of aging in the liver. Comput Struct Biotechnol J 17:1151–1161. https://doi.org/10.1016/j.csbj.2019.07.021

Inns T, Wilson D, Manley P, Harris JP, O'Brien SJ, Vivancos R (2020) What proportion of care home outbreaks are caused by norovirus? An analysis of viral causes of gastroenteritis outbreaks in care homes North East England 2016–2018. BMC Infect Dis 20(1):2. https://doi.org/10.1186/s12879-019-4726-4

Jhaveri MA, Manne V, Kowdley KV (2018) Chronic hepatitis C in elderly patients: current evidence with direct-acting antivirals. Drugs Aging 35(2):117–122. https://doi.org/10.1007/s40266-017-0515-1

Jiang S, Hillyer C, Du L (2020) Neutralizing antibodies against SARS-CoV-2 and other human coronaviruses. Trends Immunol 41(5):355–359. S1471-4906(20)30057-0 [pii]

John A, Canaday DH (2017) Herpes zoster in the older adult. Infect Dis Clin North Am 31(4):811–826. https://doi.org/10.1016/j.idc.2017.07.016

Kawai K, Yawn BP, Wollan P, Harpaz R (2016) Increasing incidence of herpes zoster over a 60-year period from a population-based study. Clin Infect Dis 63(2):221–226

Kemp L, Clare KE, Brennan PN et al (2019) New horizons in hepatitis B and C in the older adult. Age Ageing 48(1):32–37. https://doi.org/10.1093/ageing/afy170

Kim L, Liebowitz D, Lin K, Kasparek K, Pasetti MF, Garg SJ, Gottlieb K, Trager G, Tucker SN (2018) Safety and immunogenicity of an oral tablet norovirus vaccine a phase I randomized placebo-controlled trial. JCI Insight 3(13):e121077. https://doi.org/10.1172/jci.insight.121077

Kondo Y, Tsukada K, Takeuchi T et al (1993) High carrier rate after hepatitis B virus infection in the elderly. Hepatology 18(4):768–774. https://doi.org/10.1002/hep.1840180404

Kondrup J, Rasmussen HH, Hamberg O et al (2003) Nutritional risk screening (NRS 2002): a new method based on an analysis of controlled clinical trials. Clin Nutr 22(3):321–336. S0261561402002145 [pii]

Kroneman A, Vega E, Vennema H et al (2013) Proposal for a unified norovirus nomenclature and genotyping. Arch Virol 158(10):2059–2068. https://doi.org/10.1007/s00705-013-1708-5

Kuluratne SA (2015) Dengue fever. BMJ 351:h4661

Lai MM, Cavanagh D (1997) The molecular biology of coronaviruses. Adv Virus Res 48:1–100. S0065-3527(08)60286-9 [pii]

Leij-Halfwerk S, Verwijs MH, van Houdt S et al (2019) Prevalence of protein-energy malnutrition risk in European older adults in community, residential and hospital settings, according to 22 malnutrition screening tools validated for use in adults ≥65 years: a systematic review and meta-analysis. Maturitas 126:80–89. S0378-5122(19)30114-8 [pii]

Li HC, Lo SY (2015) Hepatitis C virus: virology, diagnosis and treatment. World J Hepatol 7(10):1377–1389. https://doi.org/10.4254/wjh.v7.i10.1377

Li Q, Guan X, Wu P et al (2020) Early transmission dynamics in Wuhan, China, of novel coronavirus-infected pneumonia. N Engl J Med 382(13):1199–1207. https://doi.org/10.1056/NEJMoa2001316

Liang G, Bushman FD (2021) The human virome: assembly, composition and host interactions. Nat Rev Microbiol 19:514–527

Malnutrition Advisory Group (2003) The "MUST" explanatory booklet. A guide to the 'Malnutrition Universal Screening Tool' ('MUST') for Adults.BAPEN

Mason RJ (2020) Pathogenesis of COVID-19 from a cell biology perspective. Eur Respir J 55(4):2000607. https://doi.org/10.1183/13993003.00607-2020

McKeating C, Cadden I, McDougall N et al (2018) Progression from acute to chronic hepatitis B is more common in older adults. Ulster Med J 87(3):177–180

Melhem NM (2016) Norovirus vaccines: correlates of protection challenges and limitations. Hum Vaccin Immunother 12(7):1653–1669. https://doi.org/10.1080/21645515.2015.1125054

Min L, Kerr EA, Blaum CS et al (2014) Contrasting effects of geriatric versus general medical multimorbidity on quality of ambulatory care. J Am Geriatr Soc 62(9):1714–1721. https://doi.org/10.1111/jgs.12989

Nasreddine ZS, Phillips NA, Bédirian V et al (2005) The Montreal cognitive assessment, MoCA: a brief screening tool for mild cognitive impairment. J Am Geriatr Soc 53(4):695–699. JGS53221 [pii]

Neumann SA, Miller MD, Daniels L et al (2005) Nutritional status and clinical outcomes of older patients in rehabilitation. J Hum Nutr Diet 18(2):129–136. JHN596 [pii]
Nooraei S, Bahrulolum H, Hoseini ZS, Katalani C, Hajizade A, Easton AJ, Ahmadian G (2021) Virus-like particles: preparation immunogenicity and their roles as nanovaccines and drug nanocarriers. J Nanobiotechnol 19(1):59. https://doi.org/10.1186/s12951-021-00806-7
Parra GI, Green KY (2014) Sequential gastroenteritis episodes caused by 2 norovirus genotypes. Emerg Infect Dis 20(6):1016–1018. https://doi.org/10.3201/eid2006.131627
Parrino TA, Schreiber DS, Trier JS et al (1977) Clinical immunity in acute gastroenteritis caused by Norwalk agent. N Engl J Med 297(2):86–89. https://doi.org/10.1056/NEJM197707142970204
Payne S (2017) Chapter 4—methods to study viruses, Viruses Academic Press, pp 37–52
Plotkin SA, Gilbert PB (2012) Nomenclature for immune correlates of protection after vaccination. Clin Infect Dis 54(11):1615–1617. https://doi.org/10.1093/cid/cis238
Prasad BVV, Hardy ME, Dokland T, Bella J, Rossmann MG, Estes MK (1999) X-ray crystallographic structure of the Norwalk virus capsid. Science 286(5438):287–290. https://doi.org/10.1126/science.286.5438.287
Rajapakse S (2011) Dengue shock. J Emerg Trauma Shock 4:120. https://doi.org/10.4103/0974-2700.76835
Ramani S, Estes MK, Atmar RL (2016) Chapter 3.6—norovirus vaccine development, pp 447–469
Reuben DB, Solomon DH (1989) Assessment in geriatrics. Of caveats and names. J Am Geriatr Soc 37(6):570–572. https://doi.org/10.1111/j.1532-5415.1989.tb05691.x
Rockwood K, Song X, MacKnight C et al (2005) A global clinical measure of fitness and frailty in elderly people. CMAJ 173(5):489–495. 173/5/489 [pii]
Rubenstein LZ (2006) Falls in older people: epidemiology, risk factors and strategies for prevention. Age Ageing 35(Suppl 2):ii37–ii41. 35/suppl_2/ii37 [pii]
Saito M, Goel-Apaza S, Espetia S et al (2014) Multiple norovirus infections in a birth cohort in a Peruvian Periurban community. Clin Infect Dis 58(4):483–491. https://doi.org/10.1093/cid/cit763
Sakurai R, Yasunaga M, Nishi M et al (2019) Co-existence of social isolation and homebound status increase the risk of all-cause mortality. Int Psychogeriatr 31(5):703–711. https://doi.org/10.1017/S1041610218001047
Saliba D, Elliott M, Rubenstein LZ et al (2001) The vulnerable elders survey: a tool for identifying vulnerable older people in the community. J Am Geriatr Soc 49(12):1691–1699. 49281 [pii]
Seematter-Bagnoud L, Büla C (2018) Brief assessments and screening for geriatric conditions in older primary care patients: a pragmatic approach. Public Health Rev 39(1):8. https://doi.org/10.1186/s40985-018-0086-7
Shakarchi AF, Assi L, Ehrlich JR et al (2020) Dual sensory impairment and perceived everyday discrimination in the United States. JAMA Ophthalmol 138(12):1227–1233. https://doi.org/10.1001/jamaophthalmol.2020.3982
Shirbhate E, Pandey J, Patel VK et al (2021) Understanding the role of ACE-2 receptor in pathogenesis of COVID-19 disease: a potential approach for therapeutic intervention. Pharmacol Rep 73(6):1539–1550. https://doi.org/10.1007/s43440-021-00303-6
Siebenga JJ, Vennema H, Renckens B et al (2007) Epochal evolution of GGII.4 norovirus capsid proteins from 1995 to 2006. J Virol 81(18):9932–9941. https://doi.org/10.1128/JVI.00674-07
Siebenga JJ, Vennema H, Zheng D et al (2009) Norovirus illness is a global problem: emergence and spread of norovirus GII.4 variants, 2001–2007. J Infect Dis 200(5):802–812. https://doi.org/10.1086/605127
Simning A, Fox ML, Barnett SL et al (2019) Depressive and anxiety symptoms in older adults with auditory, vision, and dual sensory impairment. J Aging Health 31(8):1353–1375. https://doi.org/10.1177/0898264318781123
Sims AC, Baric RS, Yount B et al (2005) Severe acute respiratory syndrome coronavirus infection of human ciliated airway epithelia: role of ciliated cells in viral spread in the conducting airways of the lungs. J Virol 79(24):15511–15524. 79/24/15511 [pii]

Soiza RL, Myint PK (2019) The Scottish intercollegiate guidelines network (SIGN) 157: guidelines on risk reduction and management of delirium. Medicina 55(8):491. https://doi.org/10.3390/medicina55080491
Soiza RL, Scicluna C, Thomson EC (2021) Efficacy and safety of COVID-19 vaccines in older people. Age Ageing 50(2):279–283
Stratton RJ, Green CJ, Elia M (2003) Disease-related malnutrition: an evidence-based approach to treatment. CABI Pub
Struyf T, Deeks JJ, Dinnes J et al (2020) Signs and symptoms to determine if a patient presenting in primary care or hospital outpatient settings has COVID-19 disease. Cochrane Database Syst Rev 7(7):CD013665. https://doi.org/10.1002/14651858.CD013665
Tan M, Huang P, Xia M et al (2011) Norovirus P particle, a novel platform for vaccine development and antibody production. J Virol 85(2):753–764. https://doi.org/10.1128/JVI.01835-10
Tan M, Jiang X (2012) Norovirus P particle: a subviral nanoparticle for vaccine development against norovirus, rotavirus and influenza virus. Nanomedicine (Lond) 7(6):889–897. https://doi.org/10.2217/nnm.12.62
Tan M (2021) Norovirus vaccines: current clinical development and challenges. Pathogens 10 (12):1641. https://doi.org/10.3390/pathogens10121641
Tseng YC, Liu SH, Lou MF et al (2018) Quality of life in older adults with sensory impairments: a systematic review. Qual Life Res 27(8):1957–1971. https://doi.org/10.1007/s11136-018-1799-2
Turner G, Gordon A, Keeble M et al (2019) Comprehensive geriatric assessment toolkit for primary care practitioners
van Doremalen N, Bushmaker T, Morris DH et al (2020) Aerosol and surface stability of SARS-CoV-2 as compared with SARS-CoV-1. N Engl J Med 382(16):1564–1567. https://doi.org/10.1056/NEJMc2004973
Vega E, Barclay L, Gregoricus N et al (2013) Genotypic and epidemiologic trends of norovirus outbreaks in the United States, 2009-2013. J Clin Microbiol 52. https://doi.org/10.1128/JCM.02680-13
Vekemans J, Moorthy V, Giersong B et al (2019) Respiratory syncytial virus vaccine research and development: World Health Organization technological roadmap and preferred product characteristics. Vaccine 37(50):7394–7395
Wallace M, Shelkey M, Hartford Institute for Geriatric Nursing (2007) Katz index of Independence in activities of daily living (ADL). Urol Nurs 27(1):93–94
Wang H, Yang P, Liu K et al (2008) SARS coronavirus entry into host cells through a novel clathrin- and caveolae-independent endocytic pathway. Cell Res 18(2):290–301. https://doi.org/10.1038/cr.2008.15
Wang L, He W, Yu X et al (2020) Coronavirus disease 2019 in elderly patients: characteristics and prognostic factors based on 4-week follow-up. J Infect 80(6):639–645. https://doi.org/10.1016/j.jinf.2020.03.019
Wenger NS, Solomon DH, Roth CP et al (2003) The quality of medical care provided to vulnerable community-dwelling older patients. Ann Intern Med 139(9):740–747. 139/9/740 [pii]
Whitley RJ, Roizman B (2001) Herpes simplex virus infections. Lancet 357(9267):1513–1518. https://doi.org/10.1016/S0140-6736(00)04638-9
Wolinsky FD, Coe RM, McIntosh WA et al (1990) Progress in the development of a nutritional risk index. J Nutr 120:1549–1553. https://doi.org/10.1093/jn/120.suppl_11.1549
World Health Organisation (2009) Dengue guidelines for diagnosis treatment prevention and control
World Health Organisation (2017a) Mental health of older adults [Fact sheet]
World Health Organisation (2017b) Global hepatitis report, 2017
World Health Organisation (2019) www.who.int/news/item/05-06-2019-23-million-people-falling-ill-from-unsafe-food-eachyear-in-europe-is-just-the-tip-of-the-iceberg
World Health Organisation (2021) Dementia. https://www.who.int/news-room/fact-sheets/detail/dementia. Accessed 13 May 2022

World Health Organisation (2022a) Dengue and severe dengue. In: World Health Organization. https://apps.who.int/mediacentre/factsheets/fs117/en/index.html. Accessed 25 May 2022

World Health Organisation (2022b) WHO coronavirus (COVID-19) dashboard. https://covid19.who.int/. Accessed 27 June 2022

World Health Organisation (2022c) WHO Director-General's opening remarks at the media briefing on COVID-19—11 March 2020. https://www.who.int/director-general/speeches/detail/who-director-general-s-opening-remarks-at-the-media-briefing-on-covid-19. 11 March 2020. Accessed 26 June 2022

Wu Z, McGoogan JM (2020) Characteristics of and important lessons from the coronavirus disease 2019 (COVID-19) outbreak in China: summary of a report of 72 314 cases from the Chinese Center for Disease Control and Prevention. JAMA 323(13):1239–1242. https://doi.org/10.1001/jama.2020.2648

Xia M, Tan M, Wei C et al (2011) A candidate dual vaccine against influenza and noroviruses. Vaccine 29(44):7670–7677. https://doi.org/10.1016/j.vaccine.2011.07.139

Xu Z, Shi L, Wang Y et al (2020) Pathological findings of COVID-19 associated with acute respiratory distress syndrome. Lancet Respir Med 8(4):420–422. S2213-2600(20)30076-X [pii]

Yang K, Victor C (2011) Age and loneliness in 25 European nations. Ageing Soc 31(8):1368–1388. https://doi.org/10.1017/S0144686X1000139X

Yesavage JA, Brink TL, Rose TL et al (1982) Development and validation of a geriatric depression screening scale: a preliminary report. J Psychiatr Res 17(1):37–49. https://doi.org/10.1016/0022-3956(82)90033-4

Zazove P, Plegue MA, McKee MM et al (2020) Effective hearing loss screening in primary care: the early auditory referral-primary care study. Ann Fam Med 18(6):520–527. https://doi.org/10.1370/afm.2590

Chapter 9
Models and Biomarkers for Ovarian Ageing

Tom Kelsey

Abstract The human ovarian reserve is defined by the number of non-growing follicles (NGFs) in the ovary, with the age-related decline in NGF population determining age at menopause for healthy women. In this chapter, the concept of ovarian reserve is explored in detail, with a sequence of models described that in principle allow any individual to be compared to the general population. As there is no current technology that can count the NGFs in a living ovary, we move our focus to biomarkers for the ovarian reserve. Using serum analysis and ultrasound it is possible to measure anti-Müllerian hormone (AMH), follicle-stimulating hormone (FSH), and ovarian volume (OV) and to count numbers of antral follicles (AFC). These are compared, with ovarian volume being the closest to a true biomarker for a wide range of ages and with AMH and AFC being the most popular for post-pubertal and pre-menopausal ages. The study of genetic and subcellular biomarkers for the ovarian reserve has produced less concrete results. Recent advances are described and compared in terms of limitations and potential. The chapter concludes with an overview of the future study indicated by our current knowledge and by current controversy in the field.

Keywords Ovarian reserve · Mathematical model · Reproductive age · Biomarker · Fertility · Menopause

Abbreviations

AFC	Antral follicle count
AMH	Anti-Müllerian hormone
DHEA	Dehydroepiandrosterone
FSH	Follicle-stimulating hormone

T. Kelsey (✉)
School of Computer Science, University of St Andrews, St Andrews, UK
e-mail: twk@st-andrews.ac.uk

J. R. Harris, V. I. Korolchuk (eds.), *Biochemistry and Cell Biology of Ageing: Part IV, Clinical Science*, Subcellular Biochemistry 103,
https://doi.org/10.1007/978-3-031-26576-1_9

IVF	In vitro fertilization
NGF	Non-growing follicles
OV	Ovarian volume
PACAP	Pituitary adenylate cyclase-activating polypeptide
PI3K	Phosphatidylinositol 3 kinase
POI	Premature ovarian insufficiency

Introduction

The human ovary has a population of non-growing follicles (henceforth NGFs) that develops in early foetal life, peaks at about 20–22 weeks, and then declines as age increases. NGFs are important as they are recruited towards maturation to a mature egg which can then be fertilized, hopefully leading to a live birth. However, most of the recruited NGFs do not make it this far, dying off though apoptosis and atresia at varying stages before full maturation. Once the NGF population in an ovary falls below a few hundred, there are insufficient numbers to support full maturation. No further eggs are then produced and the woman moves into post-menopausal life, with menopause being defined as the cessation of ovulation owing to a loss of NGFs (Gosden 1985).

Not all women go through menopause at the same age. A landmark study suggested an age of 50–51 years (Treloar et al. 1967), confirmed by a later large-scale study reporting 50.1 years with a standard deviation of 4.2 years, so that age at menopause for 68% of healthy women will be between 45.9 and 54.3 years (van Noord et al. 1997). Many studies have considered factors that modify expected age at menopause, such as race, parity, contraception, age at menarche, and lifestyle attributes. Much of the evidence is either of poor quality or contradictory, with only lower parity and a history of smoking being conclusively associated with slightly earlier than expected menopause (Gold 2011). Hence the key driver for age at menopause is the NGF population, and we can introduce the concept of reproductive age as an adjunct to chronological age: a woman aged 30 years (say) having an NGF population that will be sufficient for 20 years has a reproductive age of 30 years (i.e. her age at menopause will coincide with the population average). If, however, her NGF reserve is insufficient in 15 years' time, her reproductive age is 35 (i.e. she has the NGF reserve of the average 35 year old).

It should be noted that ovarian ageing is more complex than a simple decline in the population of important cells, with an early study concluding that diminished ability to absorb increasing amounts of fibrous tissue and ovarian vessel wall thickening and hyalinization were additional factors to diminishing NGF reserve (Ringrose 1963). However, the increase in fibrous tissue is directly related to atrophy of both NGFs and more mature follicles, and vessel wall thickness is not considered an important factor in remaining fertile life. It is therefore reasonable to consider NGF population to be the primary factor influencing human reproductive age.

Ultrasound scans routinely identify antral and pre-antral follicles in living ovarian tissue, but these are larger and more mature follicles, with NGFs being 30–50 μm in diameter and distributed throughout the ovarian cortex. There is no current technology for scanning a live human ovary in order to determine the NGF population. The modelling of ovarian ageing is therefore based on histological samples of human ovarian tissue from subjects with known chronological age. Pioneering work both developed the histological techniques and supplied important data (Baker 1963; Block 1951, 1952). Later studies applied these techniques to ante- and neonatal subjects (Bendsen et al. 2006; Forabosco and Sforza 2007) and to post-natal subjects up to menopausal ages (Richardson et al. 1987; Gougeon and Chainy 1987). The task of the ovarian reserve modeller is to synthesize these and similar data into datapoints (age and counted NGFs) that underpin an age-related normative model that (a) supplies an estimate of the NGFs in a human ovary of known chronological age and (b) an estimate of the levels of variation at a given age so that we can produce normal scores (also known as z-scores) and hence compare a given NGF count to the normal count for that age.

Early NGF modelling attempts were basic and based on two assumptions: There are one million NGFs in the average ovary at birth and there are 1000 at menopause. The model is then a simple exponential decay from birth to age 50. More sophisticated approaches were data driven, using combined data from histological examinations to identify a model with good fit to the existing data. Faddy and Gosden produced the first examples: one a biphasic decay with a sharp decline after age 38 years (Faddy et al. 1992) and the other the solution of a differential equation that incorporates expected age at menopause (Faddy and Gosden 1996). A power model was developed using data from 122 subjects at the same centre, predicting—rather than using—realistic ages at menopause and providing useful prediction limits that model individual variability (Hansen et al. 2008). Predictive power can be increased by combining the 122 datapoints with all other available data, and it is possible to incorporate the growth stage—from conception to 20–22 weeks' gestation—by requiring any model to show zero NGF population at conception. This resulted in a 4-parameter peak model (Wallace and Kelsey 2010) that has been externally validated by comparing its predictions to new observations with minimal differences at all ages (Depmann et al. 2015; McLaughlin et al. 2015, 2017).

This would normally be the end of any modelling research, as external validation is the gold standard for acceptance of any biomedical predictive model. However, research into how and why specific NGFs are recruited has continued, with our current understanding remaining incomplete. Part of this research has motivated an update (Fig. 9.1a) to the currently accepted best model that uses random-walk techniques applied to the same dataset (Johnson et al. 2022). It is a significant improvement on previous models as it (1) is in broad agreement with expected aspects of age-related follicle populations dynamics and hence can also be considered externally validated (Fig. 9.1c), (2) is forward looking (rather than reporting a regularized best fit to a static dataset), (3) is more closely related to the stresses and

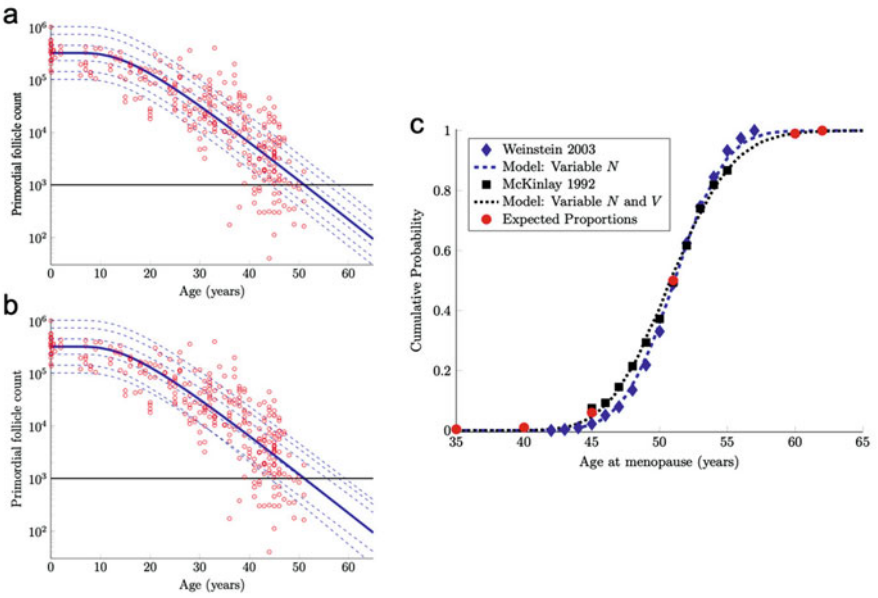


Fig. 9.1 Random-walk modelling of ovarian ageing, reproduced with permission from (Johnson et al. 2022). The continuous blue line in Panel (**a**) shows the best known age-related model of the human ovarian reserve based on data from a previous externally validated model (Wallace and Kelsey 2010), with dashed lines indicating trajectories for women having low or high NGF populations at birth. In Panel (**b**), the crossed blue lines show that the curves in (**a**) may be too simplistic and that trajectories can vary to a limited extent across individuals. Panel (**c**) illustrates that the model closely predicts age at menopause, despite this information not being used to derive the model

internal structure of the ovary, and (4) allows further detailed investigation of poorly understood aspects ovarian physiology and endocrinology (Fig. 9.1b). The random-walk model allows NGF population at birth to be augmented with drift variability, allowing the empirical testing of a key—but as yet not formally validated—assumption in previous models, namely that a large/small starting supply is linked to a late/early menopause. Initial results indicate that this assumption is not completely valid but is still a reasonable approximation, and hence we continue with the paradigm that the NGF population at birth indicates an age at menopause, and so reproductive age is determined by comparing the current NGF population in a human female to the average predicted by the random-walk model: low/high initial peak leads to early/late age at menopause.

As noted earlier, we are unable to directly count (or even estimate) the NGF populations in a living subject. We therefore seek indirect biomarkers for the remaining ovarian reserve.

Physiological and Endocrine Biomarkers

Biomarkers are closely related to the technology used to obtain values. For ovaries this means ultrasound (either transvaginal or transabdominal using the bladder as a lens in younger subjects) or blood assays that quantify levels of fertility-related hormones. Before looking at these in detail, it is important to note that most such biomarker investigation is into ovarian response with a specific focus on assisted conception. Whilst it is known that ovarian response declines with increasing age, it is overly simplistic to take the view that good/poor ovarian response is directly associated with high/low age-related NGF counts, and direct translation of biomarker results to women typically aged 25–40 years to other ages is potentially in gross error.

We first consider physiological features that can be resolved using ultrasound: antral follicle counts (AFC) and ovarian volume (OV).

The developmental pathway from NGF to ovulation—folliculogenesis—is associated with approximately a 500-fold increase in follicular diameter. Antral follicles are small cystic structures within the ovaries, at an intermediate stage of folliculogenesis between NGF and mature egg. Some antral follicles are smaller than 2 mm in diameter and are thus not readily identifiable using ultrasound. It is therefore common to define antral follicles to be 2–10 mm in diameter, representing the smallest easily identifiable follicles. Most antral follicles are lost through atresia, with only 300–400 eggs ovulated during reproductive life. The theoretical basis for using AFC as a biomarker is that the proportion of NGFs recruited towards maturation that reach the antral follicle stage is roughly constant, and hence a high/low AFC indicates high/low age-related ovarian reserve. This theory has been extensively tested and validated for ages 25–50 years, with large-scale longitudinal and cross-sectional studies showing a strong negative correlation (about 70%) between age and AFC (La Marca et al. 2011; Scheffer et al. 1999). In addition, AFC is related to age at menopause (Broekmans et al. 2004), there is moderate intercycle and interobserver variability in AFC values (Hansen et al. 2003), and infertile women are known to have smaller AFC than fertile women of the same age (Iliodromiti et al. 2016). Taken together, there is strong evidence that AFC reflects the remaining NGF pool in women aged 25 years or older. There is sparse evidence on AFC for younger ages, in particular at peri- and pre-pubertal ages. It is known that folliculogenesis proceeds at all ages for which the NGF pool is large enough, with only 22% of the original pool remaining at age 25 (Wallace and Kelsey 2010), so the above theory would suggest AFC decreasing from a maximum at birth to menopause. However, the limited evidence available suggests that AFC is low (2–5 follicles) at birth rising slowly to 10–25 follicles at 15 years (Spencer et al. 2013) and then declining towards zero at menopausal ages. This positive correlation between age and AFC for younger ages suggests that AFC does not reflect the NGF pool, with other endocrine and paracrine factors coming into play.

Ovarian volume is easily calculated in either 2D or 3D ultrasound. For 2D, the ovaries are measured in three planes and ovarian volume is calculated using a prolate

ellipsoid formula; modern 3D ultrasound has software that automatically calculates volumes. There is good evidence that adult OV decreases with increasing age as the remaining pool of NGFs becomes exhausted. As part of an ovarian cancer screening programme, 13,963 women between 25 and 91 years of age underwent annual transvaginal sonography. From 58,673 observations of ovarian volume, a statistically significant decrease in OV was shown with each decade of life from the age of 30 to 70 years. Mean OV was 4.9 mL in pre-menopausal women and 2.2 mL in post-menopausal women (Pavlik et al. 2000). Correlation between measured OV and modelled NGF populations is very strong (over 95%), allowing a direct and accurate estimate of the NGFs in an ovary from an OV measurement (Wallace and Kelsey 2004). Again, the theory that a large/small ovary contains a high/low number of NGFs breaks down for younger ages, with ovaries being undetectable at ages up to 7 years and a mean OV of 5.8 mL at age 17 (Ivarsson et al. 1983). Hence—as with AFC—it would seem that the use of OV as a direct biomarker to the ovarian reserve is limited to post-pubertal ages. This is not the case, as careful combination of age-related NGF and OV models can be used to reliably predict mean NGF density in healthy females. The hypothesis is that despite the large variation in normal NGF populations (e.g. at age 25 years 95% of ovaries will have between 8000 and 546,000 NGFs (Wallace and Kelsey 2010)) the average number contained in one cubic millilitre of the ovarian cortex is similar for most women of the same age. This hypothesis has been addressed by estimating the proportion of an ovary that forms the cortex (i.e. the physiological location of the NGFs) and then simply dividing the predicted NGF number by the predicted OV at that chronological age. This method produced good results when compared to 13 density counts from a single laboratory, with 87% correlation between observations and predictions, and was externally validated by showing 90% correlation when using 15 densities from the published literature (McLaughlin et al. 2015). The approach continues to be used when assessing ovarian reserve in the younger than 25 age group, with further model validation from studies comparing controls to subjects with Turner syndrome (Mamsen et al. 2019), galactosemia (Mamsen et al. 2018), and exposure to chemotherapy (El Issaoui et al. 2016; McLaughlin et al. 2017). OV can therefore be used to predict NGF populations in healthy females for ages ranging from childhood to post-menopausal ages, and can hence be used to compare estimated reproductive age to known chronological age in a variety of settings.

The key endocrine biomarkers are follicle-stimulating hormone (FSH) and anti-Müllerian hormone (AMH), both measured by assay from serum samples. It is known that Inhibin B falls from age 30 to age 44 and is hence correlated with NGF decline, but its significance as a biomarker is much lower than that for AMH ($p = 0.16$ for Inhibin B; $p < 0.001$ for AMH (Steiner et al. 2017).

FSH can be considered a binary biomarker: low levels indicate pre-menopause and high levels post-menopause. Serum FSH levels are constant at about 7 IU/L from ages 30 to 44 (Steiner et al. 2017) and are consistently elevated to 30 IU/L or higher in menopausal women. A single high level may be followed by a lower one in a later cycle, normally indicating perimenopause. The reasons behind this are well understood. The primary purpose of FSH is to cause ovarian follicles to enlarge and

produce estrogen. AST menopause only 500–1000 NGFs remain in an ovary, not enough to be stimulated, and thus estrogen levels decline as a woman ages. This decline in estrogen leads to an increase in FSH as there is not enough estrogen being produced to downregulate the production of FSH by the hypothalamic/pituitary axis.

AMH is a member of the transforming growth factor superfamily, similar to inhibins. In the ovary AMH is produced by the granulosa cells of developing follicles and has been shown in AMH knock-out mice to be able to inhibit the initiation of NGF maturation and FSH-induced follicle growth (Durlinger et al. 1999). AMH is widely accepted as a biomarker for ovarian response in assisted conception cycles (Iliodromiti et al. 2014; Broer et al. 2011). As with AFC, serum AMH levels are associated with numbers of growing follicles and hence both measures directly reflect ovarian activity, with a more active ovary being more likely to produce the mature egg(s) needed for a successful IVF outcome. It has been hypothesized that a more active ovary has more NGFs than a less active one, so that AMH could be considered a biomarker for the ovarian reserve, at least from its peak at about age 25 years (Kelsey et al. 2011, 2012). However, if this were true then repeated AMH measurements would be useful in predicting age at menopause, and there is conflicting evidence in the literature for this claim with one large study concluding that knowledge of the rate of AMH decline does not improve menopause prediction (de Kat et al. 2019), and two studies report the opposite view (Ramezani Tehrani et al. 2021; Freeman et al. 2012). It may be the case that the conflicts are due to cohort selection, longitudinal methodology, and/or procedure for measuring AMH. In the absence of a categorical answer, it remains unclear how useful AMH is as a biomarker for ovarian ageing for ages above 25 years. In younger females AMH is still clearly related to ovarian activity but not to the ovarian reserve (Fig. 9.2). AMH has a small rise after birth (the mini puberty of the neonate (Lanciotti et al. 2018)) and then falls before rising to pubertal ages. During puberty AMH can rise and fall as the complex para- and endocrine milieu changes. There is a rise until age about 25 years, after which the decline in AMH is strongly correlated with NGF decline (Kelsey et al. 2011). Despite these concerns, AMH is being used as biomarker for ovarian ageing in many clinical settings (Fauser and Nelson 2020), such as diagnosis of premature ovarian insufficiency (i.e. very early menopause) after cytotoxic insults delivered as therapeutic treatments, assessing chances of a spontaneous pregnancy, personalization of assisted conception treatment options, and diagnosis of polycystic ovarian syndrome.

Genetic and Subcellular Biomarkers

Much less is known about actual and potential biomarkers that are taken from serum and/or ultrasound analysis. There are several inherent problems. Genetic studies can be done with knock-out mice, giving insights into rodent and hence mammalian and hopefully into human ovarian dynamics. But evidence from rodents need not translate into the human model at all. Moreover, in the case that rodents and humans

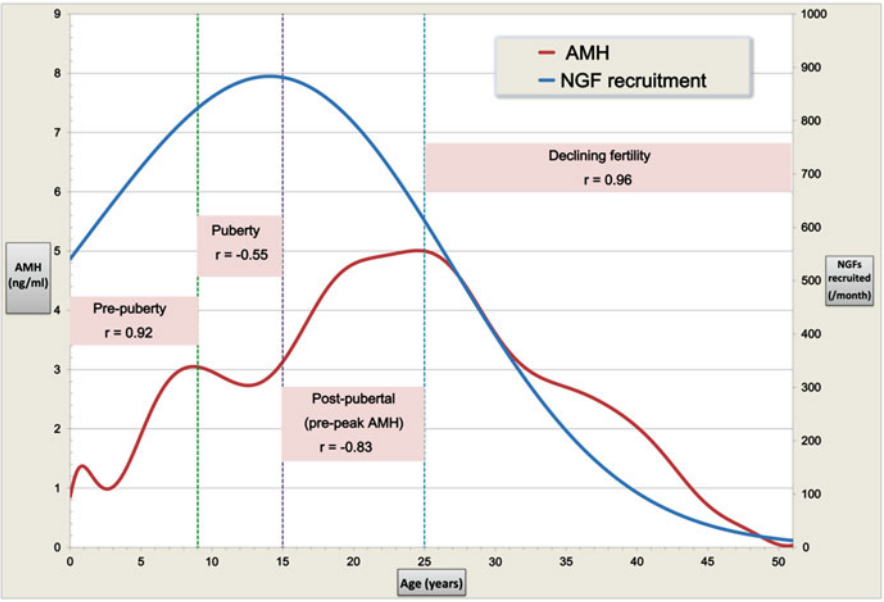


Fig. 9.2 Anti-Müllerian hormone (AMH) and follicular recruitment profile across the lifespan. Comparison of serum concentrations of AMH with recruitment rates of nongrowing follicles (NGF). The red line is the log-unadjusted validated AMH model (Kelsey et al. 2011), showing a peak at 24.5 years. The blue line denotes the numbers of NGFs recruited per month towards the maturation population of follicles (Wallace and Kelsey 2010), with peak numbers lost at age 14.2 years on average. Correlation coefficients (*r*) are given for AMH concentrations against follicular recruitment for each developmental phase; from birth to puberty (age 9 years), during puberty (9–15 years), post puberty (15–25 years), and mature adults (>25 years). Reproduced with permission from (Fleming et al. 2012)

are similar, strong evidence for similarity involves complex and expensive research. These investigations often focus on a well-defined endpoint rather than a slow ageing process, with genetic biomarkers being investigated for premature ovarian insufficiency (i.e. very early depletion of the NGF pool, often defined as less than 40 years of age; henceforth POI) or polycystic ovarian syndrome. As a result, the results are often binary in the same sense as FSH, and not easily age-related as found for AFC, AMH, and ovarian volume.

To give an example, Notch signalling is a relatively simple and evolutionarily conserved pathway associated with the recruitment, growth, migration, and death of cells. As such, it is an obvious starting point for ovarian ageing, where the recruitment of NGFs towards maturation, the growth and migration of granulosa cells (which surround ovarian follicles and both proliferate and change shape according to stage of development), and the subsequent death of most follicles before becoming eggs are all likely to be influenced by the Notch pathway. Notch receptors (Notch1 to Notch4) are encoded by different genes, with signalling induced by functional notch

ligands (Nye and Kopan 1995). Stimulation of the Notch1 pathway was associated with NGF maturation in the mouse model (Liu et al. 2016), with further studies suggesting that POI is improved by upregulation of Notch1 (Zhao and Dong 2018) and that the rare heterozygous variant in NOTCH2 may be associated with POI (Li et al. 2020). Taken together, these results suggest that the notch pathways could combine to provide a therapeutic target for POI (Guo et al. 2021). However, even if this were shown to be the case, we would still not have a biomarker for ovarian ageing at the population level. POI is rare, and its aetiology may generally involve a very low NGF population at birth but may also involve other factors not seen in the average healthy woman. A longitudinal study that measured regulation of Notch pathways in a large cohort of ageing women could provide important insights, but it would be hard to control for the contribution of other factors. We therefore know that Notch signalling is important in ovarian cell recruitment and death, but have limited means to develop this knowledge into a clinically validated and age-related model that covers the general female population.

Kisspeptin europeptides and associated KISS1 and KISS1R receptors are an important component of the hypothalamic–pituitary–gonadal axis, with both kisspeptin and its receptors expressed in the mammalian ovary and signalling associated with follicle recruitment and development towards ovulation in humans (Cejudo Roman et al. 2012). It could be the case that naturally occurring mutations (or dysfunction) are associated with faster rates of NGF recruitment and therefore reproductive age. In gene knock-out models, $Kiss1^{-/-}$ or $Kiss1R^{-/-}$ mice have shown small ovarian size and weight compared to controls, and analysis of pre- and post-pubertal mice suggests that there is an age-related expression of kisspeptin in the mouse ovary (Hu et al. 2017). There has only been partial translation to the human model, with KISS1R being the causative gene for idiopathic hypogonadotropic hypogonadism (de Roux et al. 2003) and a mutation of KISS1 probably being causative gene for the same condition (Topaloglu et al. 2012). Even though signalling between kisspeptin and its Gpr54 receptor appears to be essential for normal fertility with $Gpr54^{-/-}$ mice having no puberty and no cycles (Kirilov et al. 2013), the precise mechanisms are still obscure, and it remains to be shown that kisspeptin signalling has a measurable and reliable association with NGF recruitment and loss in healthy humans.

Other potential biomarkers include the WNT/beta-catenin pathway (Hernandez Gifford 2015; Harwood et al. 2008), pituitary adenylate cyclase-activating polypeptide (PACAP) as an NGF survival factor (Lee et al. 1999), the association of female infertility with the PI3K/PTEN/Akt and TSC/mTOR pathways (Makker et al. 2014), and phosphatidylinositol 3 kinase (PI3K)/PTEN-PDK1 signalling that controls the survival, loss, and activation of NGFs (Reddy et al. 2009). In each case, there is good initial evidence from mouse models that the pathway is important and can potentially be used in age-related models of the ovarian reserve, but the link to the healthy and general human model is not yet apparent.

Conclusions

Recent advances suggest that the use of models to identify and use biomarker information with respect to ovarian ageing is a solved problem. The key models for NGF population, ovarian volume, antral follicle count, and anti-Müllerian hormone have been externally validated by finding close agreement when matching predictions to new instances. In oncofertility and assisted conception, these biomarkers are routinely and successfully used in clinical practice for female ages 25 years and more. Antral follicle count and anti-Müllerian hormone are fully accepted as biomarkers of ovarian activity for these ages, with activity believed to be closely correlated to remaining ovarian reserve.

However there remains much that is poorly understood. The use of signalling pathways has so far been as a binary indicator (i.e. downregulation implies premature ovarian insufficiency) in the human model, instead of an age-related biomarker such as those described using blood tests and ultrasound.

The standard definition of ovarian ageing is the age-related depletion of the NGF population towards menopausal ages. However, egg quality is believed to be age-related and is known to be a strong determinant of reproductive fitness in humans. Other than preliminary studies involving zebrafish, there is little evidence for a genetic biomarker for egg quality. Dehydroepiandrosterone (DHEA) can be measured in blood samples, decreases from age 20 in women, has validated normative models, and is known to be associated with egg quality (since supplementing DHEA prior to IVF improves egg quality in general). However, DHEA has not been used to predict age at menopause, and hence its use as a putative biomarker for ovarian ageing is an avenue for future research.

Another useful future investigation would be to identify or rule out a step change in ovarian ageing that occurs in healthy women at the age of 37–38 years. Three distinct studies using different datasets have reported models that include such a step change, with strong acceleration of ovarian ageing occurring after the step change (Faddy et al. 1992; Johnson et al. 2022; Scheffer et al. 1999). These models have been deprecated for the most part since there is no known event similar to menarche or menopause that happens at this age. But it may be that these studies are reporting something real; it could be the case that our standard grouping of reproductive ages into pre-pubertal, fertile, and post-menopausal is too simplistic and that there are early- and late-reproductive ages defined by an as yet unknown event.

The mainstream literature and all textbooks posit as fact that the number of NGFs never increases after the peak at 20–22 weeks gestation. This appears to be true for the general population, as models that allowed renewal of the initial pool were found to be an inferior match to the histological data (Wallace and Kelsey 2010). In recent years, there has been a major controversy regarding ovarian stem cells, with some studies claiming to have identified and isolated them in humans (Woods and Tilly 2013; Virant-Klun et al. 2008) and other studies claiming that unequivocal evidence of their existence has yet to be produced (Horan and Williams 2017). The stakes are high: if it were possible to trigger ovarian stem cells to produce new and viable

NGFs there would be major implications for assisted conception, management of the menopause, and fertility preservation after treatment for cancer. In simple terms, ovarian ageing could be halted or even reversed. Much of the controversy is related to the types of stem cells (found in the ovary or in bone marrow) and the use of flow cytometry to sort them, with different research groups reporting contradictory outcomes. This is not unexpected, as the investigations involve isolating fixed and permeabilized cells after which live stem cells are sorted after staining for specific cell surface markers. The inherent complexity of choice for initial cell population and surface marker, together with laboratory-specific procedures and metrics, hinders both reproducibility and interpretation. Analysis of ovarian tissue after a specific chemotherapy regimen has shown a massive increase in NGFs (McLaughlin et al. 2017) thereby demonstrating that NGF regeneration can be expected after certain cytotoxic insults, but two important questions remain open. Firstly, are these new NGFs viable? Biovular and binucleated NGFs were observed in large proportions, so it may be the case that the renewed NGFs are for the most part unsuitable for maturation towards ovulation. Secondly, the histological examination is necessarily a snapshot of the population, providing no insights into which stem cells were activated and how. Much work is needed to further our understanding of the potential or impossibility for renewed and viable ovarian reserve.

We see that despite major advances, further work is needed in this important area. Ovarian ageing starts halfway between conception and birth and continues throughout life, with major impacts on puberty, fertility, the menopause, and postmenopausal life for all healthy women, and not just for conditions and illnesses affecting small percentages of women. The provision and validation of models and the identification and careful use of biomarkers are vital to the lives and relationships of all people.

References

Baker TG (1963) A quantitative and cytological study of germ cells in human ovaries. Proc R Soc Lond B Biol Sci 158:417–433. https://doi.org/10.1098/rspb.1963.0055

Bendsen E, Byskov AG, Andersen CY, Westergaard LG (2006) Number of germ cells and somatic cells in human fetal ovaries during the first weeks after sex differentiation. Hum Reprod 21(1): 30–35. https://doi.org/10.1093/humrep/dei280

Block E (1951) Quantitative morphological investigations of the follicular system in women; methods of quantitative determinations. Acta Anat (Basel) 12(3):267–285. https://doi.org/10.1159/000140549

Block E (1952) Quantitative morphological investigations of the follicular system in women; variations at different ages. Acta Anat (Basel) 14(1-2):108–123. https://doi.org/10.1159/000140595

Broekmans FJ, Faddy MJ, Scheffer G, te Velde ER (2004) Antral follicle counts are related to age at natural fertility loss and age at menopause. Menopause 11(6 Pt 1):607–614. https://doi.org/10.1097/01.gme.0000123643.76105.27

Broer SL, Dolleman M, Opmeer BC, Fauser BC, Mol BW, Broekmans FJ (2011) AMH and AFC as predictors of excessive response in controlled ovarian hyperstimulation: a meta-analysis. Hum Reprod Update 17(1):46–54. https://doi.org/10.1093/humupd/dmq034

Cejudo Roman A, Pinto FM, Dorta I, Almeida TA, Hernandez M, Illanes M, Tena-Sempere M, Candenas L (2012) Analysis of the expression of neurokinin B, kisspeptin, and their cognate receptors NK3R and KISS1R in the human female genital tract. Fertil Steril 97(5):1213–1219. https://doi.org/10.1016/j.fertnstert.2012.02.021

de Kat AC, van der Schouw YT, Eijkemans MJC, Broer SL, Verschuren WMM, Broekmans FJM (2019) Can menopause prediction be improved with multiple AMH measurements? Results from the prospective Doetinchem cohort study. J Clin Endocrinol Metab 104(11):5024–5031. https://doi.org/10.1210/jc.2018-02607

de Roux N, Genin E, Carel JC, Matsuda F, Chaussain JL, Milgrom E (2003) Hypogonadotropic hypogonadism due to loss of function of the KiSS1-derived peptide receptor GPR54. Proc Natl Acad Sci U S A 100(19):10972–10976. https://doi.org/10.1073/pnas.1834399100

Depmann M, Faddy MJ, van der Schouw YT, Peeters PH, Broer SL, Kelsey TW, Nelson SM, Broekmans FJ (2015) The relationship between variation in size of the primordial follicle pool and age at natural menopause. J Clin Endocrinol Metab 100(6):E845–E851. https://doi.org/10.1210/jc.2015-1298

Durlinger AL, Kramer P, Karels B, de Jong FH, Uilenbroek JT, Grootegoed JA, Themmen AP (1999) Control of primordial follicle recruitment by anti-Mullerian hormone in the mouse ovary. Endocrinology 140(12):5789–5796. https://doi.org/10.1210/endo.140.12.7204

El Issaoui M, Giorgione V, Mamsen LS, Rechnitzer C, Birkebaek N, Clausen N, Kelsey TW, Andersen CY (2016) Effect of first line cancer treatment on the ovarian reserve and follicular density in girls under the age of 18 years. Fertil Steril 106(7):1757–1762. e1751. https://doi.org/10.1016/j.fertnstert.2016.09.001

Faddy MJ, Gosden RG (1996) A model conforming the decline in follicle numbers to the age of menopause in women. Hum Reprod 11(7):1484–1486. https://doi.org/10.1093/oxfordjournals.humrep.a019422

Faddy MJ, Gosden RG, Gougeon A, Richardson SJ, Nelson JF (1992) Accelerated disappearance of ovarian follicles in mid-life: implications for forecasting menopause. Hum Reprod 7(10):1342–1346. https://doi.org/10.1093/oxfordjournals.humrep.a137570

Fauser B, Nelson SM (2020) Next steps toward AMH as a robust biomarker for assessing ovarian aging in individual women. J Clin Endocrinol Metab 105(7). https://doi.org/10.1210/clinem/dgaa220

Fleming R, Kelsey TW, Anderson RA, Wallace WH, Nelson SM (2012) Interpreting human follicular recruitment and antimullerian hormone concentrations throughout life. Fertil Steril 98(5):1097–1102. https://doi.org/10.1016/j.fertnstert.2012.07.1114

Forabosco A, Sforza C (2007) Establishment of ovarian reserve: a quantitative morphometric study of the developing human ovary. Fertil Steril 88(3):675–683. https://doi.org/10.1016/j.fertnstert.2006.11.191

Freeman EW, Sammel MD, Lin H, Boorman DW, Gracia CR (2012) Contribution of the rate of change of antimullerian hormone in estimating time to menopause for late reproductive-age women. Fertil Steril 98 (5):1254-1259 e1251-1252. https://doi.org/10.1016/j.fertnstert.2012.07.1139

Gold EB (2011) The timing of the age at which natural menopause occurs. Obstet Gynecol Clin North Am 38(3):425–440. https://doi.org/10.1016/j.ogc.2011.05.002

Gosden R (1985) Biology of the menopause: the causes and consequences of ovarian ageing. Academic Press, London

Gougeon A, Chainy GB (1987) Morphometric studies of small follicles in ovaries of women at different ages. J Reprod Fertil 81(2):433–442. https://doi.org/10.1530/jrf.0.0810433

Guo S, Quan S, Zou S (2021) Roles of the notch Signaling pathway in ovarian functioning. Reprod Sci 28(10):2770–2778. https://doi.org/10.1007/s43032-021-00610-6

Hansen KR, Morris JL, Thyer AC, Soules MR (2003) Reproductive aging and variability in the ovarian antral follicle count: application in the clinical setting. Fertil Steril 80(3):577–583. https://doi.org/10.1016/s0015-0282(03)00741-6

Hansen KR, Knowlton NS, Thyer AC, Charleston JS, Soules MR, Klein NA (2008) A new model of reproductive aging: the decline in ovarian non-growing follicle number from birth to menopause. Hum Reprod 23(3):699–708. https://doi.org/10.1093/humrep/dem408

Harwood BN, Cross SK, Radford EE, Haac BE, De Vries WN (2008) Members of the WNT signaling pathways are widely expressed in mouse ovaries, oocytes, and cleavage stage embryos. Dev Dyn 237(4):1099–1111. https://doi.org/10.1002/dvdy.21491

Hernandez Gifford JA (2015) The role of WNT signaling in adult ovarian folliculogenesis. Reproduction 150(4):R137–R148. https://doi.org/10.1530/REP-14-0685

Horan CJ, Williams SA (2017) Oocyte stem cells: fact or fantasy? Reproduction 154(1):R23–R35. https://doi.org/10.1530/REP-17-0008

Hu KL, Zhao H, Chang HM, Yu Y, Qiao J (2017) Kisspeptin/Kisspeptin receptor system in the ovary. Front Endocrinol (Lausanne) 8:365. https://doi.org/10.3389/fendo.2017.00365

Iliodromiti S, Kelsey TW, Wu O, Anderson RA, Nelson SM (2014) The predictive accuracy of anti-Mullerian hormone for live birth after assisted conception: a systematic review and meta-analysis of the literature. Hum Reprod Update 20(4):560–570. https://doi.org/10.1093/humupd/dmu003

Iliodromiti S, Iglesias Sanchez C, Messow CM, Cruz M, Garcia Velasco J, Nelson SM (2016) Excessive age-related decline in functional ovarian reserve in infertile women: prospective cohort of 15,500 women. J Clin Endocrinol Metab 101(9):3548–3554. https://doi.org/10.1210/jc.2015-4279

Ivarsson SA, Nilsson KO, Persson PH (1983) Ultrasonography of the pelvic organs in prepubertal and postpubertal girls. Arch Dis Child 58(5):352–354. https://doi.org/10.1136/adc.58.5.352

Johnson J, Emerson JW, Lawley SD (2022) Recapitulating human ovarian aging using random walks. PeerJ 10. https://doi.org/10.7717/peerj.13941

Kelsey TW, Wright P, Nelson SM, Anderson RA, Wallace WH (2011) A validated model of serum anti-mullerian hormone from conception to menopause. PLoS One 6(7):e22024. https://doi.org/10.1371/journal.pone.0022024

Kelsey TW, Anderson RA, Wright P, Nelson SM, Wallace WH (2012) Data-driven assessment of the human ovarian reserve. Mol Hum Reprod 18(2):79–87. https://doi.org/10.1093/molehr/gar059

Kirilov M, Clarkson J, Liu X, Roa J, Campos P, Porteous R, Schutz G, Herbison AE (2013) Dependence of fertility on kisspeptin-Gpr54 signaling at the GnRH neuron. Nat Commun 4:2492. https://doi.org/10.1038/ncomms3492

La Marca A, Spada E, Sighinolfi G, Argento C, Tirelli A, Giulini S, Milani S, Volpe A (2011) Age-specific nomogram for the decline in antral follicle count throughout the reproductive period. Fertil Steril 95(2):684–688. https://doi.org/10.1016/j.fertnstert.2010.07.1069

Lanciotti L, Cofini M, Leonardi A, Penta L, Esposito S (2018) Up-to-date review about minipuberty and overview on hypothalamic-pituitary-gonadal axis activation in Fetal and neonatal life. Front Endocrinol (Lausanne) 9:410. https://doi.org/10.3389/fendo.2018.00410

Lee J, Park HJ, Choi HS, Kwon HB, Arimura A, Lee BJ, Choi WS, Chun SY (1999) Gonadotropin stimulation of pituitary adenylate cyclase-activating polypeptide (PACAP) messenger ribonucleic acid in the rat ovary and the role of PACAP as a follicle survival factor. Endocrinology 140(2):818–826. https://doi.org/10.1210/endo.140.2.6485

Li L, Feng F, Zhao M, Li T, Yue W, Ma X, Wang B, Yin C (2020) NOTCH2 variant D1853H is mutated in two non-syndromic premature ovarian insufficiency patients from a Chinese pedigree. J Ovarian Res 13(1):41. https://doi.org/10.1186/s13048-020-00645-4

Liu TE, Wang S, Zhang L, Guo L, Yu Z, Chen C, Zheng J (2016) Growth hormone treatment of premature ovarian failure in a mouse model via stimulation of the Notch-1 signaling pathway. Exp Ther Med 12(1):215–221. https://doi.org/10.3892/etm.2016.3326

Makker A, Goel MM, Mahdi AA (2014) PI3K/PTEN/Akt and TSC/mTOR signaling pathways, ovarian dysfunction, and infertility: an update. J Mol Endocrinol 53(3):R103–R118. https://doi.org/10.1530/JME-14-0220

Mamsen LS, Kelsey TW, Ernst E, Macklon KT, Lund AM, Andersen CY (2018) Cryopreservation of ovarian tissue may be considered in young girls with galactosemia. J Assist Reprod Genet 35(7):1209–1217. https://doi.org/10.1007/s10815-018-1209-2

Mamsen LS, Charkiewicz K, Anderson RA, Telfer EE, McLaughlin M, Kelsey TW, Kristensen SG, Gook DA, Ernst E, Andersen CY (2019) Characterization of follicles in girls and young women with turner syndrome who underwent ovarian tissue cryopreservation. Fertil Steril 111(6): 1217–1225 e1213. https://doi.org/10.1016/j.fertnstert.2019.02.003

McLaughlin M, Kelsey TW, Wallace WH, Anderson RA, Telfer EE (2015) An externally validated age-related model of mean follicle density in the cortex of the human ovary. J Assist Reprod Genet 32(7):1089–1095. https://doi.org/10.1007/s10815-015-0501-7

McLaughlin M, Kelsey T, Wallace W, Anderson R, Telfer E (2017) Non-growing follicle density is increased following adriamycin, bleomycin, vinblastine and dacarbazine (ABVD) chemotherapy in the adult human ovary. Hum Reprod 32(1):165–174

Nye JS, Kopan R (1995) Developmental signaling. Vertebrate ligands for Notch. Curr Biol 5(9): 966–969. https://doi.org/10.1016/s0960-9822(95)00189-8

Pavlik EJ, DePriest PD, Gallion HH, Ueland FR, Reedy MB, Kryscio RJ, van Nagell JR Jr (2000) Ovarian volume related to age. Gynecol Oncol 77(3):410–412. https://doi.org/10.1006/gyno.2000.5783

Ramezani Tehrani F, Sheidaei A, Firouzi F, Tohidi M, Azizi F, Behboudi-Gandevani S (2021) Does the anti-Mullerian hormone decline rate improve the prediction of age at menopause? Front Endocrinol (Lausanne) 12:727229. https://doi.org/10.3389/fendo.2021.727229

Reddy P, Adhikari D, Zheng W, Liang S, Hamalainen T, Tohonen V, Ogawa W, Noda T, Volarevic S, Huhtaniemi I, Liu K (2009) PDK1 signaling in oocytes controls reproductive aging and lifespan by manipulating the survival of primordial follicles. Hum Mol Genet 18(15): 2813–2824. https://doi.org/10.1093/hmg/ddp217

Richardson SJ, Senikas V, Nelson JF (1987) Follicular depletion during the menopausal transition: evidence for accelerated loss and ultimate exhaustion. J Clin Endocrinol Metab 65(6): 1231–1237. https://doi.org/10.1210/jcem-65-6-1231

Ringrose CA (1963) The controversial and dynamic ovary. Can Med Assoc J 89:641–645

Scheffer GJ, Broekmans FJ, Dorland M, Habbema JD, Looman CW, te Velde ER (1999) Antral follicle counts by transvaginal ultrasonography are related to age in women with proven natural fertility. Fertil Steril 72(5):845–851. https://doi.org/10.1016/s0015-0282(99)00396-9

Spencer JB, Badik JR, Ryan EL, Gleason TJ, Broadaway KA, Epstein MP, Fridovich-Keil JL (2013) Modifiers of ovarian function in girls and women with classic galactosemia. J Clin Endocrinol Metab 98(7):E1257–E1265. https://doi.org/10.1210/jc.2013-1374

Steiner AZ, Pritchard D, Stanczyk FZ, Kesner JS, Meadows JW, Herring AH, Baird DD (2017) Association between biomarkers of ovarian reserve and infertility among older women of reproductive age. JAMA 318(14):1367–1376. https://doi.org/10.1001/jama.2017.14588

Topaloglu AK, Tello JA, Kotan LD, Ozbek MN, Yilmaz MB, Erdogan S, Gurbuz F, Temiz F, Millar RP, Yuksel B (2012) Inactivating KISS1 mutation and hypogonadotropic hypogonadism. N Engl J Med 366(7):629–635. https://doi.org/10.1056/NEJMoa1111184

Treloar AE, Boynton RE, Behn BG, Brown BW (1967) Variation of the human menstrual cycle through reproductive life. Int J Fertil 12(1 Pt 2):77–126

van Noord PA, Dubas JS, Dorland M, Boersma H, te Velde E (1997) Age at natural menopause in a population-based screening cohort: the role of menarche, fecundity, and lifestyle factors. Fertil Steril 68(1):95–102. https://doi.org/10.1016/s0015-0282(97)81482-3

Virant-Klun I, Zech N, Rozman P, Vogler A, Cvjeticanin B, Klemenc P, Malicev E, Meden-Vrtovec H (2008) Putative stem cells with an embryonic character isolated from the ovarian surface epithelium of women with no naturally present follicles and oocytes. Differentiation 76(8):843–856. https://doi.org/10.1111/j.1432-0436.2008.00268.x

Wallace WH, Kelsey TW (2004) Ovarian reserve and reproductive age may be determined from measurement of ovarian volume by transvaginal sonography. Hum Reprod 19(7):1612–1617. https://doi.org/10.1093/humrep/deh285

Wallace WH, Kelsey TW (2010) Human ovarian reserve from conception to the menopause. PLoS One 5(1):e8772. https://doi.org/10.1371/journal.pone.0008772

Woods DC, Tilly JL (2013) Isolation, characterization and propagation of mitotically active germ cells from adult mouse and human ovaries. Nat Protoc 8(5):966–988. https://doi.org/10.1038/nprot.2013.047

Zhao W, Dong L (2018) Long non-coding RNA HOTAIR overexpression improves premature ovarian failure by upregulating Notch-1 expression. Exp Ther Med 16(6):4791–4795. https://doi.org/10.3892/etm.2018.6750

Chapter 10
Ageing and the Autonomic Nervous System

Michael Takla, Khalil Saadeh, Gary Tse, Christopher L. -H. Huang, and Kamalan Jeevaratnam

Abstract The vertebrate nervous system is divided into central (CNS) and peripheral (PNS) components. In turn, the PNS is divided into the autonomic (ANS) and enteric (ENS) nervous systems. Ageing implicates time-related changes to anatomy and physiology in reducing organismal fitness. In the case of the CNS, there exists substantial experimental evidence of the effects of age on individual neuronal and glial function. Although many such changes have yet to be experimentally observed in the PNS, there is considerable evidence of the role of ageing in the decline of ANS function over time. As such, this chapter will argue that the ANS constitutes a paradigm for the physiological consequences of ageing, as well as for their clinical implications.

Keywords Ageing · Autonomic · Neuronal · Glial · Clinical

M. Takla
University of Cambridge, Cambridge, UK
e-mail: mt775@cam.ac.uk

K. Saadeh
University of Surrey, Guildford, UK
e-mail: ks802@cam.ac.uk

G. Tse
Kent and Medway Medical School, Canterbury, UK

University of Surrey, Guildford, UK

C. L. -H. Huang
University of Cambridge, Cambridge, UK

University of Surrey, Guildford, UK
e-mail: clh11@cam.ac.uk

K. Jeevaratnam (✉)
University of Surrey, Guildford, UK

J. R. Harris, V. I. Korolchuk (eds.), *Biochemistry and Cell Biology of Ageing: Part IV, Clinical Science*, Subcellular Biochemistry 103,
https://doi.org/10.1007/978-3-031-26576-1_10

Introduction

Ageing is the set of temporal changes of anatomy and physiology that herald a persistent decline in organismal fitness. Neurons constitute one of the groups of human cells in which such changes manifest. The vertebrate nervous system comprises central (CNS) and peripheral (PNS) components. In turn, the PNS is divided into the autonomic (ANS) and enteric (ENS) nervous systems, the latter being often but not always considered a subset of the former. (For the purposes of this chapter, however, they will be discussed as two separate, but highly interactive, parts of the PNS.)

While most experimental evidence of age-related changes in individual neurons has been accrued at the level of the CNS, it is possible—though in need of subsequent experimental verification—that such changes are extrapolatable to the PNS. In any case, at the level of system function, there is considerable evidence of ageing specific to the ANS. As such, this chapter will consider the ANS as a paradigm for the physiological effects of ageing, first discussing temporal changes in the anatomy and biochemistry of individual neurons and then exploring the physiological consequences and clinical implications of such changes.

Temporal Changes in the Nervous System

Temporal Changes in the Gross Anatomy of the Nervous System

Ageing is not associated with widespread neuronal loss; rather, selective neuronal vulnerability to the effects of advancing age results in the restricted loss of certain populations within the nervous system.

Temporal Changes in the Morphological Arrangement of the Nervous System

In regions of the nervous system vulnerable to ageing, changes in the morphological arrangement include synaptic injury, segmental demyelination, and remyelination to form shorter internodes. Along with concomitant axonal damage, this morphological rearrangement of the nervous system diminishes action potential conduction velocity (Sandell and Peters 2001, 2003; Peters and Sethares 2002) and potentially impairs interneuronal synaptic communication.

Temporal Changes in the Morphological Structure of Individual Neurons

The morphological structure of individual neurons is maintained by the cytoskeleton, which principally consists of microtubules and microtubule-associated proteins (MAP), intermediate filaments, and actin-based microfilaments (Al-Chalabi and Miller 2003; Dehmelt and Halpain 2004). Ageing is associated with the net depolymerisation of microtubules, and the accumulation of MAPs, such as (the typically axonal) tau (Blomberg et al. 2001), in neuronal somata (Trojanowski et al. 2002). Indeed, in model mammalian organisms, mutations in tau predispose to degenerative disease throughout the nervous system in an age-dependent fashion (Ishihara et al. 1999).

Temporal Changes in the Chemical Composition of Individual Neurons

Another time-related change in the nervous system is the intraneuronal accumulation of damaged molecules. This change in the chemical composition of individual neurons principally arises from the impairment of pathways involved in proteostasis, which otherwise removes such molecules to maintain the optimal concentration of correctly conformed proteins.

One proteostatic pathway implicates the proteasome, a complex of enzymes catalysing proteolysis, the degradation of unnecessary or incorrectly conformed proteins (Klein et al. 2018). There is some evidence in rodents that, in some but not all neuronal populations, proteasome activity declines with age (Keller et al. 2000). Interestingly, pharmacological inhibition of proteasomes in neuronal cell lines mimics some effects of age on neurons (Sullivan et al. 2004), including a pro-inflammatory phenotype (Rockwell et al. 2000).

Another pathway contributing to proteostasis is autophagy, an organellular process that, also being involved in regulating the dynamics of many neuronal organelles, will be discussed below.

Within the neuronal cytosol, the falling efficacy of removal mechanisms with time promotes an age-related increase in neurotoxic proteins. For instance, the proteasome, along with other enzymes including neprilysin, degrades amyloid beta (Aß) (Keller et al. 2002; Tanzi et al. 2004), such that temporal loss of proteasome function accounts, in part, for the accumulation of Aß. Likewise, even under non-pathological conditions, the fibrillar conformation of the microtubule-associated protein, tau, aggregates over time (Goedert 2005), in part owing to changes in the balance of tau kinase and phosphatase activities (Stoothoff and Johnson 2005), and in part due to impaired proteostasis (Stoothoff and Johnson 2005; Montine et al. 1996). Moreover, the elucidation of mutations giving rise to early-onset Parkinson's disease (Moore et al. 2005) and the sufficiency of (alpha)-synuclein gene triplication

in causing Parkinson's disease (Singleton et al. 2003) together imply a causal role for time-related diminished proteolytic clearance.

Within the neuronal nucleus, the time-related impairment in DNA damage repair systems raises the proportion of lesioned genetic material (Lu et al. 2004). In fact, missense or nonsense mutations in the genes encoding for DNA damage repair proteins may cause premature ageing (Kyng and Bohr 2005), implying that, rather than merely being a consequence of ageing, the accumulation of damaged DNA may also be one of its causes.

Age-Related Biochemical Changes in Individual Neurons

Age-Related Changes in Intraneuronal Organelle Dynamics

Ageing and Neuronal Autophagy

Autophagy implicates double-membrane vesicles that mature from one, and/or are assembled from many, pre-existing organellular platform(s), including the plasma membrane (Ravikumar et al. 2010) and recycling endosomes (Puri et al. 2018). These vesicles bridge the phagophore membrane elongator, LC3-II, to the LIR motifs of receptors for importing aberrantly folded, ubiquitin-tagged cytosolic proteins, such that consequent bulk and cargo-selective lysosomal degradation facilitates intracellular proteostasis.

Feeding, and nutrient deprivation and consequent low $[ATP]_i$, suppresses and triggers autophagy via mTORC1- (Kim et al. 2011) and AMPK-dependent (Egan et al. 2011) pathways, converging on the in- and activation of Ulk1, respectively.

In neurons, autophagy contributes to post-mitotic survival, and to homeostatic transport along their axons (Hara et al. 2006; Komatsu et al. 2006), as well as regulating the maturation of myelin in the PNS (Jang et al. 2015).

Ageing tends to suppress autophagic flux (Sarkis et al. 1988; Dice 1998), perhaps, as in invertebrates, partly by downregulating autophagy inducers (Simonsen et al. 2008; Demontis and Perrimon 2010; Kaushik et al. 2012). Indeed, ageing upregulates BCL-2 (Guebel and Torres 2016), which inhibits the autophagy inducer, Beclin 1. Moreover, age, in concert with other independently acting factors, impairs mitophagy in such a way as to accelerate the pathogenesis of neurodegenerative disease (Deas et al. 2011; Liu et al. 2019).

Ageing and Neuronal Mitochondrial Function

As the site of oxidative phosphorylation, mitochondria constitute the principal source of neuronal ATP, thereby necessitating mitochondrially situated DNA (mtDNA) to rapidly generate the requisite proteins that form the electron transport chain (ETC). Beyond this crucial metabolic role, mitochondria, and in particular the

formation of a mitochondrial permeability transition pore (MPTP) within their inner membranes, are also central to the common executioner pathway in neuronal apoptosis and/or necrosis (Mattson 2000).

Given their central role in neuronal metabolism, mitochondria respond to changes in intracellular indicators of nutritional state, such as the [AMP]:[ATP] and [NAD+]:[NADH] ratios. Mediating this nutritional sensitivity are the peroxisome proliferator-activated receptor gamma (PPAR-(gamma)) co-activators 1-(alpha) (PGC-1(alpha)) and -(beta) (PGC-1(beta)), which promote mitogenesis (Jäger et al. 2007; Jeninga et al. 2010). Mitochondria also respond to changes in tissue oxygenation, recruiting the transcription factor, hypoxia-inducible factor 1-(alpha) (HIF-1(alpha)) (Chandel et al. 2000), to upregulate tricarboxylic acid cycle proteins (Kim et al. 2006), and mitophagy-inducing BCL-2 family members (Zhang et al. 2008) in order to metabolically adapt to, and limit any damage incurred by, hypoxaemia (Gomes et al. 2013).

With age, there is a loss of function of the mitochondrial ETC (Yao et al. 2010; Pandya et al. 2015; Pollard et al. 2016), and a greater susceptibility to mitochondrial toxins (Kim and Chan 2001). In a fashion analogous to nuclear DNA, mtDNA in post-mitotic neurons accumulates unrepaired oxidative lesions (Chomyn and Attardi 2003; Kraytsberg et al. 2003). In turn, mtDNA mutations may play a causative role in neuronal ageing (Kujoth et al. 2005; Santos et al. 2013; Aon et al. 2016; Kauppila et al. 2017). Moreover, ageing increases the risk of mitochondrial fragmentation or excessive enlargement, as well as depolarisation (Lores-Arnaiz et al. 2016) that lowers the threshold for mPTP formation (Brown et al. 2004), perhaps predisposing to neuronal apoptosis and/or necrosis. In non-human primates, age is also associated with the downregulation of PPARs (Kayo et al. 2001; Ling et al. 2004).

Age-Related Changes in Intraneuronal Metabolism

The age-related impairment of mitochondrial respiratory chain function is part of a broader change in intraneuronal metabolism. Indeed, age is associated with a reduction in the [NAD+]:[NADH] ratio (Bai et al. 2011; Pittelli et al. 2011; X.-H. Zhu et al. 2015), and a rise in the [AMP]:[ATP] ratio, the latter activating AMP-activated protein kinase (AMPK) to stimulate alternative metabolic pathways (Burkewitz et al. 2016).

Under physiological conditions, neuronal import of glucose via plasma membrane glucose transporters (GLUTs) constitutes the dominant pathway for both glycolysis in the cytosol and oxidative phosphorylation in the mitochondria. Ageing downregulates many neuronal GLUT isoforms, reducing glucose uptake (Yin et al. 2016; Kyrtata et al. 2021). Given the especially high ATP demand for neurotransmission, both at the level of transmitter synthesis and vesicular docking, fusion, and recycling (Attwell and Laughlin 2001; Rangaraju et al. 2014), age thus increases the susceptibility of synaptic spines to degeneration (Harris et al. 2012).

Moreover, the insulin/insulin-like growth factor 1 (IGF-1) signalling (IIS) pathway is integral to neuronal metabolism and survival (Chandrasekaran et al. 2017). As such, the post-pubertal age-associated exponential decline in pituitary secretion of growth hormone (GH) (Iranmanesh et al. 1991), which diminishes hepatic IGF-1 secretion (Bando et al. 1991; Frutos et al. 2007) and thus reduces signal transduction via the IIS, contributes to age-related loss of neuronal function (Breese et al. 1991; Khan 2002).

In light of age-related impairment of neuronal glucose- and IIS-related metabolism, rising AMPK activity attempts both to compensatorily upregulate these conventional pathways and to stimulate increasing flux through alternative pathways (Burkewitz et al. 2016; Hardie 2015). For instance, AMPK induces a gain of function in GLUTs, improves insulin sensitivity, and enhances mitogenesis (Hardie et al. 2012). In parallel, the age-induced fall in [NAD+]:[NADH] ratio may impair the function of sirtuins (SIRTs) (Zhao et al. 2020), a family of class III histone deacetylases (HDACs) crucial not only for neuroprotection but also for the stimulation of AMPK, the IIS, and other metabolic pathways (Kelly 2010; Fröjdö et al. 2011; Xiong et al. 2011).

Age-Related Changes in Intraneuronal Ca^{2+} Homeostasis

In all neurons, owing to the status of Ca^{2+} as a determinant of neuronal excitability (Marty and Zimmerberg 1989) and metabolism (McCormack and Denton 1990), as well as a second messenger in neurotransmitter exocytosis (Neher and Sakaba 2008), its cytosolic concentration ($[Ca^{2+}]_c$) is maintained within a narrow range in order to increase the sensitivity of signal transduction pathways to small fluxes across cellular compartments.

Within the neuron, Ca^{2+} flows into the cytosol principally from the extracellular fluid across the plasma membrane via voltage-gated (Ca_v) channels (Catterall et al. 1990; Ma et al. 2012) and glutamate (Glu)-gated NMDA receptors (NMDARs) (MacDermott et al. 1986); and stores in the endoplasmic reticulum, via inositol-1,4,5-triphosphate ($InsP_3Rs$) and ryanodine (RyRs) receptors (Furuichi et al. 1994), and in the mitochondria, via the Na^+/Ca^{2+} exchanger (NCX) and mPTP.

From the cytosol, Ca^{2+} leaves to the extracellular fluid via the high-affinity low-turnover PMCA (Juhaszova et al. 2000), regulated by the Ca^{2+}-binding protein, calmodulin (CaM) (Brini and Carafoli 2009), and the low-affinity high-turnover NCX (Carafoli and Longoni 1987; Blaustein 1988), and stores in the endoplasmic reticulum, via sarco(endo)plasmic reticulum Ca^{2+} ATPase (SERCA) (Camello et al. 2002), and mitochondria, which act as Ca^{2+} buffers upon increases in ATP demand (Contreras et al. 2010) (Fig. 10.1).

Ageing perturbs neuronal $[Ca^{2+}]_c$ homeostasis, by reducing PMCA (Michaelis et al. 1996) and SERCA (Murchison and Griffith 1999), and mitochondrial sink capacity (Xiong et al. 2002), and augmenting Ca^{2+} efflux from the ER (Gant 2006) via $InsP_3Rs$ and RyRs (Thibault et al. 2007). The net increase in $[Ca^{2+}]_c$ impairs

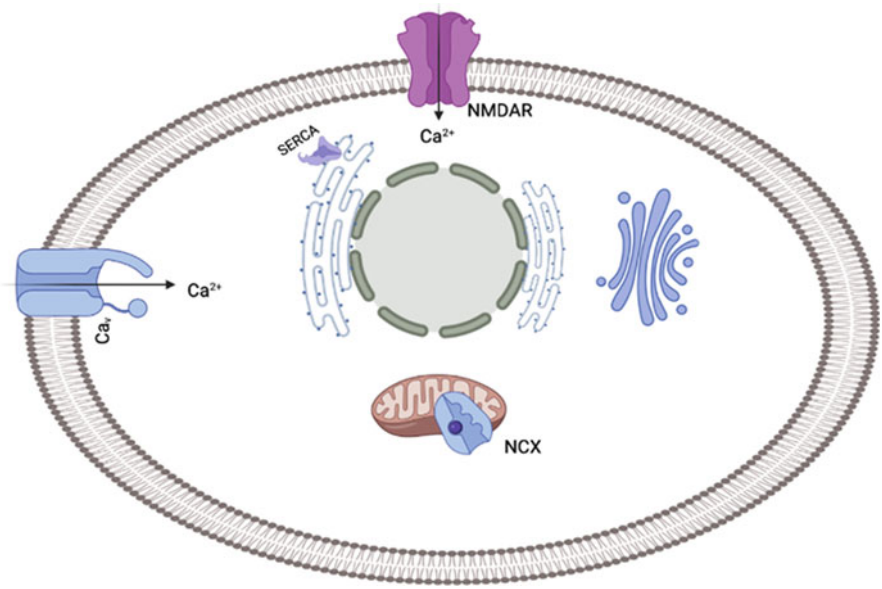


Fig. 10.1 Within the neuron, Ca^{2+} flows into the cytosol from the extracellular fluid across the plasma membrane via voltage-gated (Ca_v) channels and glutamate (Glu)-gated NMDA receptors (NMDARs), while the mitochondria may export Ca^{2+} via the Na^+/Ca^{2+} exchanger (NCX). In parallel, the mitochondria also buffer cytosolic Ca^{2+}, and the sarcoplasmic sarco(endo)plasmic reticulum Ca^{2+} ATPase (SERCA) imports Ca^{2+} for storage in the ER. Ageing neurons are characterised by dysregulated Ca^{2+} fluxes

control of neuronal excitability (Matthews et al. 2009). Furthermore, age may alter synaptic plasticity regulation by diminishing and increasing Ca^{2+} influx through NMDARs (Lehohla et al. 2008) and Ca_v (Thibault and Landfield 1996), with functional consequences (Ban et al. 1990).

The Ca^{2+} hypothesis of ageing (Khachaturian 2006) posits that such perturbations in neuronal $[Ca^{2+}]_c$ play a causative role.

Dysregulation of $[Ca^{2+}]_c$ homeostasis contributes to the selective vulnerability of neuronal populations to the effects of age. For instance, groups of neurons experiencing an age-related decline (Iacopino and Christakos 1990; Thorns et al. 2001; Geula et al. 2003) in the expression of the Ca^{2+} binding protein, calbindin, tend to be the most susceptible to neurodegeneration (Mattson et al. 1991; Magloczky and Freund 1993). Meanwhile, age-related degeneration is accelerated by neuronal populations with high basal NMDAR (Ikonomovic et al. 1999) or Ca^{2+}-permeable AMPA receptor (AMPAR) expression (Williams et al. 1997).

From these empirical findings arises the following question: how do age-related disturbances to $[Ca^{2+}]_c$ homeostasis damage neurons? The answer primarily centres on the promotion of, and diminished capacity to cope with, stressors, especially oxidation.

Age-Related Changes in Neuronal Oxidative Stress

Under physiological conditions, neurons produce reactive oxygen species (ROS), including nitric oxide (NO), hydroxyl (HO), superoxide (O_2^-), peroxynitrite ($ONOO^-$), and hydrogen peroxide (H_2O_2) (Cheung et al. 2005). These ROS are predominantly generated from mitochondrial respiration, the cytochrome P450 (CYP450) system of monooxygenases catalysing oxidation of fatty acids, steroids, and xenobiotics, NADPH oxidase, NO synthase (NOS), and xanthine oxidase (Tanaka et al. 2001; Akhtar et al. 2004). In order to counteract the potentially disruptive effect of ROS-mediated oxidation of intracellular macromolecules, antioxidants, whether non-enzymatic or enzymatic (such as superoxide dismutase (SOD), glutathione reductase, or catalase (CAT) (Griendling et al. 2000), scavenge ROS to maintain them at subtoxic concentrations (Kohen et al. 2000; Kohen and Nyska 2002).

Hartman's theory of ageing postulated a causative role for cellular macromolecules damaged by a rise in the concentrations of unscavenged ROS (Mattson 2000; Miller et al. 2000), especially secondary to increasing ROS production in light of age-related impairment of the mitochondrial ETC (Tatton et al. 2003).

Age does, in fact, tend to elevate intraneuronal ROS, in part through augmented production, and in part through impaired scavenging. Indeed, ageing reduces the ratio of glutathione (GSH) to glutathione disulfide (GSSG) throughout the nervous system (Calabrese et al. 2004; Balu et al. 2005; Donahue et al. 2006; Zhu et al. 2006) in a variety of animal models. The decline in ROS scavenging may be mediated by age-related impairment (Duan et al. 2009) of the ROS-mediated upregulation of the transcription factor, nuclear factor erythroid 2-related factor (Nrf2), which otherwise, in turn, upregulates intracellular antioxidants.

The relatively high oxidative rate and elevated lipid content of the nervous system, especially in the neuronal plasma membrane, render it susceptible to ROS-induced toxicity. In fact, malondialdehyde (MDA) (Cutler et al. 2004) and 4-hydroxynonenal (HNE), products of ROS-induced phospholipid peroxidation, may be markers of ageing in some mammalian nervous systems, in which the concentrations of polyunsaturated fatty acids (White and Barone 2001) and arachidonic acid also decline over time due to oxidation.

Age-related perturbations in neuronal $[Ca^{2+}]_c$ homeostasis potentiate those in mitochondrial ROS production. Indeed, mitochondrial Ca^{2+} overload, due to NCX loss-of-function and transfer from the ER (Rizzuto et al. 1999), opens the mPTP and thus activates neuronal apoptosis and/or necrosis (Csordás et al. 2006). Furthermore, increases in $[Ca^{2+}]_m$ augment the production of ROS (Petrosillo et al. 2004), which oxidise cardiolipin to enhance the release of cytochrome (Vercesi et al. 1997; Iverson and Orrenius 2004) that, in turn, accelerates neuronal apoptosis.

Age-Related Changes in Extraneuronal Neurotrophic Signalling

The cellular constituents and targets of the nervous system secrete neurotrophic factors, which not only promote neuronal survival but also stimulate the growth of neurites and facilitate the plasticity of synapses. Under physiological conditions, neurotrophic factors play a protective role within neuronal populations that exhibit selective vulnerability to the effects of age (Gash et al. 1998; Siegel and Chauhan 2000; Counts and Mufson 2005). In fact, falling production of, or sensitivity to, neurotrophic factors may contribute to these populations' selective vulnerability to Ageing.

Indeed, ageing downregulates both brain-derived neurotrophic factor (BDNF) and its receptor, tropomyosin receptor kinase (TrkB) (Rage et al. 2007), in those cellular groups in the nervous system (Hattiangady et al. 2005) associated with age-related functional impairment (Gooney et al. 2004) and even neurodegenerative disease (Murer et al. 2001). The concomitant upregulation of glial-derived neurotrophic factor (GDNF) may predispose some neuronal populations (Matsunaga et al. 2006) to excitotoxic degeneration (Farrand et al. 2015).

With age, the sensitivity of neurotrophic factor synthesis to endogenous and environmental stimulants may also decline. For instance, in older rodents, exercise (Adlard et al. 2005) and trauma (Yurek and Fletcher-Turner 2000) fail to upregulate BDNF to the same extent as in their younger counterparts, and the same is true for deafferentation-induced expression of IGF-1 (Editorial 1998).

Age-Related Changes in Extraneuronal Inflammation

Ageing tends to induce immunosenescence (Pawelec and Solana 1997; Gruver et al. 2007), with the senescence-associated secretory phenotype (SASP) characterised by NF-(kappa)B-mediated upregulation of pro-inflammatory cytokines (Franceschi et al. 2006), including tumour necrosis factor-(alpha) (TNF-(alpha)), and interleukins- (ILs) 1 (IL-1) and -6 (IL-6) (Salminen et al. 2012). However, along with Toll-like receptors (TLRs), these facets of the innate immune system do not uniformly inhibit neuronal function, but rather contribute to a complex microenvironment that can both beneficially modulate synaptic plasticity and exacerbate neurodegenerative disease (Okun et al. 2011).

Moreover, age, by increasing the concentration of unquenched intraneuronal ROS, also independently promotes NF-(kappa)B activation (Baldwin 1996; DiDonato et al. 1997), which upregulates anti-apoptotic genes and proteins, including SOD (Patten et al. 2010), which suppress further mitochondrial ROS release. In any case, because NF-(kappa)B upregulates TNF-(alpha), which, in turn, stimulates NF(kappa)B, ageing may establish a positive feedback loop that perpetuates a neuroinflammatory state whose effect on neuronal function depends on concurrent

signalling and other age-related changes (Song et al. 2004, 20; Chen et al. 2009; Chongthammakun et al. 2009).

The Autonomic Nervous System (ANS) as a Paradigm

The Evolutionary Origins of the ANS

Understanding the function of the ANS, and thus the physiological effects of the age-related changes in the neuronal populations that comprise it, necessitates a discussion of its evolutionary origin. Consider the vertebrate forerunner, *Hydra*, which is analogous to an elongated gastrula with an outer ectoderm and inner endoderm.

In *Hydra*, neuronal specialisations of the ectoderm cluster in a cranial ring and caudal peduncle, in addition to being distributed longitudinally in between these two sites. Despite containing both clear and dense-core neurotransmitter-filled vesicles, and expressing receptors for acetylcholine (AChRs) (Chapman et al. 2010), *Hydra* neurons predominantly express neuropeptides (Koizumi et al. 1989), which are released into *en passant* synapses to evoke prolonged postsynaptic responses (van den Pol et al. 1996).

In vertebrate embryos, a subset of ectodermal cells acquires neural competence, folding inwards to form a neural groove, whose two lips fuse together to give rise to a neural tube, the dorsal surface of which disseminates neural crest cells (NCCs) to migrate elsewhere (Copp et al. 2003). During development, the neural tube generates the central nervous system (CNS), while NCCs form almost all of the peripheral nervous system (PNS), including all ANS and chromaffin cells in the adrenal medulla. Sharing *Hydra*'s *en passant* synaptic structure and co-existence of clear and dense-core vesicles, the ANS is evolutionarily analogous to the pre-vertebrate nervous system.

The Gross Anatomy of the ANS

In general, the ANS is characterised by an afferent limb that conveys signals from the PNS to the CNS, a central integrating system, and an efferent limb that innervates most end-organs (Bankenahally and Krovvidi 2016) to elicit physiological effects necessary for homeostasis. In turn, the efferent limb consists of sympathetic and parasympathetic divisions. Within the sympathetic division, somata in the spinal cord grey matter at levels T1-L2/3 give rise to a thoraco-lumbar outflow, from which myelinated preganglionic neurons synapse to unmyelinated postganglionic neurons in ganglia arranged in paravertebral and prevertebral chains.

The two paravertebral chains can, in turn, be divided into cervical and thoracic parts, while the prevertebral chains comprise lumbar (giving rise to the coeliac

plexus) and pelvic (giving rise to the sacral plexus) parts (Kreulen 2005). However, some preganglionic neurons from the thoraco-lumbar outflow synapse to adrenal medullary chromaffin cells, which, being able to secrete adrenaline and, to a lesser extent, noradrenaline (Coupland et al. 1976; Carbone et al. 2019), are functionally analogous to postganglionic fibres, but do not form *en passant* synapses to end-organs.

Within the parasympathetic division, myelinated preganglionic neurons originate from the motor nuclei of cranial nerves III, VII, IX, and X in the medulla oblongata and midbrain, and, classically, the ventral rami of the spinal nerves arising from levels S2-4. In fact, genetic analyses suggest that the preganglionic neurons arising from S2-4 ventral rami are phenotypically and ontogenetically indistinguishable from the sympathetic thoracolumbar outflow (Espinosa-Medina et al. 2016). In any case, preganglionic neurons synapse in ganglia near or within end-organs thereafter innervated by postganglionic neurons. Although most end-organs are reciprocally innervated by both parasympathetic and sympathetic neurons, each division typically innervates distinct cellular populations therein (Jänig 2006). As such, rather than simply being antagonistic, the sympathetic and parasympathetic divisions of the ANS exert parallel control over a given end-organ.

In the brainstem and hypothalamus (Dampney 1994; Dampney et al. 2003; Kenney et al. 2003), a set of diencephalic nuclei are the principal central integrators of ANS signals. Having monitored both the internal milieu and external environment, the paraventricular nucleus (Dampney 1994, 199; Dampney et al. 2003; Kenney et al. 2003; Sun 1996) of the hypothalamus accounts for the predicted internal deficit state by encoding a response with a hormonal component, effected by the anterior pituitary gland's secretion of tropic hormones, and an autonomic component, effected by the connections between the medulla oblongata and preganglionic ANS neurons. In the medulla oblongata, the nucleus tractus solitarius (NTS) uses inputs from general visceral afferents (GVAs) to modulate preganglionic parasympathetic outflow from the caudal medulla. Meanwhile, the rostral ventral lateral medulla (RVLM) critically regulates discharge patterns in preganglionic sympathetic efferents (Dampney 1994; Dampney et al. 2003; Kenney et al. 2003; Sun 1996).

The ANS and the GI Tract

During embryonic development, NCC migration to the GI tract culminates in the formation of the enteric nervous system (ENS), which includes the submucosal ('Meissner's') and myenteric ('Auerbach's') plexuses of local polymodal intrinsic primary afferent neurons (IPANs) (Furness et al. 2004) co-expressing substance P, somatostatin (Brehmer et al. 2004), and often serotonin receptors (5-HT_4R) (Dickson et al. 2010). Within the myenteric plexus, 40% of local ENS neurons release NO, with the other 40% releasing Ach (Anlauf et al. 2003; Pimont et al. 2003; Murphy et al. 2007), while interneurons are principally cholinergic.

The ENS also consists of local efferents modulated by the ANS (Gibbins 2012). Namely, sympathetic postganglionic fibres, which emanate from the prevertebral coeliac, inferior mesenteric, and pelvic plexuses, innervate efferents of the myenteric plexus that control intestinal motility. In parallel, parasympathetic fibres synapse not only to myenteric plexus efferents but also to vasoactive intestinal peptide (VIP)- and NOS-expressing (Porter et al. 1997) efferents in the submucosal plexus controlling mucosal secretory activity and local vasodilatation (Vanner and Surprenant 1996). As discussed below, age-related changes in the ENS and ANS are thus implicated in loss of intestinal function.

Age-Related Changes in ANS Anatomy

As with the rest of the nervous system, the ANS experiences age-related changes in its gross anatomy and morphological arrangement. Compared to other neuronal populations, those of the medulla oblongata lose neurons at a slower rate with age.

While ageing does not change the number of ANS ganglia, impaired axonal regeneration and consequent accumulation of neurofilaments, which are increasingly glycosylated and phosphorylated with age, elicit neuroaxonal dystrophy (Vlassara et al. 1994; Cotman and Gómez-Pinilla 1991; Gavazzi 1995). Along with exaggerated synaptic turnover and axonal sprouting, these changes suppress synaptic neurotransmission and end-organ ANS innervation.

Age-Related Changes in the Biochemistry of Individual ANS Neurons

In all ANS ganglia, acetylcholine (ACh) is the principal neurotransmitter released by preganglionic fibres, whereupon postganglionic neurons express both ionotropic nicotinic AChRs (nAChRs), especially those composed of α3β4 subunits, and metabotropic muscarinic AChRs (MAChRs). While the opening of nAChRs tends to permit an initial fast excitatory postsynaptic potential (EPSP), the ligation of M_1AChRs and M_2AChRs initiates signal transduction pathways culminating in secondary slow EPSPs and inhibitory PSPs (IPSPs), respectively.

Within end-organs, which express $M_{1\text{-}3}$AChRs and both (alpha) and (beta) adrenergic receptors (ARs), parasympathetic and sympathetic postganglionic fibres principally release ACh and noradrenaline, respectively. However, both across ganglia and end-organs, pre- and postganglionic neurons also engage in considerable non-adrenergic non-cholinergic (NANC) and neuropeptidergic neurotransmission. This co-transmission diversifies neurochemical coding (Elfvin et al. 1993).

With age, although possible increases in postganglionic sympathetic efferent activity (Ng et al. 1993) with or without concomitant declines in synaptic reuptake by neuronal uptake 1 transporter (NET-1) (Trendelenburg 1991) raise circulating

noradrenaline concentration (Ziegler et al. 1976; Esler et al. 1990; Folkow and Svanborg 1993), end-organ (beta)-ARs become desensitised to this noradrenaline release (Lakatta 1993; Docherty 1990; Brodde and Michel 1999). In parallel, age-related falls in presynaptic oxidative metabolism and ACh synthesis and release of ACh impair postganglionic parasympathetic neuronal function (Gibson and Peterson 1981).

The Impact of Age-Related Neuronal Changes on ANS Function

Age and ANS Control of Lens Refractive Index

The parasympathetic and sympathetic divisions of the ANS exert parallel control over pupillary diameter, in order to adjust the proportion of ambient light transmitted to the retinal photoreceptors. Within the retina, intrinsically photosensitive retinal ganglion cells (ipRGCs) (Do and Yau 2010; McDougal and Gamlin 2014) signal, either directly or indirectly via the primary visual cortex, the total luminance of ambient light to the midbrain. Thereafter, the Edinger-Westphal nucleus of CN III projects preganglionic parasympathetic fibres to the ciliary ganglion, wherein post-ganglionic parasympathetic fibres form *en passant* synapses to the sphincter pupillae in the iris, as well as the ciliaris muscle in the uvea (Michael-Titus et al. 2010). In this way, large increases in ambient luminance tend to increase parasympathetically mediated pupillary constriction ('miosis') and, via slackening of the Zonule of Zinn, the refractive index of the lens, establishing a negative feedback loop (Wu et al. 2022).

In parallel, there is adrenergic inhibition of the Edinger-Westphal nucleus at the level of the midbrain, while postganglionic sympathetic fibres from the cervical part of the paravertebral ganglionic chain promote contraction of the dilator pupillae in the iris. Quantification of pupillary diameter under different conditions of gaze, accommodation, and anaesthetic suppression (Borgdorff 1975; Onorati et al. 2013) of the ascending arousal pathways suggests that the tone in such sympathetic fibres reflects the activity of central integrators, rather than negative feedback. As such, in the absence of light, the degree of arousal establishes, via sympathetic tone, a maximal pupillary diameter, which is then reduced through a negative feedback pathway mediated by the parasympathetic division of the ANS (Fig. 10.2).

While age hardens the lens (Peddie 1925), slackens the Zonule of Zinn (Weale 1962), and atrophies ciliaris (Duane 1922), it also likely increases the delay within the neural circuits controlling reflexive accommodation (Lockhart and Shi 2010). As such, a combination of age-related changes in steady-state and dynamic ANS-mediated accommodation can cause up to a 10-fold increase in the nearest point on which a patient can visually focus and thus presbyopia (Mordi and Ciuffreda 1998).

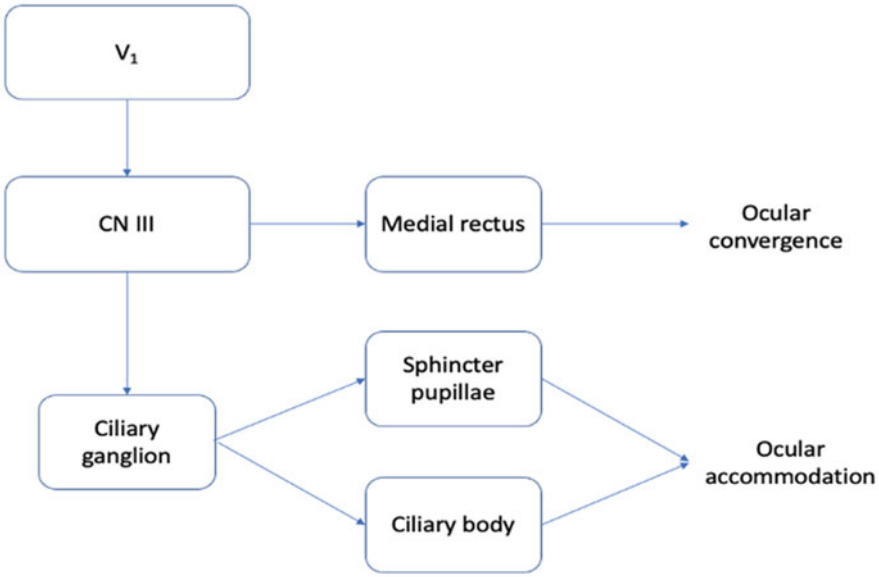


Fig. 10.2 The parasympathetic division of the autonomic nervous system (ANS) reduces the maximal pupillary diameter established by a basal sympathetic tone. In parallel, the parasympathetic division facilitates both ocular convergence and, through changes in the refractive index of the lens, accommodation to improve focus of near-field objects detected by the primary visual cortex (V_1)

Age and ANS Control of Ventilation

Parallel parasympathetic and sympathetic control over ventilation and pulmonary perfusion is necessary to minimise the difference between alveolar (P_AO_2) and arterial (P_aO_2) partial pressures of O_2, and to maintain the latter above the threshold for sufficient saturation of haemoglobin with O_2 before blood reaches the tissues.

During inspiration, postganglionic sympathetic fibres innervating the smooth muscle of the airways promote bronchodilatation via $(beta)_2$-ARs, while postganglionic parasympathetic fibres prevent collapse of the distal airways in which the intraluminal pressure is more negative than intrapleural pressure (van der Velden and Hulsmann 1999).

Arterial peripheral chemoreceptors comprise islands of NCC-derived type I glomus cells depolarised by hypoxaemia (Prabhakar 1994). Located in the carotid body and, to a lesser extent the aortic body, peripheral chemoreceptors convey afferent signals via CN IX and X, respectively, to the medulla oblongata, whereupon sympathetic efferents fire at a higher frequency to synapses in the SAN, myocardium, and vascular tunica media (Marshall 1994), and at a lower frequency to brown adipose tissue (Madden and Morrison 2005). The concomitant activation of parasympathetic efferents limits hypoxaemic-induced coronary and cerebral vasoconstriction (Marshall 1994). Most importantly, excitation of sympathetic efferents

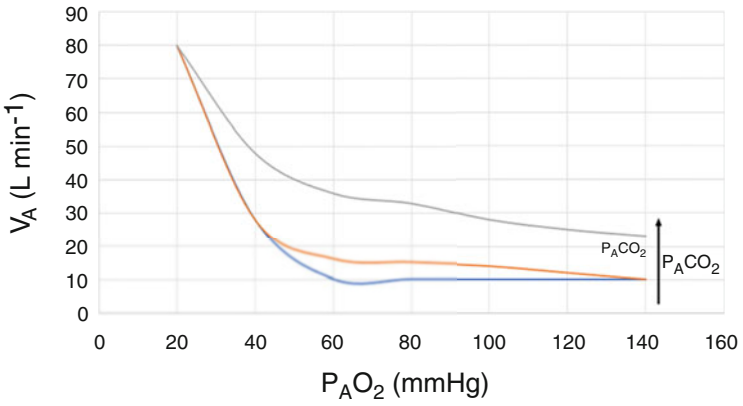


Fig. 10.3 Alveolar ventilation rate (V_A) as a function of alveolar partial pressure of O_2 (P_AO_2). Notably, elevated alveolar partial pressure of CO_2 (P_ACO_2) can potentiate the effect of hypoxaemia to maximise the increase in respiratory drive

stimulates a rise in the frequency and magnitude of phrenic nerve discharge, thereby increasing respiratory rate (Taylor et al. 1999), in a fashion partly limited by the rising tidal volume (Somers et al. 1989). Ventilatory peripheral chemoreflex output is an exponential function of P_aO_2, with gain highest when $P_aO_2 < 8$ kPa, though the range of highest reflex sensitivity can be re-set by P_aCO_2, such that hypercapnia can potentiate the effect of hypoxaemia on increasing respiratory drive (Somers et al. 1989; Gelfand and Lambertsen 1973) (Fig. 10.3).

Notably, age-related decline in vagal tone alters the physiological impact of the peripheral chemoreflex such that, although older patients exhibit the same increase in ventilatory drive, they may experience transient tachycardia to a lesser extent than their younger counterparts (Paleczny et al. 2014).

Age and ANS Control of ABP

We can define cardiac output (CO) as the product of stroke volume (SV) from the heart's left ventricle and the rate (HR) at which the ventricle contracts during systole. The classic Guyton experiments demonstrated that, although necessary to maintain CO, the heart is not its principal determinant (Guyton et al. 1957). This is because, in a closed system, CO from the heart's left ventricle is equivalent to, and thus constrained by, venous return (VR) to its right atrium. In turn, by Darcy's law, VR is determined by the difference between right atrial pressure (RAP) and the mean systemic filling pressure (MSFP) that would exist upon asystole and the distribution of blood in proportion to systemic vessel capacitance. As such, CO (= VR) can be regarded as the average flow around the entire circulatory system, rather than the volume ejected by the left ventricle alone (Beard and Feigl 2011) (Fig. 10.4).

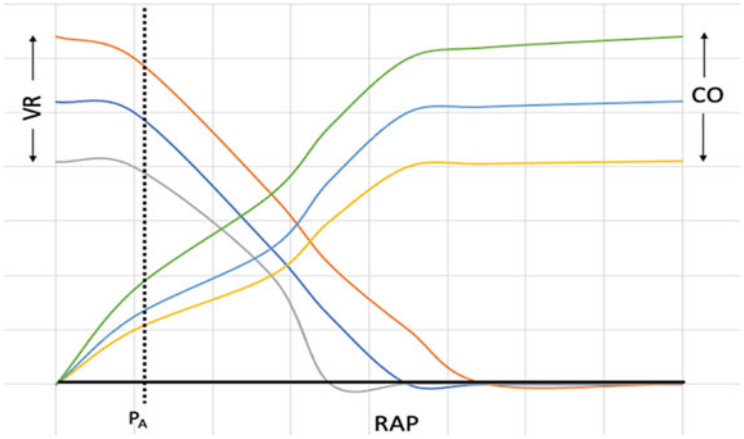


Fig. 10.4 A graphical summary of Guyton's results. Each intersection between the curves representing output for cardiac output (CO) from the left ventricle, and venous return (VR) to the right atrium, constitutes the average flow in the circulatory system. Notably, increasing right atrial pressure (RAP) such that it begins to exceed intra-alveolar pressure (P_A) tends to reduce VR for a given CO

As blood flows through vessels of the systemic circulation, the pressure exerted on the walls declines due to the vessel's resistance (R), which depends on the blood's viscosity and the vessel's radius and length. By Poiseuille's Law, and given the constant values of blood viscosity, which typically varies significantly only under conditions of dehydration or erythrocyte pathology, and of vessel length, determined by the anatomy of the adult circulatory system, the primary determinant of resistance to flow is vessel radius. Within a tissue, the artery, arteriole, capillary, venule, and vein are arranged in series. Between tissues, these vessels are arranged in parallel. The sum of the series resistances of vessels perfusing a single tissue, aggregated as a set of parallel resistances across tissues, is the total peripheral resistance (TPR).

As RAP is typically close to 0, by Darcy's law, one can thus approximate arterial blood pressure (ABP) = CO x TPR. The Frank-Starling experiments demonstrated that afterload, and, by extension, TPR, does not greatly influence CO (Frank 1959; Knowlton and Starling 1912). As such, the parameters CO and TPR are two separate and independent axes about which ABP can be homeostatically regulated, establishing a system of constant pressure and variable local flow, whereby perfusion of individual tissues depends on their rate of oxygen consumption (VO_2) and contextual physiological importance.

Understanding how age increases both mean ABP (MAP) (Zito et al. 1991) and ABP variability, therefore, necessitates an understanding of the effects of age on ANS control of CO and TPR.

Age and ANS Control of CO

Given that CO is proportional to the difference between MSFP and RAP, an increase in CO may be achieved through an increase in MSFP and/or a fall in RAP or resistance to venous return (R_{vr}).

$$VR = \frac{MSFP}{RVR} - \frac{RAP}{RVR}$$

Negligible at rest, R_{vr} is typically affected only by the action of the skeletal muscle pump and is thus not suitable as a principal means of regulating VR. The predominant strategy for reducing RAP is to create a vacuum for blood flow into the right atrium by making central venous pressure (CVP) negative. Being thin-walled vessels, veins remain patent only in the context of a positive transmural pressure gradient. Although CVP is typically more positive than intrapleural pressure (P_{pl}), changes in both CVP and P_{pl} during the respiratory cycle are such that the vena cavae would cyclically collapse if CVP became too negative (Kircher et al. 1990; R. M. Lang et al. 2005; Brennan et al. 2007; Kimura et al. 2011), limiting the extent to which a fall in RAP can increase VR (Fig. 10.5).

As such, the most effective way to increase CO is to increase MSFP, which would leave unaffected the critical pressure at which the vena cavae collapse. At rest, the systemic vasculature can accommodate, without stretching, 80% of the total volume of blood, such that the remaining 20% constitutes a stressed volume that accounts for the MSFP. Owing to a compliance much greater than that of arteries, veins,

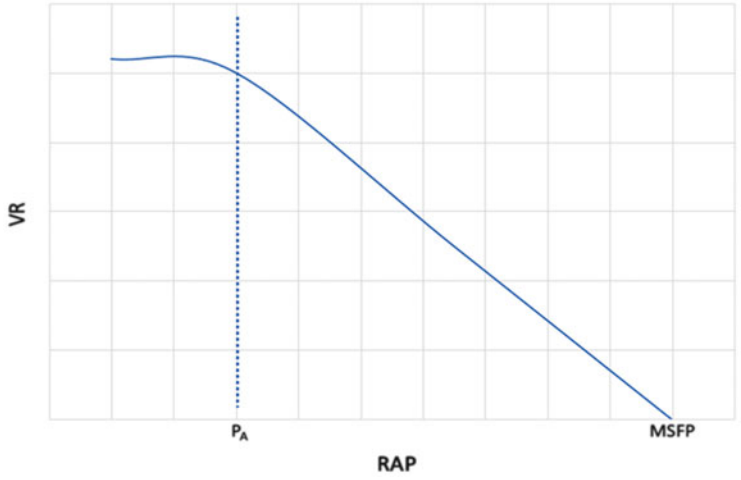


Fig. 10.5 Increasing right atrial pressure (RAP) such that it begins to exceed intra-alveolar pressure (P_A) reduces venous return (VR) to the right atrium. However, having fallen below P_A, further lowering RAP has only a limited effect on VR

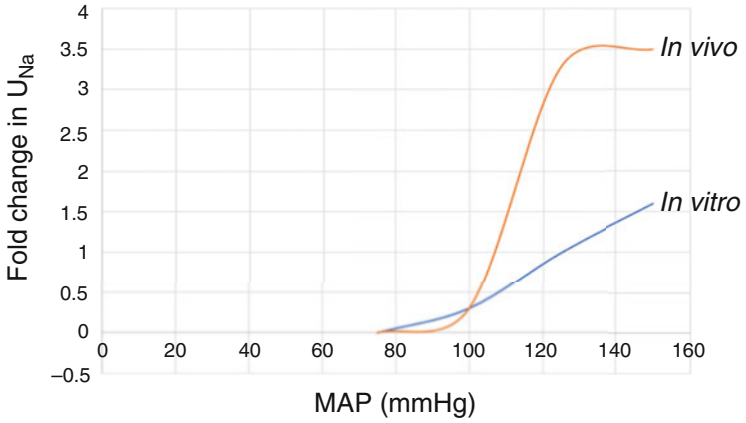


Fig. 10.6 Pressure natriuresis. As corroborated both in vitro and in vivo, a rise in mean arterial pressure (MAP) tends to reduce active Na^+ reabsorption, increasing urinary Na^+ (U_{Na}) excretion

especially in the splanchnic circulation, store the vast majority of this stressed volume.

Shifts in the MSFP and by extension CO, therefore, necessitate shifts in the stressed volume accommodated by veins, especially those in the splanchnic circulation. This raises two possibilities for increasing MSFP: either an expansion of total extracellular fluid volume (ECFV) or a rise in the proportion of ECFV accommodated by veins as stressed volume. Homeostatic regulation of ECFV is subordinate to that of plasma osmolality, the value of which is controlled much more tightly. In light of a relatively constant plasma osmolality, changes in isosmotic Na^+ excretion against an unchanged dietary Na^+ intake permit desired shifts in ECFV.

One physical means of shifting ECFV is pressure natriuresis. Given the imperfection of renal plasma flow (RPF) autoregulation, an increase in ECFV causes a slight rise in RPF and glomerular capillary hydrostatic pressure, as well as a fall in glomerular oncotic pressure. By the Starling filtration-reabsorption equation, this minimally increases GFR, while the larger rise in peritubular capillary hydrostatic pressure and larger fall in peritubular oncotic pressure reduce active Na^+ reabsorption, augmenting Na^+ back-leakage (Seeliger et al. 2001) (Fig. 10.6).

Secondly, the ANS can neurally regulate ECFV. A fall in ECFV reduces the frequency of discharge from cardiopulmonary and arterial baroreceptors to the NTS, which mediates a rise in the frequency of discharge of renal sympathetic postganglionic fibres. As there is a higher density of $(alpha)_1$-ARs in afferent arteriole (DiBona and Kopp 1997), sufficient renal sympathetic activity may override RPF autoregulation to significantly depress GFR. Furthermore, renal sympathetic noradrenaline binds $(alpha)_1$-ARs on the proximal tubule (PT) to promote translocation of Na^+/H^+ exchanger-3 (NHE3) to the apical membrane (Sonalker et al. 2008), and ligates $(beta)_1$-ARs on granular juxtaglomerular cells to increase the secretion of renin (Gordon et al. 1967; Lopez et al. 1978).

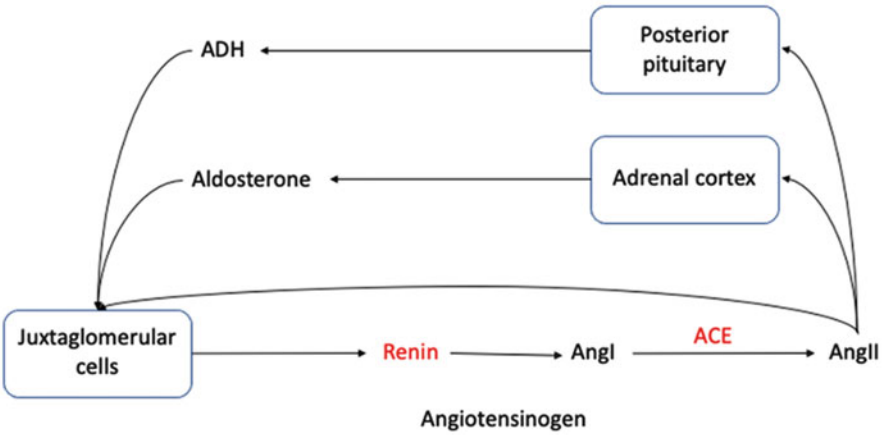


Fig. 10.7 The renin-angiotensin-aldosterone system (RAAS). The sympathetic division of the autonomic nervous system (ANS) promotes juxtaglomerular cell release of renin, which converts angiotensinogen into angiotensin I (AngI), whereupon angiotensin-converting enzyme (ACE) forms AngII. AngII acts on vascular endothelium to increase vasoconstriction, and on the renal tubules to promote Na^+ reabsorption, prolonging the rise in arterial blood pressure (ABP)

Cleaving the precursor plasma globulin, angiotensinogen, renin forms the decapeptide, angiotensin I (AngI), which is further cleaved by angiotensin-converting enzyme (ACE) on the luminal surface of endothelia and vascular myocytes of pulmonary capillaries into the 10-fold more potent octapeptide AngII (Santos et al. 2019) (Fig. 10.7). In turn, AngII acts, via the AngII receptor type 1 (AT_1R), as a dipsogen (Epstein et al. 1970), modulates central integrator influence on sympathetic outflow (Tobey et al. 1983), and permits long-term maintenance of the effects of renal sympathetic activity by increasing NHE3 and Na^+/glucose cotransporter (SGLT-2) translocation to the apical PT, and by preferentially vasoconstricting the efferent arteriole to increase the filtration fraction (Toke and Meyer 2001). Despite initially favouring Na^+ excretion, the ANS thus produces a long-term rise in intravascular oncotic pressure, promoting Na^+ reabsorption and increasing ECFV to normalise ABP.

Moreover, afferent fibres in CN X innervate myelinated B-type venoatrial stretch-activated receptors (Kappagoda et al. 1979). As such, a rise in ECFV stimulates the Bainbridge reflex, whereby stretching of the atria and ventricles stimulates not only natriuresis, but also a selective rise in the activity of sympathetic efferents to the sinoatrial node, reducing RAP. In fact, the influence of the ANS Bainbridge reflex on ECFV regulation predominates over that of atrial natriuretic peptide (ANP) release from the stretched atria and ventricles (Wang et al. 1987).

Age not only diminishes the capacity for pressure natriuresis (Y. G. Kim et al. 2022), but also reduces baseline concentrations of (Weidmann et al. 1975; Tzunoda et al. 1986) and increases vascular (Barrett-O'Keefe et al. 2013; Lang and Krajek 2019), but not intrarenal, sensitivity to ANS-stimulated renin and AngII. However,

although ageing reduces chromaffin cell adrenaline secretion, plasma noradrenaline rises (Ziegler et al. 1976; Seals and Esler 2000) and renal sympathetic outflow appears unchanged (Veith et al. 1986; Esler et al. 1995, 2002).

Age and ANS Control of TPR and Local Vascular Mechanisms

While mechanisms local to the organ in question typically contribute to vascular resistance and thus blood flow, TPR is established by the basal tone of arteriolar smooth muscle tissue, which is principally innervated by the sympathetic division of the ANS. As such, the activity of the central ANS integrator, and, by extension, overall arousal state, which can be modulated by chemoreflex and metaboreflex afferents (Dempsey et al. 2002), sets a cap on the maximal sympathetic stimulation of the vascular tunica media.

The organ-specific distribution of (alpha)-AR and (beta)-AR expression (Robertson et al. 2012) is such that the effects of postganglionic sympathetic activity on vascular tone exhibit regional dependence. In general, however, noradrenaline ligation of (alpha)-ARs tends to promote systemic arteriolar vasoconstriction (Bruno et al. 2012), elevating TPR.

Yet, in some organs, such as the brain, local autoregulation of vascular resistance predominates over ANS control to the extent that endothelial NO and neuronal NO, as well as regional arterial partial pressure of CO_2 (P_aCO_2), are much stronger influences on arteriolar diameter (Claassen et al. 2021).

Age increases resting TPR (Granath et al. 2009; Strandell 1964; Julius et al. 1967; Conway et al. 1971), as the sympathetically (Casey et al. 2012) and local endothelin-mediated (Westby et al. 2011) vasoconstrictory drive, along with vascular hypersensitivity to AngII (Wray et al. 2008), begins to outweigh vascular endothelial responsivity to, and production of, local vasodilators, such as NO (Minson et al. 2002; Black et al. 2008).

Age and ANS Control of HR

ANS modulation of CO and TPR is such that MAP is tightly regulated about a set point. But, in order to reduce the variability of ABP about the set MAP, high-pressure arterial baroreceptors, arterial peripheral chemoreceptors, and low-pressure venoatrial baroreceptors establish a negative feedback loop capable of appropriately adjusting short-term determinants of CO and/or TPR.

Vessels experiencing high blood pressure, such as the carotid sinus and aortic arch, are innervated by Piezo2-expressing mechanosensitive afferent fibres in CNs IX and X, respectively (Min et al. 2019). As such, increased mural stress triggers a rise in the frequency of discharge to the NTS, whereupon central integrator interneurons inhibit the rostral ventrolateral medulla oblongata (Boron and Boulpaep 2017). The consequent fall in sympathetic efferent discharge to the SAN, myocardium, and arteriolar tunica media temporarily reduces HR, SV, and thus, at least over

the next few cardiac cycles (Sagawa 1983; Eckberg and Sleight 1992), CO. In fact, during inspiration, alveolar expansion reduces left atrial pressure and increases the compliance of the pulmonary vasculature, thereby reducing SV. As such, the baroreflex then accelerates HR, and triggers the next inspiratory phase of the respiratory cycle, constituting a cardioventilatory coupling that phase-locks HR to RR (Larsen et al. 2003; Tzeng et al. 2007).

In the context of very low ABP, the unresponsive high-pressure arterial baroreceptors may cede to arterial peripheral chemoreceptors, which signal hypoxaemia along parasympathetic afferents in CNs IX and X, potentiating hypercapnic respiratory drive. As discussed above, there are also parts of the systemic circulation experiencing high blood pressure, such as in the terminal regions of vena cavae and right atrium, which possess stretch receptors that signal ECFV via parasympathetic afferents to the NTS.

Age-related loss of elasticity (Learoyd and Taylor 1966; Bader 1967) and increased rigidity (Abboud and Huston 1961; Gozna et al. 1974) of the arterial wall (Roach and Burton 1959), along with intimal thickening (Virmani et al. 1991), reduce vascular compliance. By restricting the range of action potential frequencies characteristic of the afferent fibres in CNs IX and X, this dampens the gain of the high-pressure baroreflex (Gribbin et al. 1971). Furthermore, despite leaving sympathetic efferents unaffected (Ebert et al. 1992; Matsukawa et al. 1994, 1998; O'Mahony et al. 2000), ageing may indeed impair the sympathetic efferents (Kingwell et al. 1992; Hunt et al. 2001) and parasympathetic ganglia (Bibevski and Dunlap 1999) and blunt the density (Brodde et al. 1998) and responsivity (Poller et al. 1997) of M_2AChR in patients; for a given fluctuation in ABP, the baroreflex is characterised by a smaller short-term vagally mediated change in HR (Laitinen et al. 1998; Monahan et al. 2001).

Age and the Interaction Between the ANS and Immunity

The immune system modulates ANS function. Indeed, interleukin-1(beta) (IL-1 (beta) modulates stress-induced sympathetic efferent discharge (Kenney et al. 2001; Shi et al. 2011), while the concentrations of the systemic inflammatory markers, IL-6 and C-reactive protein (CRP), negatively correlate to baroreflex gain (Banks and Erickson 2010; Huston and Tracey 2011). Moreover, both tumour necrosis factor (alpha) (TNF-(alpha)) (Saigusa 1990; Ohashi and Saigusa 1997) and interferon-(alpha) (Katafuchi et al. 1993a, b) influence the interaction between the central integrator and preganglionic parasympathetic outflow from the medulla. Furthermore, chronic AngII-mediated activation of, and oxidative stress (Lob et al. 2010, 2013) within, neurons within the forebrain's subfornical organ (SFO), in turn, stimulates PVN microglia to augment the pro-inflammatory cytokines that stimulate renal sympathetic outflow (Davisson and Zimmerman 2010). Finally, prostaglandin E_2 (PGE_2) in the hypothalamic preoptic area (POA) alters sympathoexcitatory regulation of the core body temperature set point, thereby contributing to brown

adipose tissue thermogenesis and pyrexia (Morrison 2011; Tanaka et al. 2009; Yoshida et al. 2009; Nakamura 2011; Saper et al. 2012).

In turn, the ANS modulates immune function. Innate and adaptive immune cells express a diverse array of adrenergic and cholinergic receptors (Rosati et al. 1986; van Esch et al. 1989). For instance, $(beta)_2$-ARs inhibit T lymphocyte (Bourne and Melmon 1971; Estes et al. 1971; Makman 1971; DeRubertis et al. 1974; Diamantstein and Ulmer 1975; Vischer 1976; Conolly and Greenacre 1977; Bishopric et al. 1980; Pochet and Delespesse 1983; Brodde et al. 1984; Khan et al. 1986; Ledbetter et al. 1986; Bartik et al. 1994; Sanders et al. 1997), and either promote or suppress B lymphocyte proliferation (Bellinger et al. 2008), while stimulating recruitment (Benschop et al. 1993) but suppressing the activity of natural killer (NK) cells (Shakhar and Ben-Eliyahu 1998; Kanemi et al. 2005), whose cytotoxicity is augmented by $(alpha)_{1/2}$-Ars (Xiao et al. 2010). Splenic sympathoexcitation (Sanders and Straub 2002) upregulates IL-1(beta) and IL-6 (Ganta et al. 2004), and downregulates TNF (Kees et al. 2003), such that acute potential brain injury may elicit peripheral immune dysfunction (Catania et al. 2009), as in post-stroke immunodepression (Dirnagl et al. 2007). Moreover, parasympathetic efferent release of ACh onto macrophages (Tracey 2002) may suppress the release of pro-inflammatory cytokines (Borovikova et al. 2000), including TNF (Tracey 2002; Rosas-Ballina et al. 2011), and in animal models facilitates symptomatic remission in arthritis, colitis, and pancreatitis (Bernik et al. 2002; Guarini et al. 2003).

Age-related loss of sympathetic efferent connections to lymphoid organs (Bellinger et al. 1992a, b, 2008; Thyagarajan et al. 2013) and alteration of (beta)-AR expression in immune cells (Schocken and Roth 1977) may alter cellular trafficking (Redwine et al. 2003) and thus immune responses to infection (Thyagarajan et al. 2000, 2013; Perez et al. 2012).

The Clinical Implications of Age-Related Changes in the ANS

The Ageing ANS and Autonomic Failure

The diminished ANS responses to physiological stressors characteristic of older patients (Collins et al. 1980) can be reproduced, at least in part, in younger patients diagnosed with diabetic neuropathy (Ewing et al. 1978). As such, ageing can be considered a predisposition to autonomic failure, manifesting as higher risks of neurocardiogenic syncope, GI dysfunction, erectile dysfunction, and thermoregulatory impairment.

The Ageing ANS and Neurocardiogenic Syncope

The effects of the ageing ANS on the cardiovascular system have significant implications for patient morbidity and mortality. For instance, the age-related blunting of the cardiovagal baroreflex not only predisposes to neurocardiogenic syncope but also increases the risk of post-myocardial infarction (MI) sudden cardiac death (La Rovere et al. 1988, 1998).

Orthostatic Hypotension

Orthostatic hypotension is a decline of 20 or more mmHg in systolic, or 10 or more mmHg in diastolic, ABP upon 3 min of standing from a sitting position. Under normal physiological conditions, adopting a standing position reduces VR and thus CO, eliciting a baroreflex-mediated temporary increase in heart rate and peripheral resistance. Owing to the aforementioned age-related reduction in the gain of the baroreflex-mediated negative feedback loop controlling short-term postural fluctuations in ABP (Zito et al. 1991; Zachariah et al. 1991; Seals et al. 1999; Maurer et al. 2000), as well as the diminished capacity of the renin-angiotensin-aldosterone system to compensate for intravascular depletion (Shannon et al. 1986), age increases the incidence of orthostatic hypotension (Harris et al. 1991; Rutan et al. 1992).

If sufficiently severe, the resultant cerebral hypoperfusion typically manifests as orthostatic intolerance, which is characterised by nausea, light-headedness and dizziness, speech disturbance, bradyphrenia, delirium (Raj et al. 2018), and even blackout (Low et al. 1995). As such, orthostatic intolerance predisposes older patients to falls (Ooi et al. 2000) and thus fragility fractures (Bulpitt et al. 2006), as well as increasing the risk of a transient ischaemic attack (TIA) (Landi et al. 1983) and MI (Luukinen et al. 2004). In fact, persistent untreated orthostatic hypotension is a risk factor for the development of vascular dementia (Román 2004).

Moreover, the unique combination of age-related baroreflex desensitisation, intravascular redistribution (Wilcox et al. 1984), and the relative rise in basal adrenergic tone (Biaggioni and Robertson 2002) is such that 50% of elderly patients diagnosed with orthostatic hypotension experience supine hypertension (J. Shannon et al. 1997), and for many, drinking water exerts a considerable vasopressor effect.

Carotid Sinus Hypersensitivity

Carotid sinus hypersensitivity is characterised by 5–10 s of a carotid sinus massage eliciting large vasodepressive, constituting a more than 50 mmHg fall in systolic ABP, and/or cardioinhibitory, constituting ventricular asystole lasting longer than 3 s, effects (McIntosh et al. 1993). Up to 40% of asymptomatic elderly patients may exhibit carotid sinus hypersensitivity (Kerr et al. 2006), while, among those

indicated for pacemaker insertion due to suffering from either the cardioinhibitory subtype or recurrent unprovoked syncopal events (Authors/Task Force Members et al. 2007), 30% present with unexplained falls, often accompanied by fragility fracture.

Cerebral Autoregulation

The aforementioned secondary role of the ANS in regulating cerebral blood flow (CBF) is such that age-related ANS dysfunction does not, in itself (Brooks et al. 1989), cause cerebral hypoperfusion (Yam et al. 2005). Consequently, in the context of ageing-induced orthostatic hypotension and carotid sinus hypersensitivity, there is a compensatory expansion of the upper and lower bounds of the range within which the set point of CBF is autoregulated (Safonova et al. 2004). The attendant maintenance of a relatively constant CBF despite considerable fluctuations, postural or otherwise, in ABP ensures that many patients with orthostatic hypotension nevertheless sustain sufficient CBF to remain asymptomatic (Novak et al. 1998).

As such, only in severe cases of orthostatic intolerance or carotid sinus hypersensitivity do prolonged standing and rapid head turning, respectively, sufficiently stimulate the cardioinhibitory and vasodepressor centres of the brainstem to elicit a ventricular asystole long enough and, via the Bezold-Jarisch Reflex (Mark 1983), hypotension significant enough to reduce CBF so much that the patient experiences a loss of consciousness.

The Ageing ANS and GI Dysfunction

Salivation is a feed-forward response to the prospect of food entry into the GI tract. The ANS almost entirely mediates extrinsic control of the salivary glands (Proctor and Carpenter 2007). Postganglionic fibres of the parasympathetic division of the ANS release vasoactive intestinal peptide (VIP) and ACh, which contract myoepithelial cells and open more apical Cl^- channels and basolateral K^+ channels in acinar cells, increasing the volume of primary secretion. In fact, the increase in $[Ca^{2+}]_i$ stimulates exocytosis of mucins and kallikrein, with lysyl-bradykinin a potent vasodilator of arterioles to the salivary gland, and increasing permeability of capillaries perfusing the acini (Garrett 1987). By contrast, while noradrenaline binding to $(beta)_1$-ARs increases $[cAMP]_i$ and PKA activity, potentiating exocytosis of amylase from the acinar cells, sympathetic neurons can promote vasoconstriction via $(alpha)_1$-Ars (Ikawa et al. 1991). As a result, while parasympathetic stimulation results in a fluid-like saliva to facilitate digestion, sympathetic stimulation instead modulates its composition to generate a more viscous saliva that facilitates respiration.

Parasympathetic general visceral efferents (GVEs) synapse with excitatory ENS efferents, promoting ACh release (Porter et al. 1996, 1997) onto M_3AChRs

expressed by interstitial cells of Cajal (ICCs) (Huizinga et al. 1997; Sanders et al. 2006), thereby increasing the amplitude of the slow waves and thus the spike potential frequency of smooth muscle cells, strengthening contractile force. By contrast, sympathetic GVEs stimulate inhibitory ENS efferent release of NO and VIP (Porter et al. 1997; Wattchow et al. 1997), which reduces the amplitude of the slow waves, eliciting smooth muscle relaxation.

In 30–40% of patients aged over 65 years, GI dysfunction (Camilleri et al. 2000) causes constipation and associated faecal impaction and overflow incontinence (Camilleri et al. 2000; Gallagher and O'Mahony 2009). In rodent models, ageing is associated with falls in mucosal secretory capacity and in the number of myenteric postganglionic parasympathetic neurons (Wade 2002; Phillips and Powley 2007), thereby limiting GI motility. In humans, ageing impairs postganglionic parasympathetically mediated smooth muscle contraction within the ascending but not descending colon while leaving total ENS and ANS neuronal populations within the large intestine unaffected (Broad et al. 2019).

The Ageing ANS and Urinary Incontinence

In the context of urothelial stretching (Mulvey et al. 2000), bladder detrusor is relaxed by $(beta)_3$-AR bound by NA from inferior mesenteric and hypogastric sympathetic efferents (Bhide et al. 2012; Khullar et al. 2013), which also binds $(alpha)_1$-ARs to relax the urethral sphincter, promoting urinary storage (Blok et al. 1995). Intense bladder filling promotes the spinobulbospinal reflex, which, while inhibiting sympathetic outflow, stimulates postganglionic parasympathetic neurons to trigger signal transduction through M_3AChRs (Daly et al. 2010). Combined with flux through ATP-bound purine 2X receptors (P2XRs) (Rapp et al. 2005), this propels urine from the full bladder through the relaxed urethral sphincter, enabling micturition.

Ageing not only augments urothelial collagen content and sensory afferent sensitivity (Siroky 2004) but also downregulates M_3AChR (Mansfield et al. 2005) such that ATP-mediated detrusor contraction begins to predominate over that instigated by parasympathetic innervation. This may contribute to the age-related rise in the incidence of urinary incontinence due to overactive bladder.

The Ageing ANS and Erectile Dysfunction

In males, penile erection is exclusively mediated by ANS-induced haemodynamic changes. Without genital arousal, sympathetic tone in the hypogastric nerve (arising from T12-L3) maintains, via $(alpha)_1$-AR, contraction of the smooth muscle in both the cavernous trabeculae and helicine arteries, as well as that of the mounds near the

tunica albuginea surrounding the corpora cavernosa (Lue et al. 1983). This forms a low-volume, low-pressure intracavernous space ($<P_a$) within the flaccid penis.

Genital arousal coincides with increased parasympathetic tone in the pelvic splanchnic nerves (S2-4), which release PGI_2 and VIP to activate nNOS in the smooth muscle mounds (Burnett et al. 1992). Along with eNOS activation in vascular endothelial cells, this stimulates an increase in the production of NO to reduce basal sympathetically mediated contraction of the vascular and mound smooth muscles (Ignarro et al. 1990), directing blood into the corpora cavernosa via arteriovenous shunts (Brindley 1983). The resultant large-volume, high-pressure intracavernous space reduces venous outflow as the rapidly developing turgor compresses the sub-albugineal venous plexus, conferring on the penis a state of tumescence.

Erection, therefore, is characterised by the almost absent blood inflow to (Banya et al. 1989), and outflow from (Fournier et al. 1987), the penis, which thus achieves full rigidity, which can be further increased by the bulbospongiosus reflex, whereby pudendal nerve (S2-4) stimulates rhythmic bulbospongiosus and ischiocavernosus contraction to compress the proximal part of the corpus cavernosum (Previnaire 2018).

Erectile dysfunction is the organic or psychogenic loss of capacity to acquire and sustain penile erection to the extent necessary for satisfactory sexual activity (NIH Consensus Conference. Impotence. NIH Consensus Development Panel on Impotence 1993).

Age is the strongest risk factor for organic erectile dysfunction, the incidence of which almost quadruples from the ages of 40 to 70 (Johannes et al. 2000). Organic causes of erectile dysfunction converge on the insufficiency of arterial inflow during tumescence and on veno-occlusive dysfunction due to loss of corporal smooth muscle function (Nehra et al. 1996) during erection. Although cavernosal veno-occlusive dysfunction is the most common cause across all age groups (Donatucci and Lue 1993), the proportion of cases caused by arterial insufficiency rises with age (Lue et al. 1989). The oxidative stress associated with ageing not only stimulates corporal smooth muscle apoptosis (Grünewald and Beal 1999), but also, at least in part through its aforementioned effects on the ANS, promotes hypertension, a risk factor for vasculogenic erectile dysfunction (Ning and Yang 2017).

The Ageing ANS and Thermoregulation

As endotherms, human's core body changes at a rate largely determined by endogenous thermogenesis, and by superimposed heat gains and losses from the skin. Endothermy confers on humans' core body temperature relative independence from ambient temperature, as well as facilitating rapid growth rates and greater muscular outputs. Yet, endothermy imposes a necessarily high metabolic rate for maintaining core body temperature within the narrow range at which organ function is optimal.

The preoptic area of the hypothalamus, which contains thermoreceptors responsive to the temperature of blood perfusing it and receives inputs from peripheral cutaneous thermoreceptive afferents, has a set point of 37 °C around which core body temperature varies diurnally (Nakamura 2011). As a central integrator of the ANS, the hypothalamus accordingly modulates tone in the parasympathetic and sympathetic divisions to compensate for insensitive heat gains or losses from the skin.

Hyperthermia is an unintentional rise in core body temperature in the absence of changes to the thermoregulatory set point. At rest, high sympathetic tone vasoconstricts dermal arterioles, preventing diversion of flow to more distal cutaneous capillaries. In the context of a very high ambient temperature causing insensible heat gains through cutaneous conduction, the hypothalamus compensatorily promotes arteriolar vasodilatation and, via sudomotor sympathetic cholinergic fibres, eccrine gland secretion of sweat onto the skin, accelerating both conductive and evaporative heat loss (Hu et al. 2018). However, ageing, in part due to the fall in NO production relative to sympathetic tone, impairs heat stress-induced redistribution of splanchnic and renal blood flow to the more superficial cutaneous vessels (Kenney et al. 1997). The resultant inhibition of conductive heat loss to compensate for high ambient temperatures is one of the reasons for which the mortality risk associated with heat stress is particularly high in elderly patients (Kenney et al. 1997).

Hypothermia is, in the absence of any alteration to the thermoregulatory set point, an unintentional fall in core body temperature to below the threshold (typically 35 °C) for maintaining a sufficient metabolic rate. In the context of very low ambient temperatures, in addition to reinforcing basal cutaneous arteriolar vasoconstriction (Wilson et al. 2007), the hypothalamus stimulates high-frequency involuntary and intermittent skeletal muscle contraction, promoting shivering thermogenesis, and the sympathetic division of the ANS, via $(beta)_3$-ARs, to promote brown adipose tissue lipolysis (Cypess et al. 2015) and thus non-shivering thermogenesis. However, with age, the volumes of skeletal muscle (Fukagawa et al. 1995) and brown adipose tissue (Zoico et al. 2019) decline, while allowing the capacity of cutaneous arteriolar smooth muscle to increase (Collins et al. 1977) and sustain (Richardson et al. 1992) tone above their basal vasoconstriction also diminishes. Moreover, ageing reduces the sensitivity of peripheral afferents to low temperatures (Collins et al. 1981).

The Ageing ANS and Sepsis

Sepsis is the life-threatening organ dysfunction that arises from a dysregulated immune response to infection (Rhodes et al. 2017). Some argue that sepsis, during which the spleen acts as the principal source of TNF (Huston et al. 2006), is a neuroendocrine disorder (Munford and Tracey 2002), in which multi-organ failure may arise from the cardiovascular lability conferred by catecholamines (H. Schmidt

et al. 2008), as well as loss of parasympathetically mediated inhibition of pro-inflammatory signalling.

Indeed, sepsis is marked by hyperexcitation of postganglionic sympathetic efferents, while the rises in TNF-(alpha) and interleukins synergistically desensitise Ars (Wu et al. 2003). Despite acting beneficially as a vasopressor to counteract septic shock, noradrenaline may also inhibit innate and adaptive immune function (Scanzano and Cosentino 2015), contributing to immunoparalysis (Stolk et al. 2020), and worsen prognosis (Boldt et al. 1995; Ostrowski et al. 2015).

Ageing, which blunts the baroreflex and suppresses parasympathetic inhibition of macrophage activity (Tracey 2002; Pavlov and Tracey 2005), provides a backdrop for a higher morbidity (Shen et al. 2004; Shi et al. 2007; Nardocci et al. 2015) in sepsis, itself a depressor of baroreflex (Schmidt et al. 2009; Rudiger 2010) and chemoreflex function (Schmidt et al. 2004; Ackland et al. 2013). As such, ANS dysfunction may be a future therapeutic target in elderly patients with sepsis (Carrara et al. 2021).

The Ageing ANS and Peri-Operative Care

To elicit unconsciousness, general anaesthetics must suppress the adrenergic, cholinergic, and orexinergic ascending arousal pathways via the ANS central integrator, the hypothalamic ventrolateral posterior nucleus (VLPO), from which they receive GABAergic projections. In depressing arousal, general anaesthetics tend to especially inhibit the sympathetic division of the ANS. For instance, potent opioids and inhalational anaesthetics, such as halothane and xenon (Xe), may reduce HR variability and augment vagal relative to sympathetic tone.

Ageing reduces the anaesthetic dose necessary to induce unconsciousness (Mapleson 1996; Schnider et al. 1999). With age, anaesthesia-induced unconsciousness requires a smaller magnitude of EEG oscillations and a lower frequency of frontal (alpha)-waves synchronised between the thalamus and neocortex (Purdon et al. 2015). This likely reflects not only neocortical thinning and grey matter atrophy (McGinnis et al. 2011; Fjell et al. 2014), but, owing to the neurochemical genesis of the frontal (alpha)-wave (Ching et al. 2010), age-related changes in GABA-dependent thalamocortical circuits. There is also some evidence in rodents that the age-mediated sensitisation of the CNS and ANS to anaesthesia delays post-anaesthetic recovery (Chemali et al. 2015).

However, many general anaesthetics also reduce ABP. Along with the risk of intraoperative blood loss, this raises the risk that a patient undergoing surgery may become hypotensive. Given that ageing impairs baroreflex function and ANS-endocrine-mediated ECFV homeostasis, this risk may be particularly high in older patients. Furthermore, age-related impairment of thermoregulation may predispose older patients to intraoperative hypothermia. As such, further research is required to confirm or refute these hypotheses, and to suggest and devise appropriate prophylactic solutions.

Conclusions

There remains considerable experimental work to be done in order to assess whether findings on ageing-induced changes in individual CNS neurons are reproducible in individual PNS neurons.

However, there is a substantial base of experimental data suggesting that age influences the overall function of the ANS, contributing to both non-pathological and pathological age-related autonomic dysfunction of mechanisms ranging from control of CO and respiratory rate to that of ocular reflexes and immunity. The clinical implications of such age-induced consequences, including orthostatic hypotension and urinary incontinence, create the possibility that patient care may be tailored not only to age but also specifically to its effects on autonomic function.

References

Abboud FM, Huston JH (1961) The effects of AGEING and degenerative vascular disease on the measurement of arterial rigidity in man*. J Clin Investig 40(6):933–939. https://doi.org/10.1172/JCI104332

Ackland GL, Kazymov V, Marina N, Singer M, Gourine AV (2013) Peripheral neural detection of danger-associated and pathogen-associated molecular patterns. Crit Care Med 41(6):e85–e92. https://doi.org/10.1097/CCM.0b013e31827c0b05

Adlard PA, Perreau VM, Cotman CW (2005) The exercise-induced expression of BDNF within the hippocampus varies across life-span. Neurobiology of Ageing 26(4):511–520. https://doi.org/10.1016/j.neurobiolAgeing.2004.05.006

Akhtar RS, Ness JM, Roth KA (2004) Bcl-2 family regulation of neuronal development and neurodegeneration. Biochim Biophys Acta 1644(2–3):189–203. https://doi.org/10.1016/j.bbamcr.2003.10.013

Al-Chalabi A, Miller CCJ (2003) Neurofilaments and neurological disease. Bioessays 25(4):346–355. https://doi.org/10.1002/bies.10251

Anlauf M, Schäfer MK-H, Eiden L, Weihe E (2003) Chemical coding of the human gastrointestinal nervous system: cholinergic, VIPergic, and catecholaminergic phenotypes. J Comp Neurol 459(1):90–111. https://doi.org/10.1002/cne.10599

Aon MA, Cortassa S, Juhaszova M, Sollott SJ (2016) Mitochondrial health, the epigenome and healthspan. Clin Sci 130(15):1285–1305. https://doi.org/10.1042/CS20160002

Attwell D, Laughlin SB (2001) An energy budget for signaling in the grey matter of the brain. J Cereb Blood Flow Metab 21(10):1133–1145. https://doi.org/10.1097/00004647-200110000-00001

Authors/Task Force Members, Vardas PE, Auricchio A, Blanc J-J, Daubert J-C, Drexler H, Ector H et al (2007) Guidelines for cardiac pacing and cardiac resynchronization therapy: the task force for cardiac pacing and cardiac resynchronization therapy of the European Society of Cardiology. Developed in collaboration with the European heart rhythm association. Eur Heart J 28(18):2256–2295. https://doi.org/10.1093/eurheartj/ehm305

Bader H (1967) Dependence of wall stress in the human thoracic aorta on age and pressure. Circ Res 20(3):354–361. https://doi.org/10.1161/01.RES.20.3.354

Bai P, Cantó C, Oudart H, Brunyánszki A, Cen Y, Thomas C, Yamamoto H et al (2011) PARP-1 inhibition increases mitochondrial metabolism through SIRT1 activation. Cell Metab 13(4):461–468. https://doi.org/10.1016/j.cmet.2011.03.004

Baldwin AS (1996) The NF-KB and IκB proteins: new discoveries and insights. Annu Rev Immunol 14(1):649–681. https://doi.org/10.1146/annurev.immunol.14.1.649

Balu M, Sangeetha P, Haripriya D, Panneerselvam C (2005) Rejuvenation of antioxidant system in central nervous system of aged rats by grape seed extract. Neurosci Lett 383(3):295–300. https://doi.org/10.1016/j.neulet.2005.04.042

Ban TA, Morey L, Aguglia E, Azzarelli O, Balsano F, Marigliano V, Caglieris N et al (1990) Nimodipine in the treatment of old age dementias. Prog Neuropsychopharmacol Biol Psychiatry 14(4):525–551. https://doi.org/10.1016/0278-5846(90)90005-2

Bando H, Zhang C, Takada Y, Yamasaki R, Saito S (1991) Impaired secretion of growth hormone-releasing hormone, growth hormone and IGF-I in elderly men. Acta Endocrinol 124(1):31–36. https://doi.org/10.1530/acta.0.1240031

Bankenahally R, Krovvidi H (2016) Autonomic nervous system: anatomy, physiology, and relevance in anaesthesia and critical care medicine. BJA Educ 16(11):381–387. https://doi.org/10.1093/bjaed/mkw011

Banks WA, Erickson MA (2010) The blood-brain barrier and immune function and dysfunction. Neurobiol Dis 37(1):26–32. https://doi.org/10.1016/j.nbd.2009.07.031

Banya Y, Ushiki T, Takagane H, Aoki H, Kubo T, Ohhori T, Ide C (1989) Two circulatory routes within the human corpus cavernosum penis: a scanning electron microscopic study of corrosion casts. J Urol 142(3):879–883. https://doi.org/10.1016/s0022-5347(17)38935-8

Barrett-O'Keefe Z, Witman MAH, McDaniel J, Fjeldstad AS, Trinity JD, Ives SJ, Conklin JD et al (2013) Angiotensin II potentiates α-adrenergic vasoconstriction in the elderly. Clin Sci (Lond) 124(6):413–422. https://doi.org/10.1042/CS20120424

Bartik MM, Bauman GP, Brooks WH, Roszman TL (1994) Costimulatory signals modulate the antiproliferative effects of agents that elevate CAMP in T cells. Cell Immunol 158(1):116–130. https://doi.org/10.1006/cimm.1994.1261

Beard DA, Feigl EO (2011) Understanding Guyton's venous return curves. Am J Physiol Heart Circ Physiol 301(3):H629–H633. https://doi.org/10.1152/ajpheart.00228.2011

Bellinger DL, Ackerman KD, Felten SY, Felten DL (1992a) A longitudinal study of age-related loss of noradrenergic nerves and lymphoid cells in the rat spleen. Exp Neurol 116(3):295–311. https://doi.org/10.1016/0014-4886(92)90009-f

Bellinger DL, Lorton D, Felten SY, Felten DL (1992b) Innervation of lymphoid organs and implications in development, ageing, and autoimmunity. Int J Immunopharmacol 14(3):329–344. https://doi.org/10.1016/0192-0561(92)90162-e

Bellinger DL, Millar BA, Perez S, Carter J, Wood C, ThyagaRajan S, Molinaro C, Lubahn C, Lorton D (2008) Sympathetic modulation of immunity: relevance to disease. Cell Immunol 252(1–2):27–56. https://doi.org/10.1016/j.cellimm.2007.09.005

Benschop RJ, Oostveen FG, Heijnen CJ, Ballieux RE (1993) Beta 2-adrenergic stimulation causes detachment of natural killer cells from cultured endothelium. Eur J Immunol 23(12):3242–3247. https://doi.org/10.1002/eji.1830231230

Bernik TR, Friedman SG, Ochani M, DiRaimo R, Ulloa L, Yang H, Sudan S, Czura CJ, Ivanova SM, Tracey KJ (2002) Pharmacological stimulation of the cholinergic antiinflammatory pathway. J Exp Med 195(6):781–788. https://doi.org/10.1084/jem.20011714

Bhide AA, Alessandro Digesu G, Fernando R, Khullar V (2012) Mirabegron—a selective B3-adrenoreceptor agonist for the treatment of overactive bladder. Res Rep Urol 4:41–45. https://doi.org/10.2147/RRU.S28930

Biaggioni I, Robertson RM (2002) Hypertension in orthostatic hypotension and autonomic dysfunction. Cardiol Clin 20(2):291–301. https://doi.org/10.1016/S0733-8651(01)00005-4

Bibevski S, Dunlap ME (1999) Ganglionic mechanisms contribute to diminished vagal control in heart failure. Circulation 99(22):2958–2963. https://doi.org/10.1161/01.cir.99.22.2958

Bishopric NH, Cohen HJ, Lefkowitz RJ (1980) Beta adrenergic receptors in lymphocyte subpopulations. J Allergy Clin Immunol 65(1):29–33. https://doi.org/10.1016/0091-6749(80)90173-6

Black MA, Green DJ, Timothy Cable N (2008) Exercise prevents age-related decline in nitric-oxide-mediated vasodilator function in cutaneous microvessels. J Physiol 586(14):3511–3524. https://doi.org/10.1113/jphysiol.2008.153742

Blaustein MP (1988) Calcium transport and buffering in neurons. Trends Neurosci 11(10): 438–443. https://doi.org/10.1016/0166-2236(88)90195-6

Blok BF, De Weerd H, Holstege G (1995) Ultrastructural evidence for a paucity of projections from the lumbosacral cord to the pontine micturition center or M-region in the cat: a new concept for the organization of the micturition reflex with the periaqueductal gray as central relay. J Comp Neurol 359(2):300–309. https://doi.org/10.1002/cne.903590208

Blomberg M, Jensen M, Basun H, Lannfelt L, Wahlund L-O (2001) Cerebrospinal fluid tau levels increase with age in healthy individuals. Dement Geriatr Cogn Disord 12(2):127–132. https://doi.org/10.1159/000051246

Boldt J, Menges T, Kuhn D, Diridis C, Hempelmann G (1995) Alterations in circulating vasoactive substances in the critically Ill—a comparison between survivors and non-survivors. Intensive Care Med 21(3):218–225. https://doi.org/10.1007/BF01701475

Borgdorff P (1975) Respiratory fluctuations in pupil size. Am J Physiol 228(4):1094–1102. https://doi.org/10.1152/ajplegacy.1975.228.4.1094

Boron WF, Boulpaep EL (eds) (2017) Medical physiology, 3rd edn. Elsevier, Philadelphia

Borovikova LV, Ivanova S, Zhang M, Yang H, Botchkina GI, Watkins LR, Wang H, Abumrad N, Eaton JW, Tracey KJ (2000) Vagus nerve stimulation attenuates the systemic inflammatory response to endotoxin. Nature 405(6785):458–462. https://doi.org/10.1038/35013070

Bourne HR, Melmon KL (1971) Adenyl cyclase in human leukocytes: evidence for activation by separate beta adrenergic and prostaglandin receptors. J Pharmacol Exp Ther 178(1):1–7

Breese CR, Ingram RL, Sonntag WE (1991) Influence of age and long-term dietary restriction on plasma insulin-like growth factor-1 (IGF-1), IGF-1 gene expression, and IGF-1 binding proteins. J Gerontol 46(5):B180–B187. https://doi.org/10.1093/geronj/46.5.B180

Brehmer A, Croner R, Dimmler A, Papadopoulos T, Schrödl F, Neuhuber W (2004) Immunohistochemical characterization of putative primary afferent (sensory) myenteric neurons in human small intestine. Auton Neurosci 112(1–2):49–59. https://doi.org/10.1016/j.autneu.2004.03.005

Brennan JM, Blair JE, Goonewardena S, Ronan A, Shah D, Vasaiwala S, Kirkpatrick JN, Spencer KT (2007) Reappraisal of the use of inferior vena cava for estimating right atrial pressure. J Am Soc Echocardiogr 20(7):857–861. https://doi.org/10.1016/j.echo.2007.01.005

Brindley GS (1983) Cavernosal alpha-blockade: a new technique for investigating and treating erectile impotence. Br J Psychiatry 143(4):332–337. https://doi.org/10.1192/bjp.143.4.332

Brini M, Carafoli E (2009) Calcium pumps in health and disease. Physiol Rev 89(4):1341–1378. https://doi.org/10.1152/physrev.00032.2008

Broad J, Kung VWS, Palmer A, Elahi S, Karami A, Darreh-Shori T, Ahmed S et al (2019) Changes in neuromuscular structure and functions of human colon during ageing are region-dependent. Gut 68(7):1210–1223. https://doi.org/10.1136/gutjnl-2018-316279

Brodde OE, Daul A, O'Hara N (1984) Beta-adrenoceptor changes in human lymphocytes, induced by dynamic exercise. Naunyn Schmiedebergs Arch Pharmacol 325(2):190–192. https://doi.org/10.1007/BF00506201

Brodde OE, Konschak U, Becker K, Rüter F, Poller U, Jakubetz J, Radke J, Zerkowski HR (1998) Cardiac muscarinic receptors decrease with age. In vitro and in vivo studies. J Clin Invest 101(2):471–478. https://doi.org/10.1172/JCI1113

Brodde OE, Michel MC (1999) Adrenergic and muscarinic receptors in the human heart. Pharmacol Rev 51(4):651–690

Brooks DJ, Redmond S, Mathias CJ, Bannister R, Symon L (1989) The effect of orthostatic hypotension on cerebral blood flow and middle cerebral artery velocity in autonomic failure, with observations on the action of ephedrine. J Neurol Neurosurg Psychiatry 52(8):962–966. https://doi.org/10.1136/jnnp.52.8.962

Brown MR, Geddes JW, Sullivan PG (2004) Brain region-specific, age-related, alterations in mitochondrial responses to elevated calcium. J Bioenerg Biomembr 36(4):401–406. https://doi.org/10.1023/B:JOBB.0000041775.10388.23

Bruno RM, Ghiadoni L, Seravalle G, Dell'Oro R, Taddei S, Grassi G (2012) Sympathetic regulation of vascular function in health and disease. Front Physiol 3. https://doi.org/10.3389/fphys.2012.00284

Bulpitt CJ, Peters R, Staessen JA, Thijs L, De Vernejoul M-C, Fletcher AE, Beckett NS (2006) Fracture risk and the use of a diuretic (indapamide Sr) ± perindopril: a substudy of the hypertension in the very elderly trial (HYVET). Trials 7(1):33. https://doi.org/10.1186/1745-6215-7-33

Burkewitz K, Weir HJM, Mair WB (2016) AMPK as a pro-longevity target. In: Cordero MD, Viollet B (eds) AMP-activated protein kinase, vol 107. Springer, Cham, pp 227–256. Experientia supplementum. https://doi.org/10.1007/978-3-319-43589-3_10

Burnett AL, Lowenstein CJ, Bredt DS, Chang TS, Snyder SH (1992) Nitric oxide: a physiologic mediator of penile erection. Science 257(5068):401–403. https://doi.org/10.1126/science.1378650

Calabrese V, Scapagnini G, Ravagna A, Colombrita C, Spadaro F, Butterfield DA, Giuffrida Stella AM (2004) Increased expression of heat shock proteins in rat brain during ageing: relationship with mitochondrial function and glutathione redox state. Mech Ageing Dev 125(4):325–335. https://doi.org/10.1016/j.mad.2004.01.003

Camello C, Lomax R, Petersen OH, Tepikin AV (2002) Calcium leak from intracellular stores—the enigma of calcium signalling. Cell Calcium 32(5–6):355–361. https://doi.org/10.1016/S0143416002001926

Camilleri M, Lee JS, Viramontes B, Bharucha AE, Tangalos EG (2000) Insights into the pathophysiology and mechanisms of constipation, irritable bowel syndrome, and diverticulosis in older people. J Am Geriatr Soc 48(9):1142–1150. https://doi.org/10.1111/j.1532-5415.2000.tb04793.x

Carafoli E, Longoni S (1987) The plasma membrane in the control of the signaling function of calcium. Soc Gen Physiol Ser 42:21–29

Carbone E, Borges R, Eiden LE, García AG, Hernández-Cruz A (2019) Chromaffin cells of the adrenal medulla: physiology, pharmacology, and disease. Compr Physiol 9(4):1443–1502. https://doi.org/10.1002/cphy.c190003

Carrara M, Ferrario M, Pinto BB, Herpain A (2021) The autonomic nervous system in septic shock and its role as a future therapeutic target: a narrative review. Ann Intensive Care 11(1):80. https://doi.org/10.1186/s13613-021-00869-7

Casey DP, Padilla J, Joyner MJ (2012) α-Adrenergic vasoconstriction contributes to the age-related increase in conduit artery retrograde and oscillatory shear. Hypertension 60(4):1016–1022. https://doi.org/10.1161/HYPERTENSIONAHA.112.200618

Catania A, Lonati C, Sordi A, Gatti S (2009) Detrimental consequences of brain injury on peripheral cells. Brain Behav Immun 23(7):877–884. https://doi.org/10.1016/j.bbi.2009.04.006

Catterall WA, Nunoki K, Lai Y, De Jongh K, Thomsen W, Rossie S (1990) Structure and modulation of voltage-sensitive sodium and calcium channels. Adv Second Messenger Phosphoprotein Res 24:30–35

Chandel NS, McClintock DS, Feliciano CE, Wood TM, Andres Melendez J, Rodriguez AM, Schumacker PT (2000) Reactive oxygen species generated at mitochondrial complex III stabilize hypoxia-inducible factor-1α during hypoxia. J Biol Chem 275(33):25130–25138. https://doi.org/10.1074/jbc.M001914200

Chandrasekaran A, del Pilar M, Idelchik S, Andrés Melendez J (2017) Redox control of senescence and age-related disease. Redox Biol 11:91–102. https://doi.org/10.1016/j.redox.2016.11.005

Chapman JA, Kirkness EF, Simakov O, Hampson SE, Mitros T, Weinmaier T, Rattei T et al (2010) The dynamic genome of hydra. Nature 464(7288):592–596. https://doi.org/10.1038/nature08830

Chemali JJ, Kenny JD, Olutola O, Taylor NE, Kimchi EY, Purdon PL, Brown EN, Solt K (2015) Ageing delays emergence from general anaesthesia in rats by increasing anaesthetic sensitivity in the brain. Br J Anaesth 115:i58–i65. https://doi.org/10.1093/bja/aev112

Chen L, Liu L, Yin J, Luo Y, Huang S (2009) Hydrogen peroxide-induced neuronal apoptosis is associated with inhibition of protein phosphatase 2A and 5, leading to activation of MAPK pathway. Int J Biochem Cell Biol 41(6):1284–1295. https://doi.org/10.1016/j.biocel.2008.10.029

Cheung ECC, Melanson-Drapeau L, Cregan SP, Vanderluit JL, Ferguson KL, McIntosh WC, Park DS, Bennett SAL, Slack RS (2005) Apoptosis-inducing factor is a key factor in neuronal cell death propagated by BAX-dependent and BAX-independent mechanisms. J Neurosci Off J Soc Neurosci 25(6):1324–1334. https://doi.org/10.1523/JNEUROSCI.4261-04.2005

Ching SN, Cimenser A, Purdon PL, Brown EN, Kopell NJ (2010) Thalamocortical model for a propofol-induced α-rhythm associated with loss of consciousness. Proc Natl Acad Sci 107(52):22665–22670. https://doi.org/10.1073/pnas.1017069108

Chomyn A, Attardi G (2003) MtDNA mutations in ageing and apoptosis. Biochem Biophys Res Commun 304(3):519–529. https://doi.org/10.1016/S0006-291X(03)00625-9

Chongthammakun V, Sanvarinda Y, Chongthammakun S (2009) Reactive oxygen species production and MAPK activation are implicated in tetrahydrobiopterin-induced SH-SY5Y cell death. Neurosci Lett 449(3):178–182. https://doi.org/10.1016/j.neulet.2008.10.106

Claassen JAHR, Thijssen DHJ, Panerai RB, Faraci FM (2021) Regulation of cerebral blood flow in humans: physiology and clinical implications of autoregulation. Physiol Rev 101(4):1487–1559. https://doi.org/10.1152/physrev.00022.2020

Collins KJ, Dore C, Exton-Smith AN, Fox RH, MacDonald IC, Woodward PM (1977) Accidental hypothermia and impaired temperature homoeostasis in the elderly. BMJ 1(6057):353–356. https://doi.org/10.1136/bmj.1.6057.353

Collins KJ, Exton-Smith AN, Dore C (1981) Urban hypothermia: preferred temperature and thermal perception in old age. BMJ 282(6259):175–177. https://doi.org/10.1136/bmj.282.6259.175

Collins KJ, Exton-Smith AN, James MH, Oliver DJ (1980) Functional changes in autonomic nervous responses with ageing. Age Ageing 9(1):17–24. https://doi.org/10.1093/ageing/9.1.17

Conolly ME, Greenacre JK (1977) The beta-adrenoceptor of the human lymphocyte and human lung parenchyma. Br J Pharmacol 59(1):17–23. https://doi.org/10.1111/j.1476-5381.1977.tb06971.x

Contreras L, Drago I, Zampese E, Pozzan T (2010) Mitochondria: the calcium connection. Biochim Biophys Acta 1797(6–7):607–618. https://doi.org/10.1016/j.bbabio.2010.05.005

Conway J, Wheeler R, Sannerstedt R (1971) Sympathetic nervous activity during exercise in relation to age. Cardiovasc Res 5(4):577–581. https://doi.org/10.1093/cvr/5.4.577

Copp AJ, Greene NDE, Murdoch JN (2003) The genetic basis of mammalian neurulation. Nat Rev Genet 4(10):784–793. https://doi.org/10.1038/nrg1181

Cotman CW, Gómez-Pinilla F (1991) Basic fibroblast growth factor in the mature brain and its possible role in Alzheimer's disease. Ann N Y Acad Sci 638:221–231. https://doi.org/10.1111/j.1749-6632.1991.tb49033.x

Counts SE, Mufson EJ (2005) The role of nerve growth factor receptors in cholinergic basal forebrain degeneration in prodromal Alzheimer disease. J Neuropathol Exp Neurol 64(4):263–272. https://doi.org/10.1093/jnen/64.4.263

Coupland RE, Fujita T, Zaidan NKKS (eds) (1976) Chromaffin, enterochromaffin, & related cells: a Naito Foundation symposium. Amsterdam. Elsevier Scientific Pub. Co., New York

Csordás G, Renken C, Várnai P, Walter L, Weaver D, Buttle KF, Balla T, Mannella CA, Hajnóczky G (2006) Structural and functional features and significance of the physical linkage between ER and mitochondria. J Cell Biol 174(7):915–921. https://doi.org/10.1083/jcb.200604016

Cutler RG, Kelly J, Storie K, Pedersen WA, Tammara A, Hatanpaa K, Troncoso JC, Mattson MP (2004) Involvement of oxidative stress-induced abnormalities in ceramide and cholesterol

metabolism in brain ageing and Alzheimer's disease. Proc Natl Acad Sci U S A 101(7): 2070–2075. https://doi.org/10.1073/pnas.0305799101
Cypess AM, Weiner LS, Roberts-Toler C, Elía EF, Kessler SH, Kahn PA, English J et al (2015) Activation of human Brown adipose tissue by a B3-adrenergic receptor agonist. Cell Metab 21(1):33–38. https://doi.org/10.1016/j.cmet.2014.12.009
Daly DM, Chess-Williams R, Chapple C, Grundy D (2010) The inhibitory role of acetylcholine and muscarinic receptors in bladder afferent activity. Eur Urol 58(1):22–28.; discussion 31–32. https://doi.org/10.1016/j.eururo.2009.12.030
Dampney RA (1994) Functional organization of central pathways regulating the cardiovascular system. Physiol Rev 74(2):323–364. https://doi.org/10.1152/physrev.1994.74.2.323
Dampney RA, Horiuchi J, Tagawa T, Fontes MA, Potts PD, Polson JW (2003) Medullary and supramedullary mechanisms regulating sympathetic vasomotor tone. Acta Physiol Scand 177(3):209–218. https://doi.org/10.1046/j.1365-201X.2003.01070.x
Davisson RL, Zimmerman MC (2010) Angiotensin II, oxidant signaling, and hypertension: down to a T? Hypertension 55(2):228–230. https://doi.org/10.1161/HYPERTENSIONAHA.109.144477
Deas E, Wood NW, Plun-Favreau H (2011) Mitophagy and Parkinson's disease: the PINK1–Parkin link. Biochim Biophys Acta 1813(4):623–633. https://doi.org/10.1016/j.bbamcr.2010.08.007
Dehmelt L, Halpain S (2004) Genome Biol 6(1):204. https://doi.org/10.1186/gb-2004-6-1-204
Demontis F, Perrimon N (2010) FOXO/4E-BP signaling in drosophila muscles regulates organism-wide proteostasis during ageing. Cell 143(5):813–825. https://doi.org/10.1016/j.cell.2010.10.007
Dempsey JA, Sheel AW, St. Croix CM, Morgan BJ (2002) Respiratory influences on sympathetic vasomotor outflow in humans. Respir Physiol Neurobiol 130(1):3–20. https://doi.org/10.1016/S0034-5687(01)00327-9
DeRubertis FR, Zenser TV, Adler WH, Hudson T (1974) Role of cyclic adenosine 3',5'--monophosphate in lymphocyte mitogenesis. J Immunol 113(1):151–161
Diamantstein T, Ulmer A (1975) The antagonistic action of cyclic GMP and cyclic AMP on proliferation of B and T lymphocytes. Immunology 28(1):113–119
DiBona GF, Kopp UC (1997) Neural control of renal function. Physiol Rev 77(1):75–197. https://doi.org/10.1152/physrev.1997.77.1.75
Dice JF (1998) How do intracellular proteolytic systems change with age. Front Biosci 3(4):d25–d43. https://doi.org/10.2741/A264
Dickson EJ, Heredia DJ, Smith TK (2010) Critical role of 5-HT1A, 5-HT3, and 5-HT7 receptor subtypes in the initiation, generation, and propagation of the murine colonic migrating motor complex. Am J Physiol Gastrointest Liver Physiol 299(1):G144–G157. https://doi.org/10.1152/ajpgi.00496.2009
DiDonato JA, Hayakawa M, Rothwarf DM, Zandi E, Karin M (1997) A cytokine-responsive IκB kinase that activates the transcription factor NF-KB. Nature 388(6642):548–554. https://doi.org/10.1038/41493
Dirnagl U, Klehmet J, Braun JS, Harms H, Meisel C, Ziemssen T, Prass K, Meisel A (2007) Stroke-induced immunodepression: experimental evidence and clinical relevance. Stroke 38(2 Suppl):770–773. https://doi.org/10.1161/01.STR.0000251441.89665.bc
Do MTH, Yau K-W (2010) Intrinsically photosensitive retinal ganglion cells. Physiol Rev 90(4): 1547–1581. https://doi.org/10.1152/physrev.00013.2010
Docherty JR (1990) Cardiovascular responses in ageing: a review. Pharmacol Rev 42(2):103–125
Donahue AN, Aschner M, Lash LH, Syversen T, Sonntag WE (2006) Growth hormone administration to aged animals reduces disulfide glutathione levels in hippocampus. Mech Ageing Dev 127(1):57–63. https://doi.org/10.1016/j.mad.2005.09.003
Donatucci CF, Lue TF (1993) Erectile dysfunction in men under 40: etiology and treatment choice. Int J Impot Res 5(2):97–103

Duan W, Zhang R, Guo Y, Jiang Y, Huang Y, Jiang H, Li C (2009) Nrf2 activity is lost in the spinal cord and its astrocytes of aged mice. In Vitro Cell Dev Biol Anim 45(7):388–397. https://doi.org/10.1007/s11626-009-9194-5

Duane A (1922) Studies in monocular and binocular accommodation, with their clinical application. Trans Am Ophthalmol Soc 20:132–157

Ebert TJ, Morgan BJ, Barney JA, Denahan T, Smith JJ (1992) Effects of ageing on baroreflex regulation of sympathetic activity in humans. Am J Physiol Heart Circ Physiol 263(3):H798–H803. https://doi.org/10.1152/ajpheart.1992.263.3.H798

Eckberg DL, Sleight P (1992) Human baroreflexes in health and disease, vol 43. Oxford Medical Publications/Clarendon Press, Oxford

Editorial (1998) Neuroscience 83(3):663–668. https://doi.org/10.1016/S0306-4522(97)00539-3

Egan DF, Shackelford DB, Mihaylova MM, Gelino S, Kohnz RA, Mair W, Vasquez DS et al (2011) Phosphorylation of ULK1 (HATG1) by AMP-activated protein kinase connects energy sensing to mitophagy. Science 331(6016):456–461. https://doi.org/10.1126/science.1196371

Elfvin L-G, Lindh B, Hökfelt T (1993) The chemical neuroanatomy of sympathetic ganglia. Annu Rev Neurosci 16(1):471–507. https://doi.org/10.1146/annurev.ne.16.030193.002351

Epstein AN, Fitzsimons JT, Rolls BJ (1970) Drinking induced by injection of angiotensin into the brain of the rat. J Physiol 210(2):457–474. https://doi.org/10.1113/jphysiol.1970.sp009220

van Esch B, Henricks PA, van Oosterhout AJ, Nijkamp FP (1989) Guinea pig alveolar macrophages possess beta-adrenergic receptors. Agents Actions 26(1–2):123–124. https://doi.org/10.1007/BF02126582

Esler MD, Turner AG, Kaye DM, Thompson JM, Kingwell BA, Morris M, Lambert GW, Jennings GL, Cox HS, Seals DR (1995) Ageing effects on human sympathetic neuronal function. Am J Physiol Regul Integr Comp Physiol 268(1):R278–R285. https://doi.org/10.1152/ajpregu.1995.268.1.R278

Esler M, Jennings G, Lambert G, Meredith I, Horne M, Eisenhofer G (1990) Overflow of catecholamine neurotransmitters to the circulation: source, fate, and functions. Physiol Rev 70(4):963–985. https://doi.org/10.1152/physrev.1990.70.4.963

Esler M, Hastings J, Lambert G, Kaye D, Jennings G, Seals DR (2002) The influence of ageing on the human sympathetic nervous system and brain norepinephrine turnover. Am J Physiol Regul Integr Comp Physiol 282(3):R909–R916. https://doi.org/10.1152/ajpregu.00335.2001

Espinosa-Medina I, Saha O, Boismoreau F, Chettouh Z, Rossi F, Richardson WD, Brunet J-F (2016) The sacral autonomic outflow is sympathetic. Science 354(6314):893–897. https://doi.org/10.1126/science.aah5454

Estes G, Solomon SS, Norton WL (1971) Inhibition of lymphocyte stimulation by cyclic and non-cyclic nucleotides. J Immunol 107(5):1489–1492

Ewing DJ, Campbell IW, Murray A, Neilson JM, Clarke BF (1978) Immediate heart-rate response to standing: simple test for autonomic neuropathy in diabetes. BMJ 1(6106):145–147. https://doi.org/10.1136/bmj.1.6106.145

Farrand AQ, Gregory RA, Scofield MD, Helke KL, Boger HA (2015) Effects of ageing on glutamate neurotransmission in the substantia nigra of Gdnf heterozygous mice. Neurobiology of Ageing 36(3):1569–1576. https://doi.org/10.1016/j.neurobiolAgeing.2014.11.017

Fjell AM, Westlye LT, Grydeland H, Amlien I, Espeseth T, Reinvang I, Raz N, Dale AM, Walhovd KB, for the Alzheimer Disease NeuroimAgeing Initiative (2014) Accelerating cortical thinning: unique to dementia or universal in ageing? Cereb Cortex 24(4):919–934. https://doi.org/10.1093/cercor/bhs379

Folkow B, Svanborg A (1993) Physiology of cardiovascular ageing. Physiol Rev 73(4):725–764. https://doi.org/10.1152/physrev.1993.73.4.725

Fournier GR, Juenemann KP, Lue TF, Tanagho EA (1987) Mechanisms of venous occlusion during canine penile erection: an anatomic demonstration. J Urol 137(1):163–167. https://doi.org/10.1016/s0022-5347(17)43911-5

Franceschi C, Bonafè M, Valensin S, Olivieri F, De Luca M, Ottaviani E, De Benedictis G (2006) Inflamm-ageing: an evolutionary perspective on immunosenescence. Ann N Y Acad Sci 908(1): 244–254. https://doi.org/10.1111/j.1749-6632.2000.tb06651.x

Frank O (1959) On the dynamics of cardiac muscle. Am Heart J 58(2):282–317. https://doi.org/10.1016/0002-8703(59)90345-X

Fröjdö S, Durand C, Molin L, Carey AL, El-Osta A, Kingwell BA, Febbraio MA, Solari F, Vidal H, Pirola L (2011) Phosphoinositide 3-kinase as a novel functional target for the regulation of the insulin signaling pathway by SIRT1. Mol Cell Endocrinol 335(2):166–176. https://doi.org/10.1016/j.mce.2011.01.008

Frutos MGS, Cacicedo L, Mendez CF, Vicent D, Gonzalez M, Sanchez-Franco F (2007) Pituitary alterations involved in the decline of growth hormone gene expression in the pituitary of ageing rats. J Gerontol Ser A Biol Med Sci 62(6):585–597. https://doi.org/10.1093/gerona/62.6.585

Fukagawa NK, Kenney WL, Buskirk ER (1995) Functional consequences of sarcopenia: effects on thermoregulation. J Gerontol Ser A Biol Sci Med Sci 50A(Special):78–85. https://doi.org/10.1093/gerona/50A.Special_Issue.78

Furness JB, Jones C, Nurgali K, Clerc N (2004) Intrinsic primary afferent neurons and nerve circuits within the intestine. Prog Neurobiol 72(2):143–164. https://doi.org/10.1016/j.pneurobio.2003.12.004

Furuichi T, Furutama D, Hakamata Y, Nakai J, Takeshima H, Mikoshiba K (1994) Multiple types of ryanodine receptor/Ca2+ release channels are differentially expressed in rabbit brain. J Neurosci 14(8):4794–4805. https://doi.org/10.1523/JNEUROSCI.14-08-04794.1994

Gallagher P, O'Mahony D (2009) Constipation in old age. Best Pract Res Clin Gastroenterol 23(6): 875–887. https://doi.org/10.1016/j.bpg.2009.09.001

Gant JC (2006) Early and simultaneous emergence of multiple hippocampal biomarkers of ageing is mediated by Ca2+-induced Ca2+ release. J Neurosci 26(13):3482–3490. https://doi.org/10.1523/JNEUROSCI.4171-05.2006

Ganta CK, Blecha F, Ganta RR, Helwig BG, Parimi S, Ning L, Fels RJ, Musch TI, Kenney MJ (2004) Hyperthermia-enhanced splenic cytokine gene expression is mediated by the sympathetic nervous system. Physiol Genomics 19(2):175–183. https://doi.org/10.1152/physiolgenomics.00109.2004

Garrett JR (1987) The proper role of nerves in salivary secretion: a review. J Dent Res 66(2): 387–397. https://doi.org/10.1177/00220345870660020201

Gash DM, Zhang Z, Gerhardt G (1998) Neuroprotective and neurorestorative properties of GDNF: GDNF in neuroprotection. Ann Neurol 44(S1):S121–S125. https://doi.org/10.1002/ana.410440718

Gavazzi I (1995) Collateral sprouting and responsiveness to nerve growth factor of ageing neurons. Neurosci Lett 189(1):47–50. https://doi.org/10.1016/0304-3940(95)11449-7

Gelfand R, Lambertsen CJ (1973) Dynamic respiratory response to abrupt change of inspired CO2 at normal and high PO2. J Appl Physiol 35(6):903–913. https://doi.org/10.1152/jappl.1973.35.6.903

Geula C, Jing B, Nagykery N, Scinto LFM, Chan J, Joseph J, Parker R, Chuang-Kuo W (2003) Loss of calbindin-D28k from ageing human cholinergic basal forebrain: relation to neuronal loss. J Comp Neurol 455(2):249–259. https://doi.org/10.1002/cne.10475

Gibbins I (2012) Peripheral autonomic pathways'. In: The human nervous system. Elsevier, pp 141–185. https://doi.org/10.1016/B978-0-12-374236-0.10005-7

Gibson GE, Peterson C (1981) Ageing decreases oxidative metabolism and the release and synthesis of acetylcholine. J Neurochem 37(4):978–984. https://doi.org/10.1111/j.1471-4159.1981.tb04484.x

Goedert M (2005) Tau gene mutations and their effects. Mov Disord 20(Suppl 12):S45–S52. https://doi.org/10.1002/mds.20539

Gomes AP, Price NL, Ling AJY, Moslehi JJ, Montgomery MK, Rajman L, White JP et al (2013) Declining NAD(+) induces a pseudohypoxic state disrupting nuclear-mitochondrial communication during ageing. Cell 155(7):1624–1638. https://doi.org/10.1016/j.cell.2013.11.037

Gooney M, Messaoudi E, Maher FO, Bramham CR, Lynch MA (2004) BDNF-induced LTP in dentate gyrus is impaired with age: analysis of changes in cell signaling events. Neurobiol Ageing 25(10):1323–1331. https://doi.org/10.1016/j.neurobiolAgeing.2004.01.003

Gordon RD, Küchel O, Liddle GW, Island DP (1967) Role of the sympathetic nervous system in regulating renin and aldosterone production in man*. J Clin Investig 46(4):599–605. https://doi.org/10.1172/JCI105561

Gozna ER, Marble AE, Shaw A, Holland JG (1974) Age-related changes in the mechanics of the aorta and pulmonary artery of man. J Appl Physiol 36(4):407–411. https://doi.org/10.1152/jappl.1974.36.4.407

Granath A, Jonsson B, Strandell T (2009) Circulation in healthy old men, studied by right heart catheterization at rest and during exercise in supine and sitting position. Acta Med Scand 176(4):425–446. https://doi.org/10.1111/j.0954-6820.1964.tb00949.x

Gribbin B, Pickering TG, Sleight P, Peto R (1971) Effect of age and high blood pressure on Barorefiex sensitivity in man. Circ Res 29(4):424–431. https://doi.org/10.1161/01.RES.29.4.424

Griendling KK, Sorescu D, Ushio-Fukai M (2000) NAD(P)H oxidase: role in cardiovascular biology and disease. Circ Res 86(5):494–501. https://doi.org/10.1161/01.res.86.5.494

Grünewald T, Beal MF (1999) NOS knockouts and neuroprotection. Nat Med 5(12):1354–1355. https://doi.org/10.1038/70918

Gruver A, Hudson L, Sempowski G (2007) Immunosenescence of ageing. J Pathol 211(2):144–156. https://doi.org/10.1002/path.2104

Guarini S, Altavilla D, Cainazzo M-M, Giuliani D, Bigiani A, Marini H, Squadrito G et al (2003) Efferent vagal fibre stimulation blunts nuclear factor-KappaB activation and protects against hypovolemic Hemorrhagic Shock. Circulation 107(8):1189–1194. https://doi.org/10.1161/01.cir.0000050627.90734.ed

Guebel DV, Torres NV (2016) Sexual dimorphism and ageing in the human hyppocampus: identification, validation, and impact of differentially expressed genes by factorial microarray and network analysis. Front Ageing Neurosci 8. https://doi.org/10.3389/fnagi.2016.00229

Guyton AC, Lindsey AW, Abernathy B, Richardson T (1957) Venous return at various right atrial pressures and the normal venous return curve. Am J Physiol 189(3):609–615. https://doi.org/10.1152/ajplegacy.1957.189.3.609

Hara T, Nakamura K, Matsui M, Yamamoto A, Nakahara Y, Suzuki-Migishima R, Yokoyama M et al (2006) Suppression of basal autophagy in neural cells causes neurodegenerative disease in mice. Nature 441(7095):885–889. https://doi.org/10.1038/nature04724

Hardie DG (2015) AMPK: positive and negative regulation, and its role in whole-body energy homeostasis. Curr Opin Cell Biol 33(April):1–7. https://doi.org/10.1016/j.ceb.2014.09.004

Hardie DG, Ross FA, Hawley SA (2012) AMPK: a nutrient and energy sensor that maintains energy homeostasis. Nat Rev Mol Cell Biol 13(4):251–262. https://doi.org/10.1038/nrm3311

Harris JJ, Jolivet R, Attwell D (2012) Synaptic energy use and supply. Neuron 75(5):762–777. https://doi.org/10.1016/j.neuron.2012.08.019

Harris T, Lipsitz LA, Kleinman JC, Cornoni-Huntley J (1991) Postural change in blood pressure associated with age and systolic blood pressure. J Gerontol 46(5):M159–M163. https://doi.org/10.1093/geronj/46.5.M159

Hattiangady B, Rao MS, Shetty GA, Shetty AK (2005) Brain-derived neurotrophic factor, phosphorylated cyclic AMP response element binding protein and neuropeptide Y decline as early as middle age in the dentate gyrus and CA1 and CA3 subfields of the hippocampus. Exp Neurol 195(2):353–371. https://doi.org/10.1016/j.expneurol.2005.05.014

Hu Y, Converse C, Lyons MC, Hsu WH (2018) Neural control of sweat secretion: a review. Br J Dermatol 178(6):1246–1256. https://doi.org/10.1111/bjd.15808

Huizinga JD, Thuneberg L, Vanderwinden J-M, Rumessen JJ (1997) Interstitial cells of Cajal as targets for pharmacological intervention in gastrointestinal motor disorders. Trends Pharmacol Sci 18(10):393–403. https://doi.org/10.1016/S0165-6147(97)01108-5

Hunt BE, Farquhar WB, Andrew Taylor J (2001) Does reduced vascular stiffening fully explain preserved cardiovagal baroreflex function in older, physically active men? Circulation 103(20): 2424–2427. https://doi.org/10.1161/01.CIR.103.20.2424

Huston JM, Tracey KJ (2011) The pulse of inflammation: heart rate variability, the cholinergic anti-inflammatory pathway and implications for therapy. J Intern Med 269(1):45–53. https://doi.org/10.1111/j.1365-2796.2010.02321.x

Huston JM, Ochani M, Rosas-Ballina M, Liao H, Ochani K, Pavlov VA, Gallowitsch-Puerta M et al (2006) Splenectomy inactivates the cholinergic antiinflammatory pathway during lethal endotoxemia and polymicrobial sepsis. J Exp Med 203(7):1623–1628. https://doi.org/10.1084/jem.20052362

Iacopino AM, Christakos S (1990) Specific reduction of calcium-binding protein (28-kilodalton calbindin-D) gene expression in ageing and neurodegenerative diseases. Proc Natl Acad Sci 87(11):4078–4082. https://doi.org/10.1073/pnas.87.11.4078

Ignarro LJ, Bush PA, Buga GM, Wood KS, Fukuto JM, Rajfer J (1990) Nitric oxide and cyclic GMP formation upon electrical field stimulation cause relaxation of corpus cavernosum smooth muscle. Biochem Biophys Res Commun 170(2):843–850. https://doi.org/10.1016/0006-291x(90)92168-y

Ikawa M, Hector M, Proctor G (1991) Parotid protein secretion from the rabbit during feeding. Exp Physiol 76(5):717–724. https://doi.org/10.1113/expphysiol.1991.sp003538

Ikonomovic MD, Mizukami K, Warde D, Sheffield R, Hamilton R, Wenthold RJ, Armstrong DM (1999) Distribution of glutamate receptor subunit NMDAR1 in the hippocampus of normal elderly and patients with Alzheimer's disease. Exp Neurol 160(1):194–204. https://doi.org/10.1006/exnr.1999.7196

Iranmanesh A, Lizarralde G, Veldhuis JD (1991) Age and relative adiposity are specific negative determinants of the frequency and amplitude of growth hormone (GH) secretory bursts and the half-life of endogenous GH in healthy men*. J Clin Endocrinol Metabol 73(5):1081–1088. https://doi.org/10.1210/jcem-73-5-1081

Ishihara T, Hong M, Zhang B, Nakagawa Y, Lee MK, Trojanowski JQ, Virginia M-Y, Lee. (1999) Age-dependent emergence and progression of a tauopathy in transgenic mice overexpressing the shortest human tau isoform. Neuron 24(3):751–762. https://doi.org/10.1016/S0896-6273(00)81127-7

Iverson SL, Orrenius S (2004) The cardiolipin–cytochrome c interaction and the mitochondrial regulation of apoptosis. Arch Biochem Biophys 423(1):37–46. https://doi.org/10.1016/j.abb.2003.12.002

Jäger S, Handschin C, St-Pierre J, Spiegelman BM (2007) AMP-activated protein kinase (AMPK) action in skeletal muscle via direct phosphorylation of PGC-1alpha. Proc Natl Acad Sci U S A 104(29):12017–12022. https://doi.org/10.1073/pnas.0705070104

Jang SY, Shin YK, Park SY, Park JY, Rha S-H, Kim JK, Lee HJ, Park HT (2015) Autophagy is involved in the reduction of myelinating Schwann cell cytoplasm during myelin maturation of the peripheral nerve. PLoS One 10(1):e0116624. https://doi.org/10.1371/journal.pone.0116624

Jänig W (2006) Integrative action of the autonomic nervous system: neurobiology of homeostasis. Cambridge University Press, Cambridge. https://doi.org/10.1017/CBO9780511541667

Jeninga EH, Schoonjans K, Auwerx J (2010) Reversible acetylation of PGC-1: connecting energy sensors and effectors to guarantee metabolic flexibility. Oncogene 29(33):4617–4624. https://doi.org/10.1038/onc.2010.206

Johannes CB, Araujo AB, Feldman HA, Derby CA, Kleinman KP, McKinlay JB (2000) Incidence of erectile dysfunction in men 40 to 69 years old: longitudinal results from the Massachusetts male ageing study. J Urol 163(2):460–463

Juhaszova M, Church P, Blaustein MP, Stanley EF (2000) Location of calcium transporters at presynaptic terminals: calcium transporters at presynaptic terminals. Eur J Neurosci 12(3): 839–846. https://doi.org/10.1046/j.1460-9568.2000.00974.x

Julius S, Amery A, Whitlock LS, Conway J (1967) Influence of age on the hemodynamic response to exercise. Circulation 36(2):222–230. https://doi.org/10.1161/01.CIR.36.2.222

Kanemi O, Zhang X, Sakamoto Y, Ebina M, Nagatomi R (2005) Acute stress reduces intraparenchymal lung natural killer cells via beta-adrenergic stimulation. Clin Exp Immunol 139(1):25–34. https://doi.org/10.1111/j.1365-2249.2005.02672.x

Kappagoda CT, Linden RJ, Sivananthan N (1979) The nature of the atrial receptors responsible for a reflex increase in heart rate in the dog. J Physiol 291(1):393–412. https://doi.org/10.1113/jphysiol.1979.sp012821

Katafuchi T, Ichijo T, Take S, Hori T (1993a) Hypothalamic modulation of splenic natural killer cell activity in rats. J Physiol 471:209–221. https://doi.org/10.1113/jphysiol.1993.sp019898

Katafuchi T, Take S, Hori T (1993b) Roles of sympathetic nervous system in the suppression of cytotoxicity of splenic natural killer cells in the rat. J Physiol 465:343–357. https://doi.org/10.1113/jphysiol.1993.sp019680

Kauppila TES, Kauppila JHK, Larsson N-G (2017) Mammalian mitochondria and ageing: an update. Cell Metab 25(1):57–71. https://doi.org/10.1016/j.cmet.2016.09.017

Kaushik S, Arias E, Kwon H, Lopez NM, Athonvarangkul D, Sahu S, Schwartz GJ, Pessin JE, Singh R (2012) Loss of autophagy in hypothalamic POMC neurons impairs lipolysis. EMBO Rep 13(3):258–265. https://doi.org/10.1038/embor.2011.260

Kayo T, Allison DB, Weindruch R, Prolla TA (2001) Influences of ageing and caloric restriction on the transcriptional profile of skeletal muscle from rhesus monkeys. Proc Natl Acad Sci 98(9):5093–5098. https://doi.org/10.1073/pnas.081061898

Kees MG, Pongratz G, Kees F, Schölmerich J, Straub RH (2003) Via beta-adrenoceptors, stimulation of extrasplenic sympathetic nerve fibers inhibits lipopolysaccharide-induced TNF secretion in perfused rat spleen. J Neuroimmunol 145(1–2):77–85. https://doi.org/10.1016/j.jneuroim.2003.09.011

Keller JN, Hanni KB, Markesbery WR (2000) Possible involvement of proteasome inhibition in ageing: implications for oxidative stress. Mech Ageing Dev 113(1):61–70. https://doi.org/10.1016/s0047-6374(99)00101-3

Keller JN, Gee J, Ding Q (2002) The proteasome in brain ageing. Ageing Res Rev 1(2):279–293. https://doi.org/10.1016/s1568-1637(01)00006-x

Kelly G (2010) A review of the sirtuin system, its clinical implications, and the potential role of dietary activators like resveratrol: part 1. Altern Med Rev 15(3):245–263

Kenney MJ, Blecha F, Morgan DA, Fels RJ (2001) Interleukin-1 beta alters Brown adipose tissue but not renal sympathetic nerve responses to hypothermia. Am J Physiol Heart Circ Physiol 281(6):H2441–H2445. https://doi.org/10.1152/ajpheart.2001.281.6.H2441

Kenney MJ, Weiss ML, Haywood JR (2003) The paraventricular nucleus: an important component of the central neurocircuitry regulating sympathetic nerve outflow. Acta Physiol Scand 177(1):7–15. https://doi.org/10.1046/j.1365-201X.2003.01042.x

Kenney WL, Morgan AL, Farquhar WB, Brooks EM, Pierzga JM, Derr JA (1997) Decreased active vasodilator sensitivity in aged skin. Am J Physiol Heart Circ Physiol 272(4):H1609–H1614. https://doi.org/10.1152/ajpheart.1997.272.4.H1609

Kerr SRJ, Pearce MS, Brayne C, Davis RJ, Kenny RA (2006) Carotid sinus hypersensitivity in asymptomatic older persons: implications for diagnosis of syncope and falls. Arch Intern Med 166(5):515. https://doi.org/10.1001/archinte.166.5.515

Khachaturian ZS (2006) Calcium hypothesis of Alzheimer's disease and brain ageing. Ann N Y Acad Sci 747(1):1–11. https://doi.org/10.1111/j.1749-6632.1994.tb44398.x

Khan A (2002) Growth hormone, insulin-like growth factor-1 and the ageing cardiovascular system. Cardiovasc Res 54(1):25–35. https://doi.org/10.1016/S0008-6363(01)00533-8

Khan MM, Sansoni P, Silverman ED, Engleman EG, Melmon KL (1986) Beta-adrenergic receptors on human suppressor, helper, and cytolytic lymphocytes. Biochem Pharmacol 35(7):1137–1142. https://doi.org/10.1016/0006-2952(86)90150-4

Khullar V, Cambronero J, Angulo JC, Wooning M, Blauwet MB, Dorrepaal C, Martin NE (2013) Efficacy of mirabegron in patients with and without prior antimuscarinic therapy for overactive bladder: a post hoc analysis of a randomized European-Australian phase 3 trial. BMC Urol 13(1):45. https://doi.org/10.1186/1471-2490-13-45

Kim GW, Chan PH (2001) Oxidative stress and neuronal DNA fragmentation mediate age-dependent vulnerability to the mitochondrial toxin, 3-nitropropionic acid, in the mouse striatum. Neurobiol Dis 8(1):114–126. https://doi.org/10.1006/nbdi.2000.0327

Kim J, Kundu M, Viollet B, Guan K-L (2011) AMPK and MTOR regulate autophagy through direct phosphorylation of Ulk1. Nat Cell Biol 13(2):132–141. https://doi.org/10.1038/ncb2152

Kim J-w, Tchernyshyov I, Semenza GL, Dang CV (2006) HIF-1-mediated expression of pyruvate dehydrogenase kinase: a metabolic switch required for cellular adaptation to hypoxia. Cell Metab 3(3):177–185. https://doi.org/10.1016/j.cmet.2006.02.002

Kim YG, Moon J-Y, Bermseok O, Chin HJ, Kim DK, Park JH, Shin SJ, Choi BS, Lim CS, Lee SH (2022) Pressure-natriuresis response is diminished in old age. Front Cardiovasc Med 9:840840. https://doi.org/10.3389/fcvm.2022.840840

Kimura BJ, Dalugdugan R, Gilcrease GW, Phan JN, Showalter BK, Wolfson T (2011) The effect of breathing manner on inferior vena Caval diameter. Eur J Echocardiogr 12(2):120–123. https://doi.org/10.1093/ejechocard/jeq157

Kingwell BA, Dart AM, Jennings GL, Korner PI (1992) Exercise training reduces the sympathetic component of the blood pressure-heart rate baroreflex in man. Clin Sci 82(4):357–362. https://doi.org/10.1042/cs0820357

Kircher BJ, Himelman RB, Schiller NB (1990) Noninvasive estimation of right atrial pressure from the inspiratory collapse of the inferior vena cava. Am J Cardiol 66(4):493–496. https://doi.org/10.1016/0002-9149(90)90711-9

Klein T, Eckhard U, Dufour A, Solis N, Overall CM (2018) Proteolytic cleavage—mechanisms, function, and "Omic" approaches for a near-ubiquitous posttranslational modification. Chem Rev 118(3):1137–1168. https://doi.org/10.1021/acs.chemrev.7b00120

Knowlton FP, Starling EH (1912) The influence of variations in temperature and blood-pressure on the performance of the isolated mammalian heart. J Physiol 44(3):206–219. https://doi.org/10.1113/jphysiol.1912.sp001511

Kohen R, Vellaichamy E, Hrbac J, Gati I, Tirosh O (2000) Quantification of the overall reactive oxygen species scavenging capacity of biological fluids and tissues. Free Radic Biol Med 28(6):871–879. https://doi.org/10.1016/s0891-5849(00)00191-x

Kohen R, Nyska A (2002) Oxidation of biological systems: oxidative stress phenomena, antioxidants, redox reactions, and methods for their quantification. Toxicol Pathol 30(6):620–650. https://doi.org/10.1080/01926230290166724

Koizumi O, Wilson JD, Grimmelikhuijzen CJP, Westfall JA (1989) Ultrastructural localization of RFamide-like peptides in neuronal dense-cored vesicles in the peduncle of hydra. J Exp Zool 249(1):17–22. https://doi.org/10.1002/jez.1402490105

Komatsu M, Waguri S, Chiba T, Murata S, Iwata J-i, Tanida I, Ueno T et al (2006) Loss of autophagy in the central nervous system causes neurodegeneration in mice. Nature 441(7095):880–884. https://doi.org/10.1038/nature04723

Kraytsberg Y, Nekhaeva E, Bodyak NB, Khrapko K (2003) Mutation and intracellular clonal expansion of mitochondrial genomes: two synergistic components of the ageing process? Mech Ageing Dev 124(1):49–53. https://doi.org/10.1016/S0047-6374(02)00169-0

Kreulen DL (2005) Neurobiology of autonomic ganglia. In: Peripheral neuropathy. Elsevier, pp 233–248. https://doi.org/10.1016/B978-0-7216-9491-7.50014-4

Kujoth GC, Hiona A, Pugh TD, Someya S, Panzer K, Wohlgemuth SE, Hofer T et al (2005) Mitochondrial DNA mutations, oxidative stress, and apoptosis in mammalian ageing. Science 309(5733):481–484. https://doi.org/10.1126/science.1112125

Kyng KJ, Bohr VA (2005) Gene expression and DNA repair in progeroid syndromes and human ageing. Ageing Res Rev 4(4):579–602. https://doi.org/10.1016/j.arr.2005.06.008

Kyrtata N, Emsley HCA, Sparasci O, Parkes LM, Dickie BR (2021) A systematic review of glucose transport alterations in Alzheimer's disease. Front Neurosci 15:626636. https://doi.org/10.3389/fnins.2021.626636

La Rovere MT, Bigger JT, Marcus FI, Mortara A, Schwartz PJ (1998) Baroreflex sensitivity and heart-rate variability in prediction of total cardiac mortality after myocardial infarction.

ATRAMI (autonomic tone and reflexes after myocardial infarction) investigators. Lancet 351(9101):478–484. https://doi.org/10.1016/s0140-6736(97)11144-8

La Rovere MT, Specchia G, Mortara A, Schwartz PJ (1988) Baroreflex sensitivity, clinical correlates, and cardiovascular mortality among patients with a first myocardial infarction. A prospective study. Circulation 78(4):816–824. https://doi.org/10.1161/01.cir.78.4.816

Laitinen T, Hartikainen J, Vanninen E, Niskanen L, Geelen G, Länsimies E (1998) Age and gender dependency of baroreflex sensitivity in healthy subjects. J Appl Physiol 84(2):576–583. https://doi.org/10.1152/jappl.1998.84.2.576

Lakatta EG (1993) Cardiovascular regulatory mechanisms in advanced age. Physiol Rev 73(2): 413–467. https://doi.org/10.1152/physrev.1993.73.2.413

Landi G, Perrone P, Guidotti M (1983) Bilateral TIAs and unilateral seizures due to orthostatic hypotension. A case report. Ital J Neurol Sci 4(2):239–241. https://doi.org/10.1007/BF02043914

Lang JA, Krajek AC (2019) Age-related differences in the cutaneous vascular response to exogenous angiotensin II. Am J Physiol Heart Circ Physiol 316(3):H516–H521. https://doi.org/10.1152/ajpheart.00509.2018

Lang RM, Bierig M, Devereux RB, Flachskampf FA, Foster E, Pellikka PA, Picard MH et al (2005) Recommendations for chamber quantification: a report from the American Society of Echocardiography's Guidelines and Standards Committee and the Chamber Quantification Writing Group, developed in conjunction with the European Association of Echocardiography, a Branch of the European Society of Cardiology. J Am Soc Echocardiogr 18(12):1440–1463. https://doi.org/10.1016/j.echo.2005.10.005

Larsen PD, Tzeng YC, Galletly DC (2003) Inspiratory coupling to cardiac activity and to somatic afferent nerve stimulation in the anaesthetised rat. Auton Neurosci 108(1–2):45–49. https://doi.org/10.1016/j.autneu.2003.08.007

Learoyd BM, Taylor MG (1966) Alterations with age in the viscoelastic properties of human arterial walls. Circ Res 18(3):278–292. https://doi.org/10.1161/01.RES.18.3.278

Ledbetter JA, Parsons M, Martin PJ, Hansen JA, Rabinovitch PS, June CH (1986) Antibody binding to CD5 (Tp67) and Tp44 T cell surface molecules: effects on cyclic nucleotides, cytoplasmic free calcium, and CAMP-mediated suppression. J Immunol 137(10):3299–3305

Lehohla M, Kellaway L, Russell VA (2008) Effect of ageing on Ca2+ uptake via NMDA receptors into barrel cortex slices of spontaneously hypertensive rats. Metab Brain Dis 23(1):1–8. https://doi.org/10.1007/s11011-007-9047-4

Ling C, Poulsen P, Carlsson E, Ridderstråle M, Almgren P, Wojtaszewski J, Beck-Nielsen H, Groop L, Vaag A (2004) Multiple environmental and genetic factors influence skeletal muscle PGC-1α and PGC-1β gene expression in twins. J Clin Investig 114(10):1518–1526. https://doi.org/10.1172/JCI21889

Liu J, Liu W, Li R, Yang H (2019) Mitophagy in Parkinson's disease: from pathogenesis to treatment. Cell 8(7):712. https://doi.org/10.3390/cells8070712

Lob HE, Marvar PJ, Guzik TJ, Sharma S, McCann LA, Weyand C, Gordon FJ, Harrison DG (2010) Induction of hypertension and peripheral inflammation by reduction of extracellular superoxide dismutase in the central nervous system. Hypertension 55(2):277–283., 6p following 283. https://doi.org/10.1161/HYPERTENSIONAHA.109.142646

Lob HE, Schultz D, Marvar PJ, Davisson RL, Harrison DG (2013) Role of the NADPH oxidases in the subfornical organ in angiotensin II-induced hypertension. Hypertension 61(2):382–387. https://doi.org/10.1161/HYPERTENSIONAHA.111.00546

Lockhart TE, Shi W (2010) Effects of age on dynamic accommodation. Ergonomics 53(7): 892–903. https://doi.org/10.1080/00140139.2010.489968

Lopez GA, Reid IA, Rose JC, Ganong WF (1978) Effect of norepinephrine on renin release and the cyclic AMP content of rat kidney slices: modification by sodium deficiency and alpha-adrenergic blockade. Neuroendocrinology 27(1–2):63–73. https://doi.org/10.1159/000122832

Lores-Arnaiz S, Lombardi P, Karadayian AG, Orgambide F, Cicerchia D, Bustamante J (2016) Brain cortex mitochondrial bioenergetics in synaptosomes and non-synaptic mitochondria during ageing. Neurochem Res 41(1–2):353–363. https://doi.org/10.1007/s11064-015-1817-5

Low PA, Opfer-Gehrking TL, McPhee BR, Fealey RD, Benarroch EE, Willner CL, Suarez GA et al (1995) Prospective evaluation of clinical characteristics of orthostatic hypotension. Mayo Clin Proc 70(7):617–622. https://doi.org/10.4065/70.7.617

Lu T, Pan Y, Kao S-Y, Li C, Kohane I, Chan J, Yankner BA (2004) Gene regulation and DNA damage in the ageing human brain. Nature 429(6994):883–891. https://doi.org/10.1038/nature02661

Lue TF, Mueller SC, Jow YR, Hwang TI (1989) Functional evaluation of penile arteries with duplex ultrasound in vasodilator-induced erection. Urol Clin North Am 16(4):799–807

Lue TF, Takamura T, Schmidt RA, Palubinskas AJ, Tanagho EA (1983) Hemodynamics of erection in the monkey. J Urol 130(6):1237–1241. https://doi.org/10.1016/s0022-5347(17)51768-1

Luukinen H, Koski K, Laippala P, Airaksinen KEJ (2004) Orthostatic hypotension and the risk of myocardial infarction in the home-dwelling elderly. J Intern Med 255(4):486–493. https://doi.org/10.1111/j.1365-2796.2004.01313.x

Ma Z, Siebert AP, Cheung K-H, Lee RJ, Johnson B, Cohen AS, Vingtdeux V, Marambaud P, Kevin Foskett J (2012) Calcium homeostasis modulator 1 (CALHM1) is the pore-forming subunit of an Ion channel that mediates extracellular Ca2+ regulation of neuronal excitability. Proc Natl Acad Sci U S A 109(28):E1963–E1971. https://doi.org/10.1073/pnas.1204023109

MacDermott AB, Mayer ML, Westbrook GL, Smith SJ, Barker JL (1986) NMDA-receptor activation increases cytoplasmic calcium concentration in cultured spinal cord neurones. Nature 321(6069):519–522. https://doi.org/10.1038/321519a0

Madden CJ, Morrison SF (2005) Hypoxic activation of arterial chemoreceptors inhibits sympathetic outflow to Brown adipose tissue in rats: hypoxia-evoked inhibition of BAT sympathetic outflow. J Physiol 566(2):559–573. https://doi.org/10.1113/jphysiol.2005.086322

Magloczky Z, Freund TF (1993) Selective neuronal death in the contralateral hippocampus following unilateral Kainate injections into the CA3 subfield. Neuroscience 56(2):317–335. https://doi.org/10.1016/0306-4522(93)90334-C

Makman MH (1971) Properties of adenylate cyclase of lymphoid cells. Proc Natl Acad Sci U S A 68(5):885–889. https://doi.org/10.1073/pnas.68.5.885

Mansfield KJ, Liu L, Mitchelson FJ, Moore KH, Millard RJ, Burcher E (2005) Muscarinic receptor subtypes in human bladder detrusor and mucosa, studied by radioligand binding and quantitative competitive RT-PCR: changes in ageing. Br J Pharmacol 144(8):1089–1099. https://doi.org/10.1038/sj.bjp.0706147

Mapleson WW (1996) Effect of age on MAC in humans: a meta-analysis. Br J Anaesth 76(2):179–185. https://doi.org/10.1093/bja/76.2.179

Mark AL (1983) The Bezold-Jarisch reflex revisited: clinical implications of inhibitory reflexes originating in the heart. J Am Coll Cardiol 1(1):90–102. https://doi.org/10.1016/s0735-1097(83)80014-x

Marshall JM (1994) Peripheral chemoreceptors and cardiovascular regulation. Physiol Rev 74(3):543–594. https://doi.org/10.1152/physrev.1994.74.3.543

Marty A, Zimmerberg J (1989) Diffusion into patch-clamp recording pipette of a factor necessary for muscarinic current response. Cell Signal 1(3):259–268. https://doi.org/10.1016/0898-6568(89)90043-0

Matsukawa T, Sugiyama Y, Iwase S, Mano T (1994) Effects of ageing on the arterial baroreflex control of muscle sympathetic nerve activity in healthy subjects. Environ Med 38(1):81–84

Matsukawa T, Sugiyama Y, Watanabe T, Kobayashi F, Mano T (1998) Baroreflex control of muscle sympathetic nerve activity is attenuated in the elderly. J Auton Nerv Syst 73(2–3):182–185. https://doi.org/10.1016/s0165-1838(98)00128-3

Matsunaga W, Isobe K, Shirokawa T (2006) Involvement of neurotrophic factors in ageing of noradrenergic innervations in hippocampus and frontal cortex. Neurosci Res 54(4):313–318. https://doi.org/10.1016/j.neures.2005.12.013

Matthews EA, Linardakis JM, Disterhoft JF (2009) The fast and slow afterhyperpolarizations are differentially modulated in hippocampal neurons by ageing and learning. J Neurosci 29(15): 4750–4755. https://doi.org/10.1523/JNEUROSCI.0384-09.2009

Mattson MP (2000) Apoptosis in neurodegenerative disorders. Nat Rev Mol Cell Biol 1(2): 120–130. https://doi.org/10.1038/35040009

Mattson MP, Rychlik B, Chu C, Christakost S (1991) Evidence for calcium-reducing and excitoprotective roles for the calcium-binding protein calbindin-1328k in cultured hippocampal neurons. Neuron 6(1):41–51. https://doi.org/10.1016/0896-6273(91)90120-O

Maurer MS, Karmally W, Rivadeneira H, Parides MK, Bloomfield DM (2000) Upright posture and postprandial hypotension in elderly persons. Ann Intern Med 133(7):533–536. https://doi.org/10.7326/0003-4819-133-7-200010030-00012

McCormack JG, Denton RM (1990) Intracellular calcium ions and intramitochondrial Ca^{2+} in the regulation of energy metabolism in mammalian tissues. Proc Nutr Soc 49(1):57–75. https://doi.org/10.1079/PNS19900009

McDougal DH, Gamlin PD (2014) Autonomic control of the eye. In: Terjung R (ed) Comprehensive physiology, 1st edn. Wiley, pp 439–473. https://doi.org/10.1002/cphy.c140014

McGinnis SM, Brickhouse M, Pascual B, Dickerson BC (2011) Age-related changes in the thickness of cortical zones in humans. Brain Topogr 24(3–4):279–291. https://doi.org/10.1007/s10548-011-0198-6

McIntosh SJ, Lawson J, Kenny RA (1993) Clinical characteristics of vasodepressor, cardioinhibitory, and mixed carotid sinus syndrome in the elderly. Am J Med 95(2):203–208. https://doi.org/10.1016/0002-9343(93)90261-M

Michaelis ML, Bigelow DJ, Schöneich C, Williams TD, Ramonda L, Yin D, Hühmer AFR, Yao Y, Gao J, Squier TC (1996) Decreased plasma membrane calcium transport activity in ageing brain. Life Sci 59(5–6):405–412. https://doi.org/10.1016/0024-3205(96)00319-0

Michael-Titus A, Revest P, Shortland P (2010) The visual system. In: The nervous system. Elsevier, pp 121–140. https://doi.org/10.1016/B978-0-7020-3373-5.00007-1

Miller FD, Pozniak CD, Walsh GS (2000) Neuronal life and death: an essential role for the P53 family. Cell Death Differ 7(10):880–888. https://doi.org/10.1038/sj.cdd.4400736

Min S, Chang RB, Prescott SL, Beeler B, Joshi NR, Strochlic DE, Liberles SD (2019) Arterial baroreceptors sense blood pressure through decorated aortic claws. Cell Rep 29(8):2192–2201.e3. https://doi.org/10.1016/j.celrep.2019.10.040

Minson CT, Holowatz LA, Wong BJ, Larry Kenney W, Wilkins BW (2002) Decreased nitric oxide- and axon reflex-mediated cutaneous vasodilation with age during local heating. J Appl Physiol 93(5):1644–1649. https://doi.org/10.1152/japplphysiol.00229.2002

Monahan KD, Dinenno FA, Seals DR, Clevenger CM, Desouza CA, Tanaka H (2001) Age-associated changes in cardiovagal baroreflex sensitivity are related to central arterial compliance. Am J Physiol Heart Circ Physiol 281(1):H284–H289. https://doi.org/10.1152/ajpheart.2001.281.1.H284

Montine TJ, Amarnath V, Martin ME, Strittmatter WJ, Graham DG (1996) E-4-hydroxy-2-Nonenal is cytotoxic and cross-links cytoskeletal proteins in P19 neuroglial cultures. Am J Pathol 148(1): 89–93

Moore DJ, West AB, Dawson VL, Dawson TM (2005) Molecular pathophysiology of Parkinson's disease. Annu Rev Neurosci 28:57–87. https://doi.org/10.1146/annurev.neuro.28.061604.135718

Mordi JA, Ciuffreda KJ (1998) Static aspects of accommodation: age and presbyopia. Vision Res 38(11):1643–1653. https://doi.org/10.1016/s0042-6989(97)00336-2

Morrison SF (2011) 2010 Carl Ludwig distinguished lectureship of the APS neural control and autonomic regulation section: central neural pathways for thermoregulatory cold defense. J Appl Physiol 110(5):1137–1149. https://doi.org/10.1152/japplphysiol.01227.2010

Mulvey MA, Schilling JD, Martinez JJ, Hultgren SJ (2000) Bad bugs and beleaguered bladders: interplay between uropathogenic Escherichia Coli and innate host defenses. Proc Natl Acad Sci U S A 97(16):8829–8835. https://doi.org/10.1073/pnas.97.16.8829

Munford RS, Tracey KJ (2002) Is severe sepsis a neuroendocrine disease? Mol Med 8(8):437–442

Murchison D, Griffith WH (1999) Age-related alterations in caffeine-sensitive calcium stores and mitochondrial buffering in rat basal forebrain. Cell Calcium 25(6):439–452. https://doi.org/10.1054/ceca.1999.0048

Murer MG, Yan Q, Raisman-Vozari R (2001) Brain-derived neurotrophic factor in the control human brain, and in Alzheimer's disease and Parkinson's disease. Prog Neurobiol 63(1):71–124. https://doi.org/10.1016/S0301-0082(00)00014-9

Murphy EMA, Defontgalland D, Costa M, Brookes SJH, Wattchow DA (2007) Quantification of subclasses of human colonic myenteric neurons by immunoreactivity to Hu, choline acetyltransferase and nitric oxide synthase. Neurogastroenterol Motil 19(2):126–134. https://doi.org/10.1111/j.1365-2982.2006.00843.x

Nakamura K (2011) Central circuitries for body temperature regulation and fever. Am J Physiol Regul Integr Comp Physiol 301(5):R1207–R1228. https://doi.org/10.1152/ajpregu.00109.2011

Nardocci G, Martin A, Abarzúa S, Rodríguez J, Simon F, Reyes EP, Acuña-Castillo C, Navarro C, Cortes PP, Fernández R (2015) Sepsis progression to multiple organ dysfunction in carotid chemo/Baro-denervated rats treated with lipopolysaccharide. J Neuroimmunol 278:44–52. https://doi.org/10.1016/j.jneuroim.2014.12.002

Neher E, Sakaba T (2008) Multiple roles of calcium ions in the regulation of neurotransmitter release. Neuron 59(6):861–872. https://doi.org/10.1016/j.neuron.2008.08.019

Nehra A, Goldstein I, Pabby A, Nugent M, Huang YH, de las Morenas A, Krane RJ, Udelson D, Saenz de Tejada I, Moreland RB (1996) Mechanisms of venous leakage: a prospective clinico-pathological correlation of corporeal function and structure. J Urol 156(4):1320–1329. https://doi.org/10.1016/s0022-5347(01)65578-2

Ng AV, Callister R, Johnson DG, Seals DR (1993) Age and gender influence muscle sympathetic nerve activity at rest in healthy humans. Hypertension 21(4):498–503. https://doi.org/10.1161/01.hyp.21.4.498

NIH Consensus Conference. Impotence. NIH Consensus Development Panel on Impotence (1993) JAMA 270(1):83–90

Ning L, Yang L (2017) Hypertension might be a risk factor for erectile dysfunction: a meta-analysis. Andrologia 49(4). https://doi.org/10.1111/and.12644

Novak V, Novak P, Spies JM, Low PA (1998) Autoregulation of cerebral blood flow in orthostatic hypotension. Stroke 29(1):104–111. https://doi.org/10.1161/01.STR.29.1.104

Ohashi K, Saigusa T (1997) Sympathetic nervous responses during cytokine-induced fever in conscious rabbits. Pflugers Arch 433(6):691–698. https://doi.org/10.1007/s004240050333

Okun E, Griffioen KJ, Mattson MP (2011) Toll-like receptor signaling in neural plasticity and disease. Trends Neurosci 34(5):269–281. https://doi.org/10.1016/j.tins.2011.02.005

O'Mahony D, Bennett C, Green A, Sinclair AJ (2000) Reduced baroreflex sensitivity in elderly humans is not due to efferent autonomic dysfunction. Clin Sci 98(1):103–110

Onorati F, Barbieri R, Mauri M, Russo V, Mainardi L (2013) Characterization of affective states by pupillary dynamics and autonomic correlates. Front Neuroeng 6. https://doi.org/10.3389/fneng.2013.00009

Ooi WL, Hossain M, Lipsitz LA (2000) The association between orthostatic hypotension and recurrent falls in nursing home residents. Am J Med 108(2):106–111. https://doi.org/10.1016/S0002-9343(99)00425-8

Ostrowski SR, Gaïni S, Pedersen C, Johansson PI (2015) Sympathoadrenal activation and endothelial damage in patients with varying degrees of acute infectious disease: an observational study. J Crit Care 30(1):90–96. https://doi.org/10.1016/j.jcrc.2014.10.006

Paleczny B, Niewiński P, Rydlewska A, Piepoli MF, Borodulin-Nadzieja L, Jankowska EA, Ponikowska B (2014) Age-related reflex responses from peripheral and central chemoreceptors in healthy men. Clin Auton Res 24(6):285–296. https://doi.org/10.1007/s10286-014-0263-9

Pandya JD, Grondin R, Yonutas HM, Haghnazar H, Gash DM, Zhang Z, Sullivan PG (2015) Decreased mitochondrial bioenergetics and calcium buffering capacity in the basal ganglia correlates with motor deficits in a nonhuman primate model of ageing. Neurobiology of Ageing 36(5):1903–1913. https://doi.org/10.1016/j.neurobiolAgeing.2015.01.018

Patten DA, Germain M, Kelly MA, Slack RS (2010) Reactive oxygen species: stuck in the middle of neurodegeneration. J Alzheimers Dis 20(s2):S357–S367. https://doi.org/10.3233/JAD-2010-100498

Pavlov VA, Tracey KJ (2005) The cholinergic anti-inflammatory pathway. Brain Behav Immun 19(6):493–499. https://doi.org/10.1016/j.bbi.2005.03.015

Pawelec G, Solana R (1997) Immunosenescence. Immunol Today 18(11):514–516. https://doi.org/10.1016/s0167-5699(97)01145-6

Peddie W (1925) Helmholtz's treatise on physiological optics. Nature 116(2907):88–89. https://doi.org/10.1038/116088a0

Perez SD, Kozic B, Molinaro CA, Thyagarajan S, Ghamsary M, Lubahn CL, Lorton D, Bellinger DL (2012) Chronically lowering sympathetic activity protects sympathetic nerves in spleens from ageing F344 rats. J Neuroimmunol 247(1–2):38–51. https://doi.org/10.1016/j.jneuroim.2012.03.022

Peters A, Sethares C (2002) Ageing and the myelinated fibers in prefrontal cortex and corpus callosum of the monkey. J Comp Neurol 442(3):277–291. https://doi.org/10.1002/cne.10099

Petrosillo G, Ruggiero FM, Pistolese M, Paradies G (2004) Ca2+-induced reactive oxygen species production promotes cytochrome c release from rat liver mitochondria via mitochondrial permeability transition (MPT)-dependent and MPT-independent mechanisms. J Biol Chem 279(51):53103–53108. https://doi.org/10.1074/jbc.M407500200

Phillips RJ, Powley TL (2007) Innervation of the gastrointestinal tract: patterns of ageing. Auton Neurosci 136(1–2):1–19. https://doi.org/10.1016/j.autneu.2007.04.005

Pimont S, Bruley Des Varannes S, Le Neel JC, Aubert P, Galmiche JP, Neunlist M (2003) Neurochemical coding of myenteric neurones in the human gastric fundus. Neurogastroenterol Motil 15(6):655–662. https://doi.org/10.1046/j.1350-1925.2003.00442.x

Pittelli M, Felici R, Pitozzi V, Giovannelli L, Bigagli E, Cialdai F, Romano G, Moroni F, Chiarugi A (2011) Pharmacological effects of exogenous NAD on mitochondrial bioenergetics, DNA repair, and apoptosis. Mol Pharmacol 80(6):1136–1146. https://doi.org/10.1124/mol.111.073916

Pochet R, Delespesse G (1983) Beta-adrenoreceptors display different efficiency on lymphocyte subpopulations. Biochem Pharmacol 32(10):1651–1655. https://doi.org/10.1016/0006-2952(83)90344-1

van den Pol AN, Obrietan K, Chen G, Belousov AB (1996) Neuropeptide Y-mediated long-term depression of excitatory activity in suprachiasmatic nucleus neurons. J Neurosci 16(18):5883–5895. https://doi.org/10.1523/JNEUROSCI.16-18-05883.1996

Pollard AK, Craig EL, Chakrabarti L (2016) Mitochondrial complex 1 activity measured by spectrophotometry is reduced across all brain regions in ageing and more specifically in neurodegeneration. PLoS One 11(6):e0157405. https://doi.org/10.1371/journal.pone.0157405

Poller U, Nedelka G, Radke J, Pönicke K, Brodde OE (1997) Age-dependent changes in cardiac muscarinic receptor function in healthy volunteers. J Am Coll Cardiol 29(1):187–193. https://doi.org/10.1016/s0735-1097(96)00437-8

Porter A, Wattchow D, Brookes S, Costa M (1997) The neurochemical coding and projections of circular muscle motor neurons in the human colon. Gastroenterology 113(6):1916–1923. https://doi.org/10.1016/S0016-5085(97)70011-8

Porter AJ, Wattchow DA, Brookes SJ, Schemann M, Costa M (1996) Choline acetyltransferase immunoreactivity in the human small and large intestine. Gastroenterology 111(2):401–408. https://doi.org/10.1053/gast.1996.v111.pm8690205

Prabhakar NR (1994) Neurotransmitters in the carotid body. In: O'Regan RG, Nolan P, McQueen DS, Paterson DJ (eds) Arterial chemoreceptors, Advances in experimental medicine and biology, vol 360. Springer, Boston, pp 57–69. https://doi.org/10.1007/978-1-4615-2572-1_6

Previnaire JG (2018) The importance of the bulbocavernosus reflex. Spinal Cord Ser Cases 4:2. https://doi.org/10.1038/s41394-017-0012-0

Proctor GB, Carpenter GH (2007) Regulation of salivary gland function by autonomic nerves. Auton Neurosci 133(1):3–18. https://doi.org/10.1016/j.autneu.2006.10.006

Purdon PL, Pavone KJ, Akeju O, Smith AC, Sampson AL, Lee J, Zhou DW, Solt K, Brown EN (2015) The ageing brain: age-dependent changes in the electroencephalogram during propofol and sevoflurane general anaesthesia. Br J Anaesth 115(July):i46–i57. https://doi.org/10.1093/bja/aev213

Puri C, Vicinanza M, Ashkenazi A, Gratian MJ, Zhang Q, Bento CF, Renna M, Menzies FM, Rubinsztein DC (2018) The RAB11A-positive compartment is a primary platform for autophagosome assembly mediated by WIPI2 recognition of PI3P-RAB11A. Dev Cell 45(1): 114–131.e8. https://doi.org/10.1016/j.devcel.2018.03.008

Rage F, Silhol M, Binamé F, Arancibia S, Tapia-Arancibia L (2007) Effect of ageing on the expression of BDNF and TrkB isoforms in rat pituitary. Neurobiology of Ageing 28(7): 1088–1098. https://doi.org/10.1016/j.neurobiolAgeing.2006.05.013

Raj V, Opie M, Arnold AC (2018) Cognitive and psychological issues in postural tachycardia syndrome. Auton Neurosci 215:46–55. https://doi.org/10.1016/j.autneu.2018.03.004

Rangaraju V, Calloway N, Ryan TA (2014) Activity-driven local ATP synthesis is required for synaptic function. Cell 156(4):825–835. https://doi.org/10.1016/j.cell.2013.12.042

Rapp DE, Lyon MB, Bales GT, Cook SP (2005) A role for the P2X receptor in urinary tract physiology and in the pathophysiology of urinary dysfunction. Eur Urol 48(2):303–308. https://doi.org/10.1016/j.eururo.2005.04.019

Ravikumar B, Moreau K, Jahreiss L, Puri C, Rubinsztein DC (2010) Plasma membrane contributes to the formation of pre-autophagosomal structures. Nat Cell Biol 12(8):747–757. https://doi.org/10.1038/ncb2078

Redwine L, Snow S, Mills P, Irwin M (2003) Acute psychological stress: effects on chemotaxis and cellular adhesion molecule expression. Psychosom Med 65(4):598–603. https://doi.org/10.1097/01.psy.0000079377.86193.a8

Rhodes A, Evans LE, Alhazzani W, Levy MM, Antonelli M, Ferrer R, Kumar A et al (2017) Surviving sepsis campaign: international guidelines for management of sepsis and septic shock: 2016. Crit Care Med 45(3):486–552. https://doi.org/10.1097/CCM.0000000000002255

Richardson D, Tyra J, McCray A (1992) Attenuation of the cutaneous vasoconstrictor response to cold in elderly men. J Gerontol 47(6):M211–M214. https://doi.org/10.1093/geronj/47.6.M211

Rizzuto R, Pinton P, Brini M, Chiesa A, Filippin L, Pozzan T (1999) Mitochondria as biosensors of calcium microdomains. Cell Calcium 26(5):193–199. https://doi.org/10.1054/ceca.1999.0076

Roach MR, Burton AC (1959) The effect of age on the elasticity of human iliac arteries. Can J Biochem Physiol 37(4):557–570

Robertson D, Biaggioni I, ProQuest (Firm). (2012) Primer on the autonomic nervous system. Elsevier, London

Rockwell P, Yuan H, Magnusson R, Figueiredo-Pereira ME (2000) Proteasome inhibition in neuronal cells induces a proinflammatory response manifested by upregulation of cyclooxygenase-2, its accumulation as ubiquitin conjugates, and production of the prostaglandin PGE(2). Arch Biochem Biophys 374(2):325–333. https://doi.org/10.1006/abbi.1999.1646

Román GC (2004) Brain hypoperfusion: a critical factor in vascular dementia. Neurol Res 26(5): 454–458. https://doi.org/10.1179/016164104225017686

Rosas-Ballina M, Olofsson PS, Ochani M, Valdés-Ferrer SI, Levine YA, Reardon C, Tusche MW et al (2011) Acetylcholine-synthesizing T cells relay neural signals in a vagus nerve circuit. Science 334(6052):98–101. https://doi.org/10.1126/science.1209985

Rosati C, Hannaert P, Dausse JP, Braquet P, Garay R (1986) Stimulation of beta-adrenoceptors inhibits calcium-dependent potassium-channels in mouse macrophages. J Cell Physiol 129(3): 310–314. https://doi.org/10.1002/jcp.1041290307

Rudiger A (2010) Beta-block the septic heart. Crit Care Med 38(10 Suppl):S608–S612. https://doi.org/10.1097/CCM.0b013e3181f204ca

Rutan GH, Hermanson B, Bild DE, Kittner SJ, LaBaw F, Tell GS (1992) Orthostatic hypotension in older adults. The cardiovascular health study. CHS collaborative research group. Hypertension 19(6 Pt 1):508–519. https://doi.org/10.1161/01.hyp.19.6.508

Safonova LP, Michalos A, Wolf U, Wolf M, Hueber DM, Choi JH, Gupta R, Polzonetti C, Mantulin WW, Gratton E (2004) Age-correlated changes in cerebral hemodynamics assessed by near-infrared spectroscopy. Arch Gerontol Geriatr 39(3):207–225. https://doi.org/10.1016/j.archger.2004.03.007

Sagawa K (1983) Baroreflex control of systemic arterial pressure and vascular bed. In: Terjung R (ed) Comprehensive physiology, 1st edn. Wiley, pp 453–496. https://doi.org/10.1002/cphy.cp020314

Saigusa T (1990) Participation of interleukin-1 and tumor necrosis factor in the responses of the sympathetic nervous system during lipopolysaccharide-induced fever. Pflugers Arch 416(3):225–229. https://doi.org/10.1007/BF00392057

Salminen A, Kauppinen A, Kaarniranta K (2012) Emerging role of NF-KB signaling in the induction of senescence-associated secretory phenotype (SASP). Cell Signal 24(4):835–845. https://doi.org/10.1016/j.cellsig.2011.12.006

Sandell JH, Peters A (2001) Effects of age on nerve fibers in the rhesus monkey optic nerve. J Comp Neurol 429(4):541–553. https://doi.org/10.1002/1096-9861(20010122)429:4<541::aid-cne3>3.0.co;2-5

Sandell JH, Peters A (2003) Disrupted myelin and axon loss in the anterior commissure of the aged rhesus monkey. J Comp Neurol 466(1):14–30. https://doi.org/10.1002/cne.10859

Sanders KM, Koh SD, Ward SM (2006) Interstitial cells of CAJAL as pacemakers in the gastrointestinal tract. Annu Rev Physiol 68(1):307–343. https://doi.org/10.1146/annurev.physiol.68.040504.094718

Sanders VM, Baker RA, Ramer-Quinn DS, Kasprowicz DJ, Fuchs BA, Street NE (1997) Differential expression of the Beta2-adrenergic receptor by Th1 and Th2 clones: implications for cytokine production and B cell help. J Immunol 158(9):4200–4210

Sanders VM, Straub RH (2002) Norepinephrine, the beta-adrenergic receptor, and immunity. Brain Behav Immun 16(4):290–332. https://doi.org/10.1006/brbi.2001.0639

Santos RX, Correia SC, Zhu X, Smith MA, Moreira PI, Castellani RJ, Nunomura A, Perry G (2013) Mitochondrial DNA oxidative damage and repair in ageing and Alzheimer's disease. Antioxid Redox Signal 18(18):2444–2457. https://doi.org/10.1089/ars.2012.5039

Santos RA, Souza GY, Oudit TV-B, Canta G, Steckelings UM, Bader M (2019) The renin-angiotensin system: going beyond the classical paradigms. Am J Physiol Heart Circ Physiol 316(5):H958–H970. https://doi.org/10.1152/ajpheart.00723.2018

Saper CB, Romanovsky AA, Scammell TE (2012) Neural circuitry engaged by prostaglandins during the sickness syndrome. Nat Neurosci 15(8):1088–1095. https://doi.org/10.1038/nn.3159

Sarkis G, Ashcom J, Hawdon J, Jacobson L (1988) Decline in protease activities with age in the nematode Caenorhabditis elegans. Mech Ageing Dev 45(3):191–201. https://doi.org/10.1016/0047-6374(88)90001-2

Scanzano A, Cosentino M (2015) Adrenergic regulation of innate immunity: a review. Front Pharmacol 6:171. https://doi.org/10.3389/fphar.2015.00171

Schmidt C, Kurt B, Höcherl K, Bucher M (2009) Inhibition of NF-kappaB activity prevents downregulation of alpha1-adrenergic receptors and circulatory failure during CLP-induced sepsis. Shock 32(3):239–246. https://doi.org/10.1097/SHK.0b013e3181994752

Schmidt H, Hoyer D, Hennen R, Heinroth K, Rauchhaus M, Prondzinsky R, Hottenrott K, Buerke M, Müller-Werdan U, Werdan K (2008) Autonomic dysfunction predicts both 1- and 2-month mortality in middle-aged patients with multiple organ dysfunction syndrome*. Crit Care Med 36(3):967–970. https://doi.org/10.1097/CCM.0B013E3181653263

Schmidt H, Müller-Werdan U, Nuding S, Hoffmann T, Francis DP, Hoyer D, Rauchhaus M, Werdan K (2004) Impaired chemoreflex sensitivity in adult patients with multiple organ dysfunction syndrome—the potential role of disease severity. Intensive Care Med 30(4):665–672. https://doi.org/10.1007/s00134-003-2131-2

Schnider TW, Minto CF, Shafer SL, Gambus PL, Andresen C, Goodale DB, Youngs EJ (1999) The influence of age on propofol pharmacodynamics. Anesthesiology 90(6):1502–1516. https://doi.org/10.1097/00000542-199906000-00003

Schocken DD, Roth GS (1977) Reduced beta-adrenergic receptor concentrations in ageing man. Nature 267(5614):856–858. https://doi.org/10.1038/267856a0

Seals DR, Esler MD (2000) Human ageing and the sympathoadrenal system. J Physiol 528(Pt 3): 407–417. https://doi.org/10.1111/j.1469-7793.2000.00407.x

Seals DR, Stevenson ET, Jones PP, DeSouza CA, Tanaka H, With the Technical Assistance of Cyndi Long and Mary Jo Reiling (1999) Lack of age-associated elevations in 24-h systolic and pulse pressures in women who exercise regularly. Am J Physiol Heart Circ Physiol 277(3): H947–H955. https://doi.org/10.1152/ajpheart.1999.277.3.H947

Seeliger E, Safak E, Persson PB, Reinhardt HW (2001) Contribution of pressure natriuresis to control of total body sodium: balance studies in freely moving dogs. J Physiol 537(3):941–947. https://doi.org/10.1113/jphysiol.2001.012561

Shakhar G, Ben-Eliyahu S (1998) In vivo beta-adrenergic stimulation suppresses natural killer activity and compromises resistance to tumor metastasis in rats. J Immunol 160(7):3251–3258

Shannon J, Jordan J, Costa F, Robertson RM, Biaggioni I (1997) The hypertension of autonomic failure and its treatment. Hypertension 30(5):1062–1067. https://doi.org/10.1161/01.HYP.30.5.1062

Shannon RP, Wei JY, Rosa RM, Epstein FH, Rowe JW (1986) The effect of age and sodium depletion on cardiovascular response to orthostasis. Hypertension 8(5):438–443. https://doi.org/10.1161/01.hyp.8.5.438

Shen F-M, Guan Y-F, Xie H-H, Ding-Feng S (2004) Arterial baroreflex function determines the survival time in lipopolysaccharide-induced shock in rats. Shock 21(6):556–560. https://doi.org/10.1097/01.shk.0000126647.51109.5c

Shi K-Y, Shen F-M, Liu A-J, Chu Z-X, Cao Y-L, Ding-Feng S (2007) The survival time post-Cecal ligation and puncture in sinoaortic denervated rats. J Cardiovasc Pharmacol 50(2):162–167. https://doi.org/10.1097/FJC.0b013e31805c942d

Shi Z, Gan X-B, Fan Z-D, Zhang F, Zhou Y-B, Gao X-Y, De W, Zhu G-Q (2011) Inflammatory cytokines in paraventricular nucleus modulate sympathetic activity and cardiac sympathetic afferent reflex in rats. Acta Physiol (Oxf) 203(2):289–297. https://doi.org/10.1111/j.1748-1716.2011.02313.x

Siegel GJ, Chauhan NB (2000) Neurotrophic factors in Alzheimer's and Parkinson's disease brain. Brain Res Rev 33(2–3):199–227. https://doi.org/10.1016/S0165-0173(00)00030-8

Simonsen A, Cumming RC, Brech A, Isakson P, Schubert DR, Finley KD (2008) Promoting basal levels of autophagy in the nervous system enhances longevity and oxidant resistance in adult drosophila. Autophagy 4(2):176–184. https://doi.org/10.4161/auto.5269

Singleton AB, Farrer M, Johnson J, Singleton A, Hague S, Kachergus J, Hulihan M et al (2003) α-Synuclein Locus triplication causes Parkinson's disease. Science 302(5646):841–841. https://doi.org/10.1126/science.1090278

Siroky MB (2004) The ageing bladder. Rev Urol 6(Suppl 1):S3–S7

Somers VK, Mark AL, Zavala DC, Abboud FM (1989) Contrasting effects of hypoxia and hypercapnia on ventilation and sympathetic activity in humans. J Appl Physiol 67(5): 2101–2106. https://doi.org/10.1152/jappl.1989.67.5.2101

Sonalker PA, Tofovic SP, Bastacky SI, Jackson EK (2008) Chronic noradrenaline increases renal expression of NHE-3, NBC-1, BSC-1 and aquaporin-2. Clin Exp Pharmacol Physiol 35(5–6): 594–600. https://doi.org/10.1111/j.1440-1681.2007.04846.x

Song YS, Park HJ, Kim SY, Lee SH, Yoo HS, Lee HS, Lee MK et al (2004) Protective role of Bcl-2 on β-amyloid-induced cell death of differentiated PC12 cells: reduction of NF-KB and P38 MAP kinase activation. Neurosci Res 49(1):69–80. https://doi.org/10.1016/j.neures.2004.01.010

Stolk RF, van der Pasch E, Naumann F, Schouwstra J, Bressers S, van Herwaarden AE, Gerretsen J et al (2020) Norepinephrine dysregulates the immune response and compromises host defense

during sepsis. Am J Respir Crit Care Med 202(6):830–842. https://doi.org/10.1164/rccm.202002-0339OC

Stoothoff WH, Johnson GVW (2005) Tau phosphorylation: physiological and pathological consequences. Biochim Biophys Acta 1739(2–3):280–297. https://doi.org/10.1016/j.bbadis.2004.06.017

Strandell T (1964) Circulatory studies on healthy old men with special reference to the limitation of the maximal physical working capacity. Acta Med Scand Suppl 414(Suppl 414):1–44

Sullivan PG, Dragicevic NB, Deng J-H, Bai Y, Dimayuga E, Ding Q, Chen Q, Bruce-Keller AJ, Keller JN (2004) Proteasome inhibition alters neural mitochondrial homeostasis and mitochondria turnover. J Biol Chem 279(20):20699–20707. https://doi.org/10.1074/jbc.M313579200

Sun MK (1996) Pharmacology of reticulospinal vasomotor neurons in cardiovascular regulation. Pharmacol Rev 48(4):465–494

Tanaka M, McKinley MJ, McAllen RM (2009) Roles of two preoptic cell groups in tonic and febrile control of rat tail sympathetic fibers. Am J Physiol Regul Integr Comp Physiol 296(4):R1248–R1257. https://doi.org/10.1152/ajpregu.91010.2008

Tanaka Y, Engelender S, Igarashi S, Rao RK, Wanner T, Tanzi RE, Sawa A, Dawson VL, Dawson TM, Ross CA (2001) Inducible expression of mutant alpha-synuclein decreases proteasome activity and increases sensitivity to mitochondria-dependent apoptosis. Hum Mol Genet 10(9):919–926. https://doi.org/10.1093/hmg/10.9.919

Tanzi RE, Moir RD, Wagner SL (2004) Clearance of Alzheimer's abeta peptide: the many roads to perdition. Neuron 43(5):605–608. https://doi.org/10.1016/j.neuron.2004.08.024

Tatton WG, Chalmers-Redman R, Brown D, Tatton N (2003) Apoptosis in Parkinson's disease: signals for neuronal degradation. Ann Neurol 53 Suppl 3:S61–S70.; discussion S70–72. https://doi.org/10.1002/ana.10489

Taylor EW, Jordan D, Coote JH (1999) Central control of the cardiovascular and respiratory systems and their interactions in vertebrates. Physiol Rev 79(3):855–916. https://doi.org/10.1152/physrev.1999.79.3.855

Thibault O, Gant JC, Landfield PW (2007) Expansion of the calcium hypothesis of brain Ageing and Alzheimer's disease: minding the store. Ageing Cell 6(3):307–317. https://doi.org/10.1111/j.1474-9726.2007.00295.x

Thibault O, Landfield PW (1996) Increase in single L-type calcium channels in hippocampal neurons during ageing. Science 272(5264):1017–1020. https://doi.org/10.1126/science.272.5264.1017

Thorns V, Licastro F, Masliah E (2001) Locally reduced levels of acidic FGF Lead to decreased expression of 28-KDa calbindin and contribute to the selective vulnerability of the neurons in the entorhinal cortex in Alzheimer's disease. Neuropathology 21(3):203–211. https://doi.org/10.1046/j.1440-1789.2001.00399.x

Thyagarajan S, Madden KS, Stevens SY, Felten DL (2000) Restoration of splenic noradrenergic nerve fibers and immune reactivity in old F344 rats: a comparison between L-deprenyl and L-desmethyldeprenyl. Int J Immunopharmacol 22(7):523–536. https://doi.org/10.1016/s0192-0561(00)00016-3

Thyagarajan S, Madden KS, Boehm GW, Stevens SY, Felten DL, Bellinger DL (2013) L-deprenyl reverses age-associated decline in splenic norepinephrine, Interleukin-2 and interferon-γ production in old female F344 rats. Neuroimmunomodulation 20(2):72–78. https://doi.org/10.1159/000345043

Tobey JC, Fry HK, Mizejewski CS, Fink GD, Weaver LC (1983) Differential sympathetic responses initiated by angiotensin and sodium chloride. Am J Physiol 245(1):R60–R68. https://doi.org/10.1152/ajpregu.1983.245.1.R60

Toke A, Meyer TW (2001) Hemodynamic effects of angiotensin II in the kidney. In: Wolf G (ed) Contributions to nephrology, vol 135. Karger, Basel, pp 34–46. https://doi.org/10.1159/000060155

Tracey KJ (2002) The inflammatory reflex. Nature 420(6917):853–859. https://doi.org/10.1038/nature01321

Trendelenburg U (1991) The TiPS lecture: functional aspects of the neuronal uptake of noradrenaline. Trends Pharmacol Sci 12(9):334–337. https://doi.org/10.1016/0165-6147(91)90592-g

Trojanowski JQ, Virginia M-Y, Lee. (2002) The role of tau in Alzheimer's disease. Med Clin N Am 86(3):615–627. https://doi.org/10.1016/S0025-7125(02)00002-0

Tzeng YC, Larsen PD, Galletly DC (2007) Mechanism of cardioventilatory coupling: insights from cardiac pacing, vagotomy, and sinoaortic denervation in the anesthetized rat. Am J Physiol Heart Circ Physiol 292(4):H1967–H1977. https://doi.org/10.1152/ajpheart.01049.2006

Tzunoda K, Abe K, Goto T, Yasujima M, Sato M, Omata K, Seino M, Yoshinaga K (1986) Effect of age on the renin-angiotensin-aldosterone system in normal subjects: simultaneous measurement of active and inactive renin, renin substrate, and aldosterone in plasma. J Clin Endocrinol Metabol 62(2):384–389. https://doi.org/10.1210/jcem-62-2-384

Vanner S, Surprenant A (1996) Neural reflexes controlling intestinal microcirculation. Am J Physiol 271(2 Pt 1):G223–G230. https://doi.org/10.1152/ajpgi.1996.271.2.G223

Veith RC, Featherstone JA, Linares OA, Halter JB (1986) Age differences in plasma norepinephrine kinetics in humans. J Gerontol 41(3):319–324. https://doi.org/10.1093/geronj/41.3.319

van der Velden VH, Hulsmann AR (1999) Autonomic innervation of human airways: structure, function, and pathophysiology in asthma. Neuroimmunomodulation 6(3):145–159. https://doi.org/10.1159/000026376

Vercesi AE, Kowaltowski AJ, Grijalba MT, Meinicke AR, Castilho RF (1997) The role of reactive oxygen species in mitochondrial permeability transition. Biosci Rep 17(1):43–52. https://doi.org/10.1023/A:1027335217774

Virmani R, Avolio AP, Mergner WJ, Robinowitz M, Herderick EE, Cornhill JF, Guo SY, Liu TH, Ou DY, O'Rourke M (1991) Effect of ageing on aortic morphology in populations with high and Low prevalence of hypertension and atherosclerosis. Comparison between occidental and Chinese communities. Am J Pathol 139(5):1119–1129

Vischer TL (1976) The differential effect of cyclic AMP on lymphocyte stimulation by T- or B-cell mitogens. Immunology 30(5):735–739

Vlassara H, Bucala R, Striker L (1994) Pathogenic effects of advanced glycosylation: biochemical, biologic, and clinical implications for diabetes and ageing. Lab Invest 70(2):138–151

Wade PR (2002) Ageing and neural control of the GI tract. I. Age-related changes in the enteric nervous system. Am J Physiol Gastrointest Liver Physiol 283(3):G489–G495. https://doi.org/10.1152/ajpgi.00091.2002

Wang BC, Ginter GF, Goetz KL (1987) Augmented pressor response to vasopressin in awake dogs after cardiac denervation. Am J Physiol Regul Integr Comp Physiol 252(1):R145–R152. https://doi.org/10.1152/ajpregu.1987.252.1.R145

Wattchow D, Porter A, Brookes S, Costa M (1997) The polarity of neurochemically defined myenteric neurons in the human colon. Gastroenterology 113(2):497–506. https://doi.org/10.1053/gast.1997.v113.pm9247469

Weale RA (1962) Presbyopia. Br J Ophthalmol 46(11):660–668. https://doi.org/10.1136/bjo.46.11.660

Weidmann P, De Myttenaere-Bursztein S, Maxwell MH, de Lima J (1975) Effect of ageing on plasma renin and aldosterone in normal man. Kidney Int 8(5):325–333. https://doi.org/10.1038/ki.1975.120

Westby CM, Weil BR, Greiner JJ, Stauffer BL, Desouza CA (2011) Endothelin-1 vasoconstriction and the age-related decline in endothelium-dependent vasodilatation in men. Clin Sci 120(11):485–491. https://doi.org/10.1042/CS20100475

White LD, Barone S (2001) Qualitative and quantitative estimates of apoptosis from birth to senescence in the rat brain. Cell Death Differ 8(4):345–356. https://doi.org/10.1038/sj.cdd.4400816

Wilcox CS, Puritz R, Lightman SL, Bannister R, Aminoff MJ (1984) Plasma volume regulation in patients with progressive autonomic failure during changes in salt intake or posture. J Lab Clin Med 104(3):331–339

Williams TL, Day NC, Ince PG, Kamboj RK, Shaw PJ (1997) Calcium-permeable?-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptors: a molecular determinant of selective vulnerability in amyotrophic lateral sclerosis. Ann Neurol 42(2):200–207. https://doi.org/10.1002/ana.410420211

Wilson TE, Sauder CL, Kearney ML, Kuipers NT, Leuenberger UA, Monahan KD, Ray CA (2007) Skin-surface cooling elicits peripheral and visceral vasoconstriction in humans. J Appl Physiol 103(4):1257–1262. https://doi.org/10.1152/japplphysiol.00401.2007

Wray DW, Nishiyama SK, Harris RA, Richardson RS (2008) Angiotensin II in the elderly: impact of angiotensin II type 1 receptor sensitivity on peripheral hemodynamics. Hypertension 51(6):1611–1616. https://doi.org/10.1161/HYPERTENSIONAHA.108.111294

Wu F, Zhao Y, Zhang H (2022) Ocular autonomic nervous system: an update from anatomy to physiological functions. Vision 6(1):6. https://doi.org/10.3390/vision6010006

Wu L-L, Yang S-L, Yang R-C, Hsu H-K, Hsu C, Dong L-W, Liu M-S (2003) G protein and adenylate cyclase complex-mediated signal transduction in the rat heart during sepsis. Shock 19(6):533–537. https://doi.org/10.1097/01.shk.0000055816.40894.cd

Xiao J, Huang H-W, Peng Y-P, Bao J-Y, Huang Y, Qiu Y-H (2010) Modulation of natural killer cell function by alpha-adrenoreceptor-coupled signalling. Neuro Endocrinol Lett 31(5):635–644

Xiong J, Verkhratsky A, Toescu EC (2002) Changes in mitochondrial status associated with altered Ca2+ homeostasis in aged cerebellar granule neurons in brain slices. J Neurosci Off J Soc Neurosci 22(24):10761–10771

Xiong S, Salazar G, Patrushev N, Wayne Alexander R (2011) FoxO1 mediates an autofeedback loop regulating SIRT1 expression. J Biol Chem 286(7):5289–5299. https://doi.org/10.1074/jbc.M110.163667

Yam AT, Lang EW, Lagopoulos J, Yip K, Griffith J, Mudaliar Y, Dorsch NWC (2005) Cerebral autoregulation and ageing. J Clin Neurosci 12(6):643–646. https://doi.org/10.1016/j.jocn.2004.08.017

Yao J, Hamilton RT, Cadenas E, Brinton RD (2010) Decline in mitochondrial bioenergetics and shift to ketogenic profile in brain during reproductive senescence. Biochim Biophys Acta 1800(10):1121–1126. https://doi.org/10.1016/j.bbagen.2010.06.002

Yin F, Sancheti H, Patil I, Cadenas E (2016) Energy metabolism and inflammation in brain ageing and Alzheimer's disease. Free Radic Biol Med 100:108–122. https://doi.org/10.1016/j.freeradbiomed.2016.04.200

Yoshida K, Li X, Cano G, Lazarus M, Saper CB (2009) Parallel preoptic pathways for thermoregulation. J Neurosci Off J Soc Neurosci 29(38):11954–11964. https://doi.org/10.1523/JNEUROSCI.2643-09.2009

Yurek DM, Fletcher-Turner A (2000) Lesion-induced increase of BDNF is greater in the striatum of young versus old rat brain. Exp Neurol 161(1):392–396. https://doi.org/10.1006/exnr.1999.7274

Zachariah PK, Krier J, Schwartz GL (1991) Orthostatic hypotension and ambulatory blood pressure monitoring. J Hypertens Suppl 9(8):S78–S80

Zhang H, Bosch-Marce M, Shimoda LA, Tan YS, Baek JH, Wesley JB, Gonzalez FJ, Semenza GL (2008) Mitochondrial autophagy is an HIF-1-dependent adaptive metabolic response to hypoxia. J Biol Chem 283(16):10892–10903. https://doi.org/10.1074/jbc.M800102200

Zhao L, Cao J, Hu K, He X, Yun D, Tong T, Han L (2020) Sirtuins and their biological relevance in ageing and age-related diseases. Ageing Dis 11(4):927–945. https://doi.org/10.14336/AD.2019.0820

Zhu X-H, Ming L, Lee B-Y, Ugurbil K, Chen W (2015) In vivo NAD assay reveals the intracellular NAD contents and redox state in healthy human brain and their age dependences. Proc Natl Acad Sci 112(9):2876–2881. https://doi.org/10.1073/pnas.1417921112

Zhu Y, Carvey PM, Ling Z (2006) Age-related changes in glutathione and glutathione-related enzymes in rat brain. Brain Res 1090(1):35–44. https://doi.org/10.1016/j.brainres.2006.03.063
Ziegler MG, Lake CR, Kopin IJ (1976) Plasma noradrenaline increases with age. Nature 261(5558): 333–335. https://doi.org/10.1038/261333a0
Zito M, Parati G, Omboni S, Cervone C, Ulian L, D'Aviero M, Abate G, Mancia G (1991) Effect of ageing on blood pressure variability. J Hypertens Suppl 9(6):S328–S329
Zoico E, Rubele S, De Caro A, Nori N, Mazzali G, Fantin F, Rossi A, Zamboni M (2019) Brown and Beige adipose tissue and ageing. Front Endocrinol 10:368. https://doi.org/10.3389/fendo.2019.00368

Chapter 11
Astrocytes in Ageing

Alexei Verkhratsky and Alexey Semyanov

Abstract Ageing is associated with a morphological and functional decline of astrocytes with a prevalence of morphological atrophy and loss of function. In particular, ageing is manifested by the shrinkage of astrocytic processes: branches and leaflets, which decreases synaptic coverage. Astrocytic dystrophy affects multiple functions astrocytes play in the brain active milieu. In particular, and in combination with an age-dependent decline in the expression of glutamate transporters, astrocytic atrophy translates into deficient glutamate clearance and K^+ buffering. Decreased astrocyte presence may contribute to age-dependent remodelling of brain extracellular space, hence affecting extrasynaptic signalling. Old astrocytes lose endfeet polarisation of AQP4 water channels, thus limiting the operation of the glymphatic system. In ageing, astrocytes down-regulate their antioxidant capacity leading to decreased neuroprotection. All these changes may contribute to an age-dependent cognitive decline.

A. Verkhratsky (✉)
Faculty of Biology, Medicine and Health, The University of Manchester, Manchester, UK

Achucarro Center for Neuroscience, IKERBASQUE, Basque Foundation for Science, Bilbao, Spain

Department of Neurosciences, University of the Basque Country UPV/EHU and CIBERNED, Leioa, Spain

Department of Forensic Analytical Toxicology, School of Forensic Medicine, China Medical University, Shenyang, China

Department of Stem Cell Biology, State Research Institute Centre for Innovative Medicine, Vilnius, Lithuania
e-mail: Alexej.Verkhratsky@manchester.ac.uk

A. Semyanov
Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of Sciences, Moscow, Russia

Sechenov First Moscow State Medical University, Moscow, Russia

Department of Physiology, Jiaxing University College of Medicine, Jiaxing, Zhejiang Pro, China

J. R. Harris, V. I. Korolchuk (eds.), *Biochemistry and Cell Biology of Ageing: Part IV, Clinical Science*, Subcellular Biochemistry 103,
https://doi.org/10.1007/978-3-031-26576-1_11

Keywords Ageing · Astrocyte · Astroglial perisynaptic cradle · Astroglial function · Synaptic transmission · Reactive astrogliosis · Neuroplasticity

Brain Ageing, Cognitive Reserve and Neuroglia

The human brain sustains ageing substantially better than other organs and systems (Humphry 1889; Verkhratsky et al. 2021), which reflects the combination of the unique plastic capacity of the nervous tissue which provides for lifelong learning and an exceptional degree of neuroprotection and homeostatic support provided by highly elaborated neuroglia (Oberheim et al. 2009; Verkhratsky et al. 2019; Verkhratsky and Nedergaard 2016). The brain activity presents exceptional demands on logistical support, from providing the energy substrates, to removing reactive oxygen species, controlling extracellular concentration of ions and neuroactive molecules, removing waste and redundant cellular elements, maintaining brain connectome, controlling neurogenesis and mounting brain defence against environmental stresses. All these functions are the responsibility of neuroglia (Verkhratsky and Butt 2013; Verkhratsky and Nedergaard 2018).

The outcome of brain ageing is different between individuals; broadly we can distinguish between physiological ageing with preserved cognitive capacity and pathological ageing in which cognitive function is impaired due to progressive neurodegeneration. Nonetheless, even neurodegenerative processes produce highly different cognitive outcomes. The spectrum of the neurological and cognitive outcomes of ageing and age-dependent brain pathologies (as well as other brain disorders including, for example, traumatic injury or stroke) is defined by the individual cognitive reserve. The cognitive reserve reflects the lifelong interaction of genetic factors with environment (also known as the exposome), cumulative neuronal plasticity, accumulated pathological damage and regenerative capacity of every given organism. Several components contributing to the cognitive reserve were proposed in recent decades. These include brain reserve, brain maintenance, brain resilience and brain compensation. Brain reserve is considered from a purely anatomical view as a resource determined by the structural properties of every given brain acquired through lifelong plasticity and learning (Stern 2009; Zorec et al. 2018; Song et al. 2022). The brain reserve is defined by the functional organisation of the brain as a result of lifelong learning and adaptation, whereas brain resilience and compensation are determined by the capability of the nervous tissue and the brain as an organ to defend itself, to limit damage and to regenerate. Neuroglia contributes to every aspect of cognitive reserve. As the main homeostatic system of the central nervous system (CNS) neuroglia define the brain maintenance and hence functional plasticity. As the main defensive element of the CNS neuroglia defines the brain resilience and the brain compensation.

The process of ageing impacts all types of neuroglial cells. In particular, physiological ageing is associated with substantial deterioration of the white matter, the volume of which is reduced by ~10%. In contrast, the grey matter shrinks, in physiological ageing, by a mere 3% (Haug and Eggers 1991). Decline of the white

matter reflects the decrease in the number of oligodendrocytes and the reduced capacity of oligodendrocyte precursor cells to proliferate, differentiate and remyelinate (Rivera et al. 2021; Vanzuli et al. 2015). Human ageing causes a substantial decrease in the brain defence due to the accumulation of dystrophic microglial cells, which lose their protective capabilities. Old human microglia are characterised by shorter and less complex processes, which often demonstrate fragmentation (Davies et al. 2017; Streit et al. 2004, 2009, 2014). Arguably this deterioration of microglia lessens the anti-inflammatory capabilities of the old brain, reduces neuroprotection and facilitates neurodegeneration (Streit et al. 2020). Of note, such dystrophic microglial ageing is not observed in rodents, which led to a misleading concept of an inflamed old brain. Astrocytes similarly deteriorate with age, leading to a reduced homeostatic support and neuroprotection as discussed in detail in this chapter.

Astrocytes and the Brain Active Milieu

Astrocytes belong to the extended class of astroglia, which also includes radial glia, radial stem cells, several types of radial astrocytes such as tanycytes, Bergmann and Müller glia, pityicites, ependymocytes, choroid plexus cells and retinal pigment epithelial cells (Verkhratsky and Nedergaard 2018). Astrocytes, being the essential homeostatic cells of the CNS, perform highly diverse tasks supporting the proper functional activity of the nervous system. Together with other brain cells and non-cellular elements, astrocytes form the active milieu of the nervous tissue (Fig. 11.1), see Semyanov and Verkhratsky (2021, 2022). The main components of the active milieu are represented by neurones, their axons, dendrites and synapses, by astrocyte somata and arborisation interposed between synapses, by microglia and their motile surveilling processes, by the brain vessels, glia limitans and perivascular space, and by extracellular space hosting extracellular matrix. These elements are constantly interacting, thus creating a highly plastic multicomponent system underlying cognition. Astrocytes, due to their exceptionally developed processes, contact all elements of the active milieu. Numerous astrocytic transporters (Verkhratsky and Rose 2020), which concentrate at perisynaptic sites and in the endfeet, provide for homeostasis of ions, neurotransmitters, neurohormones and supply of energy substrates. In addition, astrocytes secrete factors regulating synaptogenesis, thus contributing to neuroplasticity through the rewiring and remodelling of neuronal ensembles (Augusto-Oliveira et al. 2020).

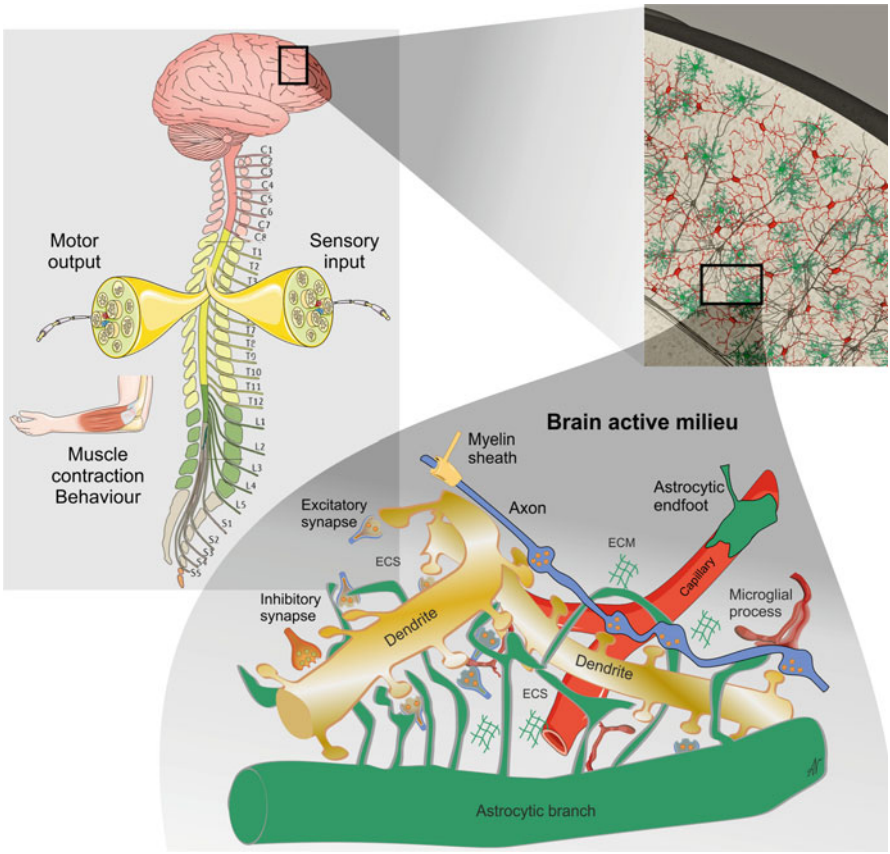


Fig. 11.1 Astrocyte-neurone communication in the active milieu. The active milieu concept integrates multiple theories that address different aspects of local functional organisation of the brain: multipartite synapse, neuro(glio)vascular unit, extrasynaptic signalling and volume transmission. The active milieu is formed through dynamic interactions between neuronal elements (somata, axons, dendrites and spines), non-neuronal cell elements (astrocytic and microglial processes), vasculature (capillary), extracellular space (ECS) and extracellular matrix (ECM). In an active milieu, synapses can contact, signal and be homeostatically controlled by astrocyte branches, by single or by several leaflets. A single astrocytic branch or leaflet may be contacted by several synapses. Dynamic changes in the morphology of astrocytic processes affect diffusional barriers, neurotransmitter clearance and K^+ dynamics, the supply of glutamine or energy substrates, thus regulating neuronal plasticity. Astrocytic processes form loop-like structures through reciprocal gap junctions. Reproduced from Semyanov and Verkhratsky (2021)

Protoplasmic Astrocytes

Protoplasmic astrocytes of the grey matter are characterised by complex morphology. It is generally believed that murine protoplasmic astrocytes occupy non-overlapping territorial domains, thus parcellating the grey matter into relatively

independent functional units (Bushong et al. 2002; Aten et al. 2022). This might not be applicable to all brains; in particular pan-electron microscopy of a 1 mm^3 fragment of human brain shows a substantial overlap of neighbouring astrocytes (Shapson-Coe et al. 2021). Morphology of protoplasmic astrocytes is dominated by highly elaborated arborisation. Several branching primary processes emanate from the relatively small soma; these branches give rise to fine processes called leaflets that bestow on astrocytes characteristic spongiform appearance (Fig. 11.2). Leaflets account for 70–80% of astrocyte surface area while occupying less than 10% of total cell volume (Chao et al. 2002; Ventura and Harris 1999). According to the current classification (Semyanov and Verkhratsky 2021; Khakh and Sofroniew 2015; Gavrilov et al. 2018), the arbor of protoplasmic astrocytes is represented by (1) branches or main (primary, secondary, etc.) astrocytic processes, which can be visualised with diffraction-limited optical microscopy, (2) leaflets that are below the resolution of diffraction-limited optical microscopy, and (3) endfeet which are specialised extensions of astrocytic branches plastering blood vessels. Branches and leaflets are distinguished based in surface to volume ratio (SVR) and the presence of organelles (Fig. 11.2). The rod-like branches have relatively low SVR and contain endoplasmic reticulum and mitochondria, whereas exceedingly thin (~100 nm) leaflets have high SVR ($>25\ \mu m^{-1}$) and are devoid of extracellular organelles. In addition, leaflets do not contain glial fibrillary acidic protein (GFAP) filaments.

Visualising astrocytes is a challenging task. In vivo or in situ diffraction-limited microscopy using various forms of fluorescent makers either expressed by or perfused into astrocytes cannot resolve leaflets because of their small size. Super-resolution techniques have sufficient resolution but often rely on intense laser illumination which can potentially distort the structure of delicate leaflets in vivo (Marx 2013). Tissue fixation can sort this issue but itself can affect the structure of the leaflets (Korogod et al. 2015). Immunocytochemistry is limited because of the absence of a universal marker, and relatively poor staining of small compartments, whereas electron microscopy is associated with various fixation artefacts. The most common astrocytic marker GFAP is expressed by the minority of astrocytes in the healthy brain, and moreover, antibodies against GFAP label only cytoskeleton present in branches and does not visualise the leaflets (Verkhratsky and Nedergaard 2018). An additional increase in GFAP expression and hence GFAP immunoreactivity may reflect both pathological (reactive astrogliosis (Escartin et al. 2021)) and physiological astrocytic states. Expression of GFAP, for example, fluctuates with circadian rhythms in the supraoptic nucleus (Becquet et al. 2008). Similarly, GFAP expression is increased by physical exercise and environmental stimulation (Diniz et al. 2016; Rodriguez et al. 2013; Sampedro-Piquero et al. 2014).

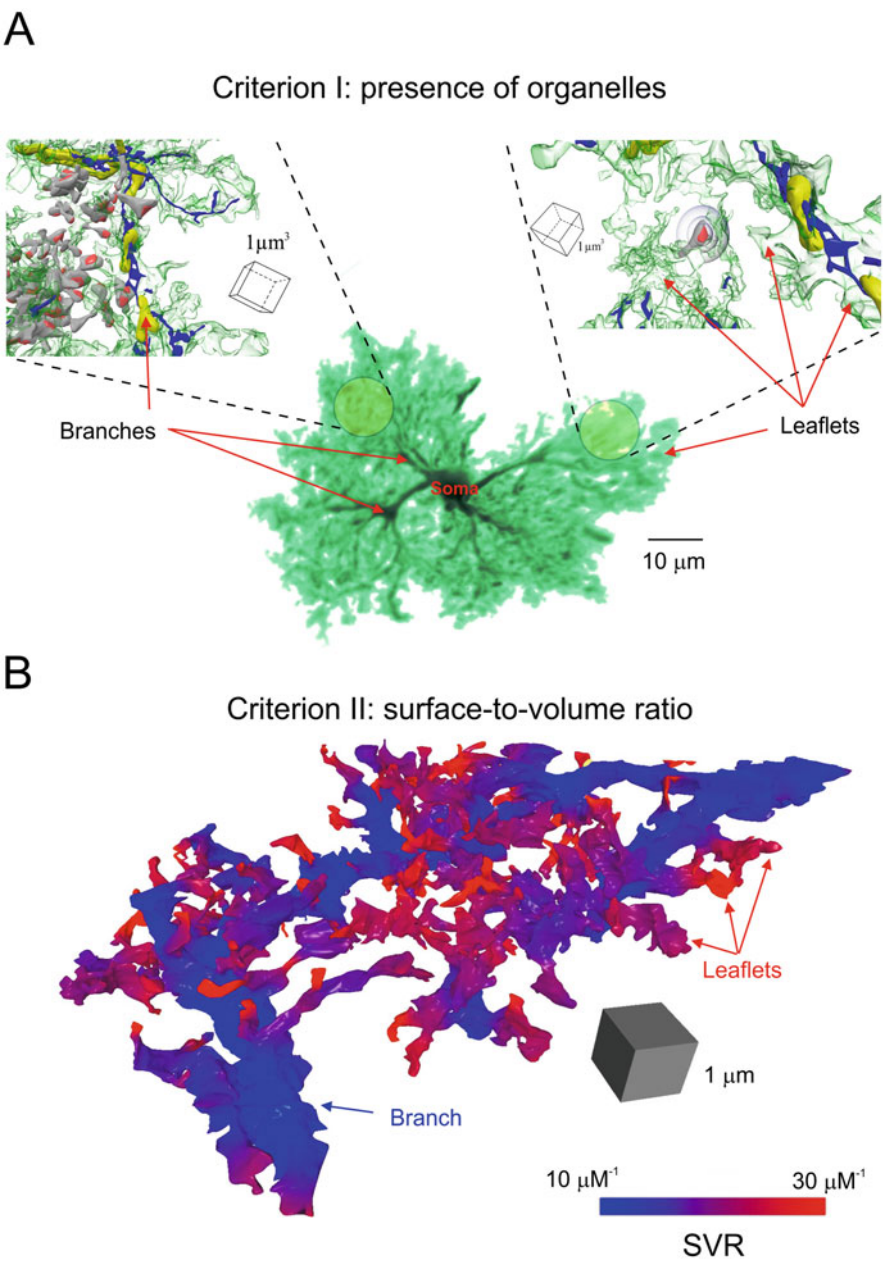


Fig. 11.2 Morphological classification of astrocytic processes. (**a**) Three-dimensional reconstruction of an astrocyte loaded with fluorescent dye Alexa Fluo 594 through a patch-pipette (*centre*). The cell consists of soma with optically resolved branches of primary, secondary and higher orders. These branches are surrounded by fine terminal leaflets that cannot be resolved with diffraction-limited optical imaging and appear as a spongiform cloud. Astrocytic leaflets occupy most of the astrocyte territory also known as an anatomic domain. Two insets (*left* and *right*) are zooming onto branches and leaflets reconstructed from serial section transmission electron microscopy (TEM) image stacks. Green—astrocyte membrane, blue—endoplasmic reticulum (ER), yellow—

Ageing and Morphological Decline of Astrocytes

Most morphological analyses of old astrocytes utilised GFAP as the only marker. There is no obvious decrease in the number of astrocytes in the old brain, although the data are not uniform (Table 11.1). Similarly, morphometry of astrocytes yielded contradictory results as both increase and decrease in size and complexity of astrocytes were reported (Table 11.1). Immunostaining of astrocytes in 3, 9, 18 and 24 month old mice with antibodies against GFAP, glutamate synthetase and protein S100B demonstrated complex and region-dependent changes (Rodriguez et al. 2014). The size of GFAP-positive profiles was increased in the CA1 region and in dentate gyrus of old hippocampus but substantially decreased in the entorhinal cortex. Astrocytes labelled with antibody against glutamate synthetase were smaller in old hippocampus but somewhat larger in old entorhinal cortex. Old astrocytes immunopositive for S100B were larger in entorhinal cortex but demonstrated no age-dependent changes in hippocampus. Neocortical astrocytes, labelled by EGFP under the control of Adlh1l1 astrocyte-specific promoter, demonstrate morphological atrophy with age as evidenced by the decrease of their surface area and complexity (Yang et al. 2022).

In-depth morphological analysis was performed on astrocytes from young, adult and old mice (3, 9 and 24 months). Astrocytes were filled with the fluorescent probe Alexa Fluor 594 for confocal imaging and subsequent 3D reconstruction (Popov et al. 2021). This fluorescent probe diffuses through the cytosol and reveals the full extent of arborisation. Transition from young to adult animals was accompanied by a substantial increase in the size and complexity of astrocytes. In contrast, ageing led to a significant decrease in astrocytic size, volume, territorial domain and complexity in mice (Fig. 11.3). The very similar decrease in astrocytic morphological profiles and astrocytic complexity was observed in astrocytes in older humans, using access tissue obtained during neurosurgery (own unpublished data). To access the state of leaflets the volume fraction of peripheral processes (defined as the fluorescence ratio of unresolved processes area to the astrocyte soma (Plata et al. 2018; Minge et al.

Fig. 11.2 (continued) mitochondria, grey—dendritic spine, red—postsynaptic density. Astrocytic branches contain organelles, whereas leaflets are organelle-free. Leaflets are interspaced with dendritic spines, filling the space between them like paper cushions around pears packed for shipping. Thus, most of the synapses hosted by dendritic spines interact with organelle-free leaflets, while individual leaflets may interact with several synapses. Some dendritic spines, however, reside in the vicinity of astrocytic branches and soma, and those synapses may have a different effect on astrocytic Ca^{2+} activity. (**b**) Analysis of local astrocytic surface-to-volume ratio (SVR) with the method of spheres in serial section TEM reconstruction. Astrocytic branches and leaflets have significantly different SVR. High SVR correlates with a low volume of cytoplasm in astrocytic leaflets, which significantly affects the dynamics of ionic gradients. Ions (e.g. Na^+, Ca^{2+}) entering the leaflet through plasma membrane achieve high concentration faster than those entering branches with lower SVR. Hence, the amplitude and time course of intracellular Ca^{2+} and Na^+ transients representing a major form of astrocytic excitability is determined by local SVR. Reproduced from Semyanov and Verkhratsky (2021)

Table 11.1 Numbers and morphological appearance of aged astrocytes

Specie/Age/brain region	Experimental techniques	Main findings	References
Rhesus macaques/4 groups: juvenile (5 months–2 years), adolescent (3–5 years), adult (7–12 years) and geriatrics (>20 years)/frontal lobe	Immunocutochemistry, morphometry, neurolucida, Sholl analysis	Astroglial density does not change with age; astroglial complexity increases from juvenile to adult animals and decreases in ageing	Robillard et al. (2016)
Rat (Sprague-Dawley and Fisher-344)/ 1–18 months (SD) 1–30 months (F-344)/cerebrum and cerebellum	GFAP immunohistochemistry, morphometry	Perimeter and surface area of GFAP-positive astrocytes significantly increased during attaining adulthood; at advanced age an increase was much smaller	Bjorklund et al. (1985)
Rat (Fisher-344)/ 3 and 25 months/dentate gyrus of the hippocampus	Electron microscopy	Increase in astroglial processes profile (41% in numbers and 43% in volume fraction) were detected in old specimens; the mean number of astrocytes per square area did not change with age	Geinisman et al. (1978)
Rat (Fisher-344)/2–3 and 24–25 months/ hippocampus	Cajal gold chloride stain	Hypertrophic astrocytes were located near the areas of neurodegeneration and neuronal loss	Landfield et al. (1977)
Rat/3 to 29.6 months/ cerebral cortex	Electron microscopy	No age-dependent morphological changes in astrocytes were observed, save an increase in membrane-bound inclusions	Vaughan and Peters (1974)
Mice/2 weeks, 8 weeks, 18 weeks, 40–42 weeks and 50–59 weeks/hippocampus CA1	GFAP immunohistochemistry	Significant increase in the number of GFAP-positive cells was observed in the CA1 area of old mice	Hayakawa et al. (2007)
Rats, male (Wistar)/3 and 22 months/hippocampus	GFAP immunohistochemistry; GFAP Western blot LPS infusion for 4 weeks intraventricularly	GFAP protein increased in aged mice by 108%; in LPS-treated by 129%. The density of GFAP-positive astrocytes was significantly decreased in old rats, they demonstrate atrophic morphology with shorter processes; some processes show signs of clasmatodendrosis. On the contrary LPS triggered	Cerbai et al. (2012)

(continued)

Table 11.1 (continued)

Specie/Age/brain region	Experimental techniques	Main findings	References
		hypertrophy of GFAP profiles	
Humans (18 females, 13 males)/18–93 years/ neocortex	Stereological cell count	The number of astrocytes was stable throughout the lifespan	Pelvig et al. (2008)
Humans (23 females) 65–105 years/neocortex	Stereological cell count	The trend for reduction of astrocyte numbers in the oldest subjects was noted; although it did not reach statistical significance	Fabricius et al. (2013)
Mice, females (C57Bl/ 6NNIA, B6)/3–4, 13–14, 20–24 months/ hippocampus	GFAP immunolabelling, stereological cell count	Ageing increased numbers of GFAP-positive astrocytes by 20%	Mouton et al. (2002)
Mice/males (C57BL/6J)/ 4–5 s, 13–14, 27–-28 months/hippocampus	Stereological cell count	No significant differences in astrocytes numbers at different ages were found	Long et al. (1998)
Rats, males (Sprague-Dawley) 12, 24 months/ frontal cortex, hippocampus	GFAP immunohistochemistry;	Ageing was associated with an increase in number of GFAP-positive astrocytes and an increase in size of GFAP-positive profiles	Amenta et al. (1998)
Resus monkey/males 9–10, 14–17; females 22–29 years/cortex, putamen, globus pallidus, hippocampus	GFAP immunohistochemistry; unbiased stereology	Astrocytes in old animals do not show age-dependent changes	Kanaan et al. (2010)
Mice, female (albino Swiss)/6, 20 months/ hippocampus	GFAP immunohistochemistry, behavioural tests	Number of astrocytes increased with ageing in the molecular layer and in the polymorphic layer, it remained unchanged in the granular layer. Astrocytes in molecular layer show hypertrophy of GFAP-positive profiles	Diniz et al. (2010)
Mice, males, females (C57BL/6)/3 months, 1, 2 years/olfactory bulb	GFAP immunohistochemistry	Olfactory bulb astrocytes increase in complexity between 3 months and 1 year; while no change in astroglial morphology or numbers was detected in aged animals	Klein et al. (2020)
Humans/28 weeks of gestation—88 years/ Subsntatia nigra	Nissl staining, GFAP, s100B immunohistochemistry	No significant change in total number of glial cells was observed in old age.	Jyothi et al. (2015)

(continued)

Table 11.1 (continued)

Specie/Age/brain region	Experimental techniques	Main findings	References
		Similarly, no major changes in astrocyte morphology were detected	
Mice (C57BL6)/ 0.6, 6, 19, 24 months/ hippocampus	Golgi staining; caloric restriction	No significant changes in astrocytes size in old age have been detected. Somata of astrocytes in calorically restricted animals were smaller than in *ad libidum* fed mice	Castiglioni Jr. et al. (1991)
Mice(Swiss)/6, 20 months/hippocampus	GFAP immunohistochemistry, behavioural tests, 3D astrocytes morphometry	Both ageing and environmental deprivation reduced complexity of astrocytes; environmental enrichment in contrast increases astroglial complexity in all ages	Diniz et al. (2016)
Rats (Wistar)/3, 6–12, 18–25 months/retina	GFAP, S100B immunohistochemistry, TUNEL assay	The number of astrocytes decreases in old age due to an age-dependent increase in astroglial apoptosis	Mansour et al. (2008)
Mice, males (SV129/ C57BL6)/3, 9, 18, 24 months/hippocampus, entorhinal cortex	GFAP, GS, S100B immunohistochemsitry	GFAP profiles in old mice showed hypertrophy in CA1 region and in DG. In contrast in EC GFAP-positive profiles show a substantial decrease in size and complexity. GD-positive profiles were smaller in old hippocampus and were unchanged in EC. Finally, S100B profiles were larger in old EC, displayed moderate changes in DG and no changes in CA1	Rodriguez et al. (2014)
Mice (Aldh1l1-Cre/ER2), 20 months/neocortex	GFAP, EGFP expressed in astrocytes	Aged astrocytes have reduced surface area and decreases complexity	Yang et al. (2022)

2021; Popov et al. 2021)) was estimated. Volume fraction measurements are based on the presumption that fluorescence measured from the soma reflects 100% of astrocyte space occupancy, whereas the fluorescence measured from an optically unresolved area is proportional to the volume fraction of astrocyte processes in any given area (Medvedev et al. 2014). The volume fraction of astrocytic leaflets decreases with age, which translates into decreased astrocytic coverage of synapses (Popov et al. 2021). At the same time shrinkage of astrocytic processes and decrease

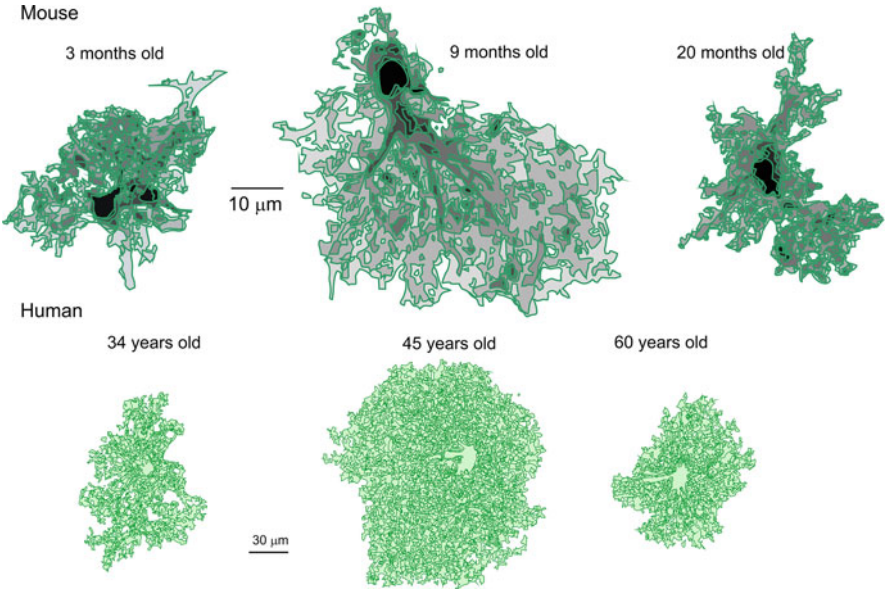


Fig. 11.3 Age-dependent morphological decline of astrocytes. Reconstructions of mouse and human astrocytes filled with fluorescent die Alexa Fluor 594. Human samples were access tissue obtained during surgery. All images are authors' own unpublished data

in their territories affects the neuropil and opens diffusion channels which may explain an increase in the mean diffusivity of the grey matter in elderly human demonstrated by diffusion tensor imaging (Salminen et al. 2016). Ageing also affects astrocytic syncytia by decreasing gap junctional coupling (Peters et al. 2009; Popov et al. 2021). Connexin-based gap junctions connect astrocytes into functional syncytia with a prominent degree of region-specific anatomical segregation (Giaume et al. 2021). This gap junction connectivity maintains isopotentiality of astroglial syncytia (Ma et al. 2016). Disruption of astrocytic syncytia may lead to multiple effects on astrocytic physiology. In particular, a decrease in intercellular coupling may lead to a loss of syncytium isopotentiality. Without isopotentiality the astrocytes become prone to membrane depolarisation caused by K^+ accumulation during synaptic transmission (Shih et al. 2013) that may further suppress glutamate uptake (Tyurikova et al. 2022).

In conclusion, astrocytic changes in the ageing brain are complex and region-specific. At the same time, overall decrease in astrocytic complexity and size of their territorial domains seems to predominate in the old brains. These atrophic changes may impact various aspects of brain functions, affecting synaptic and volume transmission, homeostatic support of the nervous tissue and neuroprotection. Astrocytic morphological decline translates into functional deficits discussed in the ensuing chapters.

Physiology of Old Astrocytes

Astrocytes are non-excitable cells, characterised by a highly hyperpolarised (−80 to −85 mV) resting membrane potential (reflecting high K^+ permeability of their plasmalemma), by high activity of plasmalemmal transporters providing for various aspects of homoeostatic support and by intracellular ionic excitability (Verkhratsky and Nedergaard 2018; Verkhratsky et al. 2020a, b). Physiological stimulation of astrocytes triggers ion fluxes through plasmalemma and endomembranes, thus generating ionic signals that provide a substrate for astrocytic excitability (Verkhratsky and Nedergaard 2018; Verkhratsky et al. 1998). High resting membrane potential and ionic gradients create an electrochemical driving force critical for the normal function of astrocytic homeostatic transporters. Most of these transporters are particularly sensitive to transmembrane Na^+ and are the targets for astrocytic Na^+ signalling (Rose and Verkhratsky 2016; Kirischuk et al. 2012).

There have been only a few studies of physiological properties of aged astrocytes (Table 11.2). The resting membrane potential of astrocytes is not affected by ageing (Lalo et al. 2011; Popov et al. 2021). Similarly, membrane input resistance is not affected by ageing in hippocampal astrocytes (Lalo et al. 2011); however, it increases in cortical astrocytes (Popov et al. 2021). An increase in the input resistance reflects an age-dependent decrease in the size of astrocytes and decrease in the gap junctional connectivity and uncoupling through the gap junctions. Astrocytes in aged brains of humans and rodents express functional receptors to neurotransmitters and generate spontaneous and induced Ca^{2+} signals in response to appropriate stimulation (Lalo et al. 2011; Gomez-Gonzalo et al. 2017; Navarrete et al. 2013; Popov et al. 2021), indicating preservation of the basic mechanism of astrocytic excitability. Functional expression of major receptors and glutamate transporters however does change during the lifespan. In mice, the density of AMPA, NMDA and P2X receptors as well as the density of plasmalemmal glutamate transporter membrane currents increases in the first 6 months of life and then sharply decreases at the more advanced ages (Lalo et al. 2011). There are sporadic reports on age-dependent changes in astrocytic Ca^{2+} signalling. For example, there are some indications of aberrant Ca^{2+} signalling in aged astroglia. Spontaneous Ca^{2+} oscillations in Bergmann glia occur 20 times more frequently in 20 months old mice than in young 2.5 months old controls (Mathiesen et al. 2013). Similarly, ATP-induced astrocytic Ca^{2+} responses seem to decrease with age (Lalo et al. 2019).

Functional Decline of Ageing Astrocytes

Ageing is associated with a progressive decline of main astrocytic functions (Fig. 11.4) Waning astrocytic support and neuroprotection impacts on brain active milieu and considerably affects various neuronal functions, synaptic transmission,

Table 11.2 Physiology of aged astrocytes

Specie/Age/Brain region	Experimental techniques	Main findings	References
Mice/8–14, 48–80 weeks (~2.5, 20 months) old/cerebellum, Bergmann glia	In vivo transcranial confocal microscopy, Oregon Green BAPTA-1 $[Ca^{2+}]_i$ recordings	Old Bergmann glial cells demonstrated much higher (almost 20 times) spontaneous $[Ca^{2+}]_i$ activity	Mathiesen et al. (2013)
GFAP-EGFP mice/4, 10, 21 months/cortex	In situ recording from astrocytes in acute cortical slices, whole-cell voltage-clamp, two-photon microscopy, Oregon Green BAPTA-1 $[Ca^{2+}]_i$ recordings, Enriched environment together with physical exercise, calorie restriction (15%)	Old astrocytes demonstrated decreased P2X receptors-mediated miniature EPSCs and suppressed purinergic Ca^{2+} signalling. Exposure to enriched environment or to calorie restrictive diet rescued purinergic signalling	Lalo et al. (2019)
GFAP-EGFP mice/1, 3, 6, 9, 12, 18–-21 months/Somato-sensory cortex	In situ recording from astrocytes in acute cortical slices, whole-cell voltage-clamp, fura-2 / monochromator-based $[Ca^{2+}]_i$ recordings	The density of P2X, NMDA and AMPA ionotropic receptor currents, as well as the density of glutamate transporter currants showed bell-shaped age dependence. All densities peaked at 3–6 months, and then steeply declined and stayed unchanged in old age. Neurotransmitter-evoked astroglial Ca^{2+} signalling showed same age dependence	Lalo et al. (2011)
Mice (C57BL/6)/05, 5, 12, 20 months/ Somato-sensory cortex, hippocampus	In situ recording from astrocytes in acute cortical slices, whole-cell voltage-clamp, fluo-4 / monochromator based $[Ca^{2+}]_i$ recordings	No major changes in astroglial basic electrophysiological parameters as well as in astroglial Ca^{2+} signalling were detected	Gomez-Gonzalo et al. (2017)
Humans, males and females/28–59 years old/ excess tissue for temporal lobe drug-resistant epilepsy surgery	In situ recording from astrocytes in acute cortical slices, whole-cell voltage-clamp, fluo-4 / monochromator based $[Ca^{2+}]_i$ recordings	No major differences in electrophysiological parameters or Ca^{2+} signals were found in aged tissues	Navarrete et al. (2013)
Rats (Sprague–Dawley)/ 3–6, 24–27 months/ hippocampus	GLAST/GLT-1 immunoblotting; d-[^{3}H]aspartate uptake (measure of glutamate transport) In situ extracellular	Protein levels of both astroglial transporters GLAST and GLT-1 (EAAT1/2) are significantly decreased in old age. This facilitates	Potier et al. (2010)

(continued)

Table 11.2 (continued)

Specie/Age/Brain region	Experimental techniques	Main findings	References
	recordings from acute slices	glutamate spillover, activation of extrasynaptic NMDA and mGluR receptors, which modulate synaptic plasticity	
Rats (fisher F-344)/3, 24 months/hippocampus	qPCR, immunoblotting, Morris water maze	mRNA for GLT-1 was decreased in aged rats which correlated with memory impairment; treatment with Riluzole significantly increased GLT-1 expression and improved memory	Brothers et al. (2013)
Mice (C57BL/6)/3, 12, 24 months/ hippocampus	Whole-cell patch clamp, Ca^{2+} imaging, confocal microscopy	In old animals, astrocytic membrane current associated with K^+ and glutamate uptake were reduced indicating compromised glutamate clearance and K^+ buffering	Popov et al. (2021)

neuroplasticity, and neuroprotection. Thus, age-dependent functional decline of astrocytes contributes to cognitive deficits of the senescent brain.

Neurotransmitter Homeostasis

Astrocytes are fundamental for glutamate metabolism in the CNS. The de novo synthesis of glutamate from glucose is confined to astrocytes; which subsequently convert newly synthesised glutamate to glutamine, which is shuttled to neurones. Similarly, astrocytes are the major sink for glutamate released during synaptic transmission—astrocytes accumulate glutamate by the activity of EAAT1/2 glutamate transporters. In astrocytes, glutamate undergoes conversion to glutamine (by astrocyte-specific glutamine synthetase), which again is shuttled back to neurones to replenish synaptic pools of glutamate and gamma-aminobutyric acid (GABA)—the famous glutamate (GABA)glutamine shuttle (Andersen et al. 2022).

The glutamate-to-glutamine ratio increases with age, thus indicating deficiencies in the operation of the glutamate (GABA) glutamine shuttle (Duarte et al. 2014; Harris et al. 2014). Indeed, the expression of glutamate transporters is decreased in astrocytes from old (24–27 months) rats, which translates into decreased glutamate uptake (Potier et al. 2010). Similarly, glutamate transporter currents are also decreased in hippocampal and cortical astrocytes in mice (Popov et al. 2021; Lalo et al. 2011). In 18–21 month-old mice the density of glutamate transporter current was only ~10–15% of that in adult animals (Lalo et al. 2011). Physiological ageing

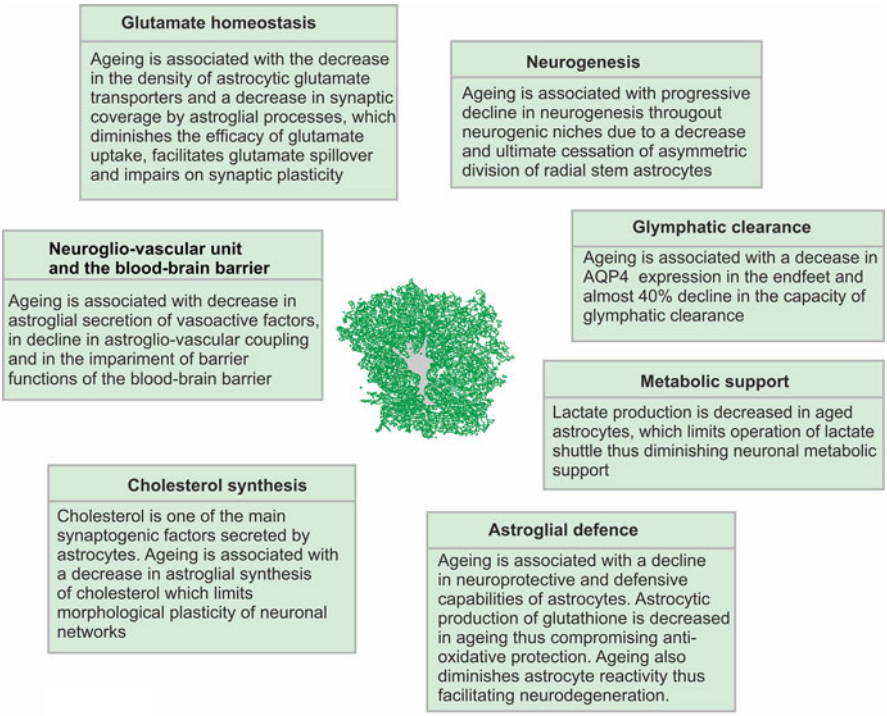


Fig. 11.4 Age-dependent functional decline of astrocytes

also leads to a slight decrease in the expression of astrocytic glutamine synthetase (Olabarria et al. 2011). In addition, a decrease in astrocytic territorial domains and synaptic coverage may further compromise glutamate uptake and promote glutamate spillover, thus affecting synaptic plasticity (Popov et al. 2021; Verkhratsky et al. 2021).

Astrocytes are the main possessors of monoaminoxidase-B (MAO-B) which is the central enzyme of catabolism of monoamines (Levitt et al. 1982; Westlund et al. 1988). Ageing is associated with a substantial (two- to threefold—Kumar and Andersen 2004) increase in the levels of MAO-B, which has several detrimental consequences for brain function. An increase in MAO-B substantially lowers the levels of monoamines and in particular noradrenaline. This parallels the age-dependent decline of locus coeruleus that provides for the bulk of noradrenergic innervation of the CNS and is particularly vulnerable to age-dependent neurodegeneration (Zorec et al. 2018). Increased levels of MAO-B promote GABA synthesis from putrescine, thus making astrocytes a prominent source of GABA which mediates aberrant tonic inhibition, thus affecting synaptic transmission (Jo et al. 2014; Garaschuk and Verkhratsky 2019). Finally, the major

by-product of MAO-B activity is hydrogen peroxide which may mediate neuronal damage (Chun et al. 2020).

Neuroglio-Vascular Unit and the Blood-Brain Barrier

Astrocytes integrate neurones, synapses, microglial cells and neighbouring asculature into the neuro-gliovascular (or neurovascular) unit (Iadecola 2017). Astrocytes in particular provide for dynamic regulation of the blood flow and for control of the endothelial blood-brain barrier (BBB) by numerous mediators released from astrocytic endfeet plastering brain vasculature (Pivoriunas and Verkhratsky 2021). Furthermore, endfeet are endowed with baroreceptors, which allow astrocytes to sense and regulate local blood pressure (Marina et al. 2020). Age-dependent shrinkage of astrocytes may affect their interactions with brain vessels and limit their ability to monitor and control local functional hyperaemia. Astrocytes are in close functional relation with endotheliocytes and pericytes, continuously supporting the integrity of the BBB (Sweeney et al. 2019; Pivoriunas and Verkhratsky 2021).

Ageing is associated with the restructuring of blood vessels and a decrease in blood supply (Amin-Hanjani et al. 2015), which affects energy metabolism of the nervous tissue. There are some indications that ageing affects the astrocytic secretion of vasoactive agents arachidonic acid and eicosanoids, which may affect local neurovascular coupling (Keleshian et al. 2013; Tarantini et al. 2017). Ageing also leads to a decline in the BBB function (Montagne et al. 2015; Nation et al. 2019).

Interrogating aged BBB at the ultrastructural level demonstrates the increased thickness of capillary walls and basement membranes; in addition, the area of the astrocyte endfeet surrounding the capillaries increases with age (Bors et al. 2018). There are also some indications that astrocytes lose their ability to regulate tight junction expression in the context of neurodegeneration (Kriauciunaite et al. 2020); whether a similar loss of function occurs in physiological ageing remains to be investigated. In conclusion, brain ageing is accompanied by a progressive decline in the functional performance of the neurogliovascular unit.

Neurogenesis

The neural stem cells responsible for adult neurogenesis are radial stem astrocytes and tanycytes (Verkhratsky and Nedergaard 2018) localised in several neurogenic niches. These cells combine features of stem cells and homeostatic astrocytes: they form perivascular endfeet and, through distal processes and leaflets, provide for synaptic coverage (Mirzadeh et al. 2008; Moss et al. 2016). Ageing reduces neurogenic capacity in all neurogenic zones due to a suppression of asymmetric division of radial stem astrocytes (Ben Abdallah et al. 2010; Bouab et al. 2011). Of note,

pathological ageing and neurodegenerative processes cause much more prominent suppression of radial stem astrocytes associated with prominent and rapid fall of neurogenesis (Rodriguez et al. 2008; Rodriguez and Verkhratsky 2011).

Age-Dependent Decline of the Glymphatic System

Astrocytes, through their endfeet, glia limitans and endfeet-associated aquaporin 4 (AQP4) water channels make a fundamental component of the pan-CNS waste-collecting mechanism known as a glymphatic system (Nedergaard 2013). Ageing is associated with the loss of specific attachment of AQP4 to the endfeet which causes a 40% decline in the operational capacity of the glymphatic system (Kress et al. 2014), with an obvious impact on brain health. Of note, even more severe decline of the glymphatic system accompanies neurodegenerative diseases (Peng et al. 2016).

Ageing Impairs Astroglial Metabolic Support

Ageing increases astroglial oxidative metabolism, which may limit their ability to produce intermediates of glycolysis and to supply neurones with energy substrates (Jiang and Cadenas 2014). This is further corroborated by evidence indicating reduced astrocytic lactate production and hence reduced operation of lactate shuttle. Furthermore, there are indications that ageing reduces the astroglial ability to produce lactate and hence to operate the lactate shuttle (Harris et al. 2015).

Ageing and Astrocytic Mitochondria

Astrocytes contribute to neuronal well-being and energy metabolism through transmitophagy, in which astrocytes accumulate and destroy damaged neuronal mitochondria and possibly supply neurons with healthy organelles (Davis et al. 2014; Hayakawa et al. 2016). Age-dependent astroglial atrophy may conceivably affect this pathway, thus limiting neuroprotection.

Cholesterol Synthesis

Astrocytes are the main source of cholesterol in the CNS being thus indispensable for feeding neurons with building blocks for synaptogenesis and morphological plasticity (Mauch et al. 2001). Cholesterol synthesis declines with ageing reflecting

down-regulation of the main synthesising enzyme HMG-CoA reductase (Boisvert et al. 2018), which limits neuronal remodelling and plasticity.

Age-Dependent Decline of Astroglial Neuroprotection

Astrocytic homeostatic support and defensive capabilities are critical elements for neuroprotection (Pekny et al. 2016). Astrocytes are, for example, the main providers for CNS defence against reactive oxygen species (ROS). Astrocytes are indispensable elements for the synthesis of glutathione and reduction of ascorbic acid, both being the main ROS scavengers in the nervous tissue (Makar et al. 1994). In ageing, astrocytic production of glutathione is decreased thus increasing neuronal vulnerability to oxidative stress (Emir et al. 2011; Maher 2005). Ageing is also associated with a decline in astrocytic reactivity, which together with age-dependent microglial dystrophy paves the way for neurodegenerative diseases (Verkhratsky et al. 2015).

Conclusions

Ageing is associated with the decline of astrocytes: they become smaller, less complex and lose their ability to support and protect the nervous tissue.

References

Amenta F, Bronzetti E, Sabbatini M, Vega JA (1998) Astrocyte changes in aging cerebral cortex and hippocampus: a quantitative immunohistochemical study. Microsc Res Tech 43(1):29–33. https://doi.org/10.1002/(SICI)1097-0029(19981001)

Amin-Hanjani S, Du X, Pandey DK, Thulborn KR, Charbel FT (2015) Effect of age and vascular anatomy on blood flow in major cerebral vessels. J Cereb Blood Flow Metab 35(2):312–318. https://doi.org/10.1038/jcbfm.2014.203

Andersen JV, Schousboe A, Verkhratsky A (2022) Astrocyte energy and neurotransmitter metabolism in Alzheimer's disease: integration of the glutamate/GABA-glutamine cycle. Prog Neurobiol 217:102331. https://doi.org/10.1016/j.pneurobio.2022.102331

Aten S, Kiyoshi CM, Arzola EP, Patterson JA, Taylor AT, Du Y, Guiher AM, Philip M, Camacho EG, Mediratta D, Collins K, Boni K, Garcia SA, Kumar R, Drake AN, Hegazi A, Trank L, Benson E, Kidd G, Terman D, Zhou M (2022) Ultrastructural view of astrocyte arborization, astrocyte-astrocyte and astrocyte-synapse contacts, intracellular vesicle-like structures, and mitochondrial network. Prog Neurobiol 213:102264. https://doi.org/10.1016/j.pneurobio.2022.102264

Augusto-Oliveira M, Arrifano GP, Takeda PY, Lopes-Araujo A, Santos-Sacramento L, Anthony DC, Verkhratsky A, Crespo-Lopez ME (2020) Astroglia-specific contributions to the regulation of synapses, cognition and behaviour. Neurosci Biobehav Rev 118:331–357. https://doi.org/10.1016/j.neubiorev.2020.07.039

Becquet D, Girardet C, Guillaumond F, Francois-Bellan AM, Bosler O (2008) Ultrastructural plasticity in the rat suprachiasmatic nucleus. Possible involvement in clock entrainment. Glia 56(3):294–305. https://doi.org/10.1002/glia.20613

Ben Abdallah NM, Slomianka L, Vyssotski AL, Lipp HP (2010) Early age-related changes in adult hippocampal neurogenesis in C57 mice. Neurobiol Aging 31(1):151–161. https://doi.org/10.1016/j.neurobiolaging.2008.03.002

Bjorklund H, Eriksdotter-Nilsson M, Dahl D, Rose G, Hoffer B, Olson L (1985) Image analysis of GFA-positive astrocytes from adolescence to senescence. Exp Brain Res 58(1):163–170. https://doi.org/10.1007/BF00238964

Boisvert MM, Erikson GA, Shokhirev MN, Allen NJ (2018) The aging astrocyte transcriptome from multiple regions of the mouse brain. Cell Rep 22(1):269–285. https://doi.org/10.1016/j.celrep.2017.12.039

Bors L, Toth K, Toth EZ, Bajza A, Csorba A, Szigeti K, Mathe D, Perlaki G, Orsi G, Toth GK, Erdo F (2018) Age-dependent changes at the blood-brain barrier. A comparative structural and functional study in young adult and middle aged rats. Brain Res Bull 139:269–277. https://doi.org/10.1016/j.brainresbull.2018.03.001

Bouab M, Paliouras GN, Aumont A, Forest-Berard K, Fernandes KJ (2011) Aging of the subventricular zone neural stem cell niche: evidence for quiescence-associated changes between early and mid-adulthood. Neuroscience 173:135–149. https://doi.org/10.1016/j.neuroscience.2010.11.032

Brothers HM, Bardou I, Hopp SC, Kaercher RM, Corona AW, Fenn AM, Godbout JP, Wenk GL (2013) Riluzole partially rescues age-associated, but not LPS-induced, loss of glutamate transporters and spatial memory. J Neuroimmune Pharmacol 8(5):1098–1105. https://doi.org/10.1007/s11481-013-9476-2

Bushong EA, Martone ME, Jones YZ, Ellisman MH (2002) Protoplasmic astrocytes in CA1 stratum radiatum occupy separate anatomical domains. J Neurosci 22(1):183–192

Castiglioni AJ Jr, Legare ME, Busbee DL, Tiffany-Castiglioni E (1991) Morphological changes in astrocytes of aging mice fed normal or caloric restricted diets. Age 14:102–106

Cerbai F, Lana D, Nosi D, Petkova-Kirova P, Zecchi S, Brothers HM, Wenk GL, Giovannini MG (2012) The neuron-astrocyte-microglia triad in normal brain ageing and in a model of neuroinflammation in the rat hippocampus. PLoS One 7(9):e45250. https://doi.org/10.1371/journal.pone.0045250

Chao TI, Rickmann M, Wolff JR (2002) The synapse-astrocyte boundary: anatomical basis for an integrative role of glia in synaptic transmission. In: Volterra A, Magistretti P, Haydon PG (eds) The tripartite synapses: glia in synaptic transmission. OUP, Oxford, pp 3–23

Chun H, Im H, Kang YJ, Kim Y, Shin JH, Won W, Lim J, Ju Y, Park YM, Kim S, Lee SE, Lee J, Woo J, Hwang Y, Cho H, Jo S, Park JH, Kim D, Kim DY, Seo JS, Gwag BJ, Kim YS, Park KD, Kaang BK, Cho H, Ryu H, Lee CJ (2020) Severe reactive astrocytes precipitate pathological hallmarks of Alzheimer's disease via $H_2O_2^-$ production. Nat Neurosci 23(12):1555–1566. https://doi.org/10.1038/s41593-020-00735-y

Davies DS, Ma J, Jegathees T, Goldsbury C (2017) Microglia show altered morphology and reduced arborization in human brain during aging and Alzheimer's disease. Brain Pathol 27(6):795–808. https://doi.org/10.1111/bpa.12456

Davis CH, Kim KY, Bushong EA, Mills EA, Boassa D, Shih T, Kinebuchi M, Phan S, Zhou Y, Bihlmeyer NA, Nguyen JV, Jin Y, Ellisman MH, Marsh-Armstrong N (2014) Transcellular degradation of axonal mitochondria. Proc Natl Acad Sci U S A 111(26):9633–9638. https://doi.org/10.1073/pnas.1404651111

Diniz DG, Foro CA, Rego CM, Gloria DA, de Oliveira FR, Paes JM, de Sousa AA, Tokuhashi TP, Trindade LS, Turiel MC, Vasconcelos EG, Torres JB, Cunnigham C, Perry VH, Vasconcelos PF, Diniz CW (2010) Environmental impoverishment and aging alter object recognition, spatial learning, and dentate gyrus astrocytes. Eur J Neurosci 32(3):509–519. https://doi.org/10.1111/j.1460-9568.2010.07296.x

Diniz DG, de Oliveira MA, de Lima CM, Foro CA, Sosthenes MC, Bento-Torres J, da Costa Vasconcelos PF, Anthony DC, Diniz CW (2016) Age, environment, object recognition and morphological diversity of GFAP-immunolabeled astrocytes. Behav Brain Funct 12(1):28. https://doi.org/10.1186/s12993-016-0111-2

Duarte JM, Do KQ, Gruetter R (2014) Longitudinal neurochemical modifications in the aging mouse brain measured in vivo by ^{1}H magnetic resonance spectroscopy. Neurobiol Aging 35(7): 1660–1668. https://doi.org/10.1016/j.neurobiolaging.2014.01.135

Emir UE, Raatz S, McPherson S, Hodges JS, Torkelson C, Tawfik P, White T, Terpstra M (2011) Noninvasive quantification of ascorbate and glutathione concentration in the elderly human brain. NMR Biomed 24(7):888–894. https://doi.org/10.1002/nbm.1646

Escartin C, Galea E, Lakatos A, O'Callaghan JP, Petzold GC, Serrano-Pozo A, Steinhauser C, Volterra A, Carmignoto G, Agarwal A et al (2021) Reactive astrocyte nomenclature, definitions, and future directions. Nat Neurosci 24(3):312–325. https://doi.org/10.1038/s41593-020-00783-4

Fabricius K, Jacobsen JS, Pakkenberg B (2013) Effect of age on neocortical brain cells in 90+ year old human females--a cell counting study. Neurobiol Aging 34(1):91–99. https://doi.org/10.1016/j.neurobiolaging.2012.06.009

Garaschuk O, Verkhratsky A (2019) GABAergic astrocytes in Alzheimer's disease. Aging 11(6): 1602–1604. https://doi.org/10.18632/aging.101870

Gavrilov N, Golyagina I, Brazhe A, Scimemi A, Turlapov V, Semyanov A (2018) Astrocytic coverage of dendritic spines, dendritic shafts, and axonal boutons in hippocampal neuropil. Front Cell Neurosci 12:248. https://doi.org/10.3389/fncel.2018.00248

Geinisman Y, Bondareff W, Dodge JT (1978) Hypertrophy of astroglial processes in the dentate gyrus of the senescent rat. Am J Anat 153(4):537–543. https://doi.org/10.1002/aja.1001530405

Giaume C, Naus CC, Saez JC, Leybaert L (2021) Glial connexins and pannexins in the healthy and diseased brain. Physiol Rev 101(1):93–145. https://doi.org/10.1152/physrev.00043.2018

Gomez-Gonzalo M, Martin-Fernandez M, Martinez-Murillo R, Mederos S, Hernandez-Vivanco A, Jamison S, Fernandez AP, Serrano J, Calero P, Futch HS, Corpas R, Sanfeliu C, Perea G, Araque A (2017) Neuron-astrocyte signaling is preserved in the aging brain. Glia 65(4): 569–580. https://doi.org/10.1002/glia.23112

Harris JL, Yeh HW, Swerdlow RH, Choi IY, Lee P, Brooks WM (2014) High-field proton magnetic resonance spectroscopy reveals metabolic effects of normal brain aging. Neurobiol Aging 35(7): 1686–1694. https://doi.org/10.1016/j.neurobiolaging.2014.01.018

Harris JL, Choi IY, Brooks WM (2015) Probing astrocyte metabolism in vivo: proton magnetic resonance spectroscopy in the injured and aging brain. Front Aging Neurosci 7:202. https://doi.org/10.3389/fnagi.2015.00202

Haug H, Eggers R (1991) Morphometry of the human cortex cerebri and corpus striatum during aging. Neurobiol Aging 12(4):336–338; discussion 352–335

Hayakawa N, Kato H, Araki T (2007) Age-related changes of astorocytes, oligodendrocytes and microglia in the mouse hippocampal CA1 sector. Mech Ageing Dev 128(4):311–316. https://doi.org/10.1016/j.mad.2007.01.005

Hayakawa K, Esposito E, Wang X, Terasaki Y, Liu Y, Xing C, Ji X, Lo EH (2016) Transfer of mitochondria from astrocytes to neurons after stroke. Nature 535(7613):551–555. https://doi.org/10.1038/nature18928

Humphry GM (1889) The old age. The results of information received respecting nearly nine hundred persons who had attained the age of 80 years, including seventy-four centenarians. MacMillan & Bowes, Cambridge

Iadecola C (2017) The neurovascular unit coming of age: a journey through neurovascular coupling in health and disease. Neuron 96(1):17–42. https://doi.org/10.1016/j.neuron.2017.07.030

Jiang T, Cadenas E (2014) Astrocytic metabolic and inflammatory changes as a function of age. Aging Cell 13(6):1059–1067. https://doi.org/10.1111/acel.12268

Jo S, Yarishkin O, Hwang YJ, Chun YE, Park M, Woo DH, Bae JY, Kim T, Lee J, Chun H, Park HJ, Lee DY, Hong J, Kim HY, Oh SJ, Park SJ, Lee H, Yoon BE, Kim Y, Jeong Y, Shim I, Bae

YC, Cho J, Kowall NW, Ryu H, Hwang E, Kim D, Lee CJ (2014) GABA from reactive astrocytes impairs memory in mouse models of Alzheimer's disease. Nat Med 20(8):886–896. https://doi.org/10.1038/nm.3639

Jyothi HJ, Vidyadhara DJ, Mahadevan A, Philip M, Parmar SK, Manohari SG, Shankar SK, Raju TR, Alladi PA (2015) Aging causes morphological alterations in astrocytes and microglia in human substantia nigra pars compacta. Neurobiol Aging 36(12):3321–3333. https://doi.org/10.1016/j.neurobiolaging.2015.08.024

Kanaan NM, Kordower JH, Collier TJ (2010) Age-related changes in glial cells of dopamine midbrain subregions in rhesus monkeys. Neurobiol Aging 31(6):937–952. https://doi.org/10.1016/j.neurobiolaging.2008.07.006

Keleshian VL, Modi HR, Rapoport SI, Rao JS (2013) Aging is associated with altered inflammatory, arachidonic acid cascade, and synaptic markers, influenced by epigenetic modifications, in the human frontal cortex. J Neurochem 125(1):63–73. https://doi.org/10.1111/jnc.12153

Khakh BS, Sofroniew MV (2015) Diversity of astrocyte functions and phenotypes in neural circuits. Nat Neurosci 18(7):942–952. https://doi.org/10.1038/nn.4043

Kirischuk S, Parpura V, Verkhratsky A (2012) Sodium dynamics: another key to astroglial excitability? Trends Neurosci 35(8):497–506. https://doi.org/10.1016/j.tins.2012.04.003

Klein M, Lohr C, Droste D (2020) Age-dependent heterogeneity of murine olfactory bulb astrocytes. Front Aging Neurosci 12:172. https://doi.org/10.3389/fnagi.2020.00172

Korogod N, Petersen CC, Knott GW (2015) Ultrastructural analysis of adult mouse neocortex comparing aldehyde perfusion with cryo fixation. elife 4:e05793. https://doi.org/10.7554/eLife.05793

Kress BT, Iliff JJ, Xia M, Wang M, Wei HS, Zeppenfeld D, Xie L, Kang H, Xu Q, Liew JA, Plog BA, Ding F, Deane R, Nedergaard M (2014) Impairment of paravascular clearance pathways in the aging brain. Ann Neurol 76(6):845–861. https://doi.org/10.1002/ana.24271

Kriauciunaite K, Kausyle A, Pajarskiene J, Tunaitis V, Lim D, Verkhratsky A, Pivoriunas A (2020) Immortalised hippocampal astrocytes from 3xTG-AD mice fail to support BBB integrity in vitro: role of extracellular vesicles in glial-endothelial communication. Cell Mol Neurobiol 41(3):551–562. https://doi.org/10.1007/s10571-020-00871-w

Kumar MJ, Andersen JK (2004) Perspectives on MAO-B in aging and neurological disease: where do we go from here? Mol Neurobiol 30(1):77–89. https://doi.org/10.1385/MN:30:1:077

Lalo U, Palygin O, North RA, Verkhratsky A, Pankratov Y (2011) Age-dependent remodelling of ionotropic signalling in cortical astroglia. Aging Cell 10(3):392–402. https://doi.org/10.1111/j.1474-9726.2011.00682.x

Lalo U, Bogdanov A, Pankratov Y (2019) Age- and experience-related plasticity of ATP-mediated signaling in the neocortex. Front Cell Neurosci 13:242. https://doi.org/10.3389/fncel.2019.00242

Landfield PW, Rose G, Sandles L, Wohlstadter TC, Lynch G (1977) Patterns of astroglial hypertrophy and neuronal degeneration in the hippocampus of ages, memory-deficient rats. J Gerontol 32(1):3–12. https://doi.org/10.1093/geronj/32.1.3

Levitt P, Pintar JE, Breakefield XO (1982) Immunocytochemical demonstration of monoamine oxidase B in brain astrocytes and serotonergic neurons. Proc Natl Acad Sci U S A 79(20):6385–6389. https://doi.org/10.1073/pnas.79.20.6385

Long JM, Kalehua AN, Muth NJ, Calhoun ME, Jucker M, Hengemihle JM, Ingram DK, Mouton PR (1998) Stereological analysis of astrocyte and microglia in aging mouse hippocampus. Neurobiol Aging 19(5):497–503. https://doi.org/10.1016/s0197-4580(98)00088-8

Ma B, Buckalew R, Du Y, Kiyoshi CM, Alford CC, Wang W, McTigue DM, Enyeart JJ, Terman D, Zhou M (2016) Gap junction coupling confers isopotentiality on astrocyte syncytium. Glia 64(2):214–226. https://doi.org/10.1002/glia.22924

Maher P (2005) The effects of stress and aging on glutathione metabolism. Ageing Res Rev 4(2):288–314. https://doi.org/10.1016/j.arr.2005.02.005

Makar TK, Nedergaard M, Preuss A, Gelbard AS, Perumal AS, Cooper AJ (1994) Vitamin E, ascorbate, glutathione, glutathione disulfide, and enzymes of glutathione metabolism in cultures

of chick astrocytes and neurons: evidence that astrocytes play an important role in antioxidative processes in the brain. J Neurochem 62(1):45–53

Mansour H, Chamberlain CG, Weible MW 2nd, Hughes S, Chu Y, Chan-Ling T (2008) Aging-related changes in astrocytes in the rat retina: imbalance between cell proliferation and cell death reduces astrocyte availability. Aging Cell 7(4):526–540. https://doi.org/10.1111/j.1474-9726.2008.00402.x

Marina N, Christie IN, Korsak A, Doronin M, Brazhe A, Hosford PS, Wells JA, Sheikhbahaei S, Humoud I, Paton JFR, Lythgoe MF, Semyanov A, Kasparov S, Gourine AV (2020) Astrocytes monitor cerebral perfusion and control systemic circulation to maintain brain blood flow. Nat Commun 11(1):131. https://doi.org/10.1038/s41467-019-13956-y

Marx V (2013) Is super-resolution microscopy right for you? Nat Methods 10(12):1157–1163. https://doi.org/10.1038/nmeth.2756

Mathiesen C, Brazhe A, Thomsen K, Lauritzen M (2013) Spontaneous calcium waves in Bergman glia increase with age and hypoxia and may reduce tissue oxygen. J Cereb Blood Flow Metab 33(2):161–169. https://doi.org/10.1038/jcbfm.2012.175

Mauch DH, Nagler K, Schumacher S, Goritz C, Muller EC, Otto A, Pfrieger FW (2001) CNS synaptogenesis promoted by glia-derived cholesterol. Science 294(5545):1354–1357. https://doi.org/10.1126/science.294.5545.1354

Medvedev N, Popov V, Henneberger C, Kraev I, Rusakov DA, Stewart MG (2014) Glia selectively approach synapses on thin dendritic spines. Philos Trans R Soc Lond Ser B Biol Sci 369(1654):20140047. https://doi.org/10.1098/rstb.2014.0047

Minge D, Domingos C, Unichenko P, Behringer C, Pauletti A, Anders S, Herde MK, Delekate A, Gulakova P, Schoch S, Petzold GC, Henneberger C (2021) Heterogeneity and development of fine astrocyte morphology captured by diffraction-limited microscopy. Front Cell Neurosci 15:669280. https://doi.org/10.3389/fncel.2021.669280

Mirzadeh Z, Merkle FT, Soriano-Navarro M, Garcia-Verdugo JM, Alvarez-Buylla A (2008) Neural stem cells confer unique pinwheel architecture to the ventricular surface in neurogenic regions of the adult brain. Cell Stem Cell 3(3):265–278. https://doi.org/10.1016/j.stem.2008.07.004

Montagne A, Barnes SR, Sweeney MD, Halliday MR, Sagare AP, Zhao Z, Toga AW, Jacobs RE, Liu CY, Amezcua L, Harrington MG, Chui HC, Law M, Zlokovic BV (2015) Blood-brain barrier breakdown in the aging human hippocampus. Neuron 85(2):296–302. https://doi.org/10.1016/j.neuron.2014.12.032

Moss J, Gebara E, Bushong EA, Sanchez-Pascual I, O'Laoi R, El M'Ghari I, Kocher-Braissant J, Ellisman MH, Toni N (2016) Fine processes of nestin-GFP-positive radial glia-like stem cells in the adult dentate gyrus ensheathe local synapses and vasculature. Proc Natl Acad Sci U S A 113(18):E2536–E2545. https://doi.org/10.1073/pnas.1514652113

Mouton PR, Long JM, Lei DL, Howard V, Jucker M, Calhoun ME, Ingram DK (2002) Age and gender effects on microglia and astrocyte numbers in brains of mice. Brain Res 956(1):30–35. https://doi.org/10.1016/s0006-8993(02)03475-3

Nation DA, Sweeney MD, Montagne A, Sagare AP, D'Orazio LM, Pachicano M, Sepehrband F, Nelson AR, Buennagel DP, Harrington MG, Benzinger TLS, Fagan AM, Ringman JM, Schneider LS, Morris JC, Chui HC, Law M, Toga AW, Zlokovic BV (2019) Blood-brain barrier breakdown is an early biomarker of human cognitive dysfunction. Nat Med 25(2):270–276. https://doi.org/10.1038/s41591-018-0297-y

Navarrete M, Perea G, Maglio L, Pastor J, Garcia de Sola R, Araque A (2013) Astrocyte calcium signal and gliotransmission in human brain tissue. Cereb Cortex 23(5):1240–1246. https://doi.org/10.1093/cercor/bhs122

Nedergaard M (2013) Neuroscience. Garbage truck of the brain. Science 340(6140):1529–1530. https://doi.org/10.1126/science.1240514

Oberheim NA, Takano T, Han X, He W, Lin JH, Wang F, Xu Q, Wyatt JD, Pilcher W, Ojemann JG, Ransom BR, Goldman SA, Nedergaard M (2009) Uniquely hominid features of adult human astrocytes. J Neurosci 29(10):3276–3287. https://doi.org/10.1523/JNEUROSCI.4707-08.2009

Olabarria M, Noristani HN, Verkhratsky A, Rodriguez JJ (2011) Age-dependent decrease in glutamine synthetase expression in the hippocampal astroglia of the triple transgenic Alzheimer's disease mouse model: mechanism for deficient glutamatergic transmission? Mol Neurodegener 6:55. https://doi.org/10.1186/1750-1326-6-55

Pekny M, Pekna M, Messing A, Steinhauser C, Lee JM, Parpura V, Hol EM, Sofroniew MV, Verkhratsky A (2016) Astrocytes: a central element in neurological diseases. Acta Neuropathol 131(3):323–345. https://doi.org/10.1007/s00401-015-1513-1

Pelvig DP, Pakkenberg H, Stark AK, Pakkenberg B (2008) Neocortical glial cell numbers in human brains. Neurobiol Aging 29(11):1754–1762. https://doi.org/10.1016/j.neurobiolaging.2007.04.013

Peng W, Achariyar TM, Li B, Liao Y, Mestre H, Hitomi E, Regan S, Kasper T, Peng S, Ding F, Benveniste H, Nedergaard M, Deane R (2016) Suppression of glymphatic fluid transport in a mouse model of Alzheimer's disease. Neurobiol Dis 93:215–225. https://doi.org/10.1016/j.nbd.2016.05.015

Peters O, Schipke CG, Philipps A, Haas B, Pannasch U, Wang LP, Benedetti B, Kingston AE, Kettenmann H (2009) Astrocyte function is modified by Alzheimer's disease-like pathology in aged mice. J Alzheimers Dis 18(1):177–189. https://doi.org/10.3233/JAD-2009-1140

Pivoriunas A, Verkhratsky A (2021) Astrocyte-endotheliocyte axis in the regulation of the blood-brain barrier. Neurochem Res 46(10):2538–2550. https://doi.org/10.1007/s11064-021-03338-6

Plata A, Lebedeva A, Denisov P, Nosova O, Postnikova TY, Pimashkin A, Brazhe A, Zaitsev AV, Rusakov DA, Semyanov A (2018) Astrocytic atrophy following status epilepticus parallels reduced Ca^{2+} activity and impaired synaptic plasticity in the rat hippocampus. Front Mol Neurosci 11:215. https://doi.org/10.3389/fnmol.2018.00215

Popov A, Brazhe A, Denisov P, Sutyagina O, Li L, Lazareva N, Verkhratsky A, Semyanov A (2021) Astrocyte dystrophy in ageing brain parallels impaired synaptic plasticity. Aging Cell 20(3):e13334. https://doi.org/10.1111/acel.13334

Potier B, Billard JM, Riviere S, Sinet PM, Denis I, Champeil-Potokar G, Grintal B, Jouvenceau A, Kollen M, Dutar P (2010) Reduction in glutamate uptake is associated with extrasynaptic NMDA and metabotropic glutamate receptor activation at the hippocampal CA1 synapse of aged rats. Aging Cell 9(5):722–735. https://doi.org/10.1111/j.1474-9726.2010.00593.x

Rivera AD, Chacon-De-La-Rocha I, Pieropan F, Papanikolau M, Azim K, Butt AM (2021) Keeping the ageing brain wired: a role for purine signalling in regulating cellular metabolism in oligodendrocyte progenitors. Pflugers Archiv 473(5):775–783. https://doi.org/10.1007/s00424-021-02544-z

Robillard KN, Lee KM, Chiu KB, MacLean AG (2016) Glial cell morphological and density changes through the lifespan of rhesus macaques. Brain Behav Immun 55:60–69. https://doi.org/10.1016/j.bbi.2016.01.006

Rodriguez JJ, Verkhratsky A (2011) Neurogenesis in Alzheimer's disease. J Anat 219(1):78–89. https://doi.org/10.1111/j.1469-7580.2011.01343.x

Rodriguez JJ, Jones VC, Tabuchi M, Allan SM, Knight EM, LaFerla FM, Oddo S, Verkhratsky A (2008) Impaired adult neurogenesis in the dentate gyrus of a triple transgenic mouse model of Alzheimer's disease. PLoS One 3(8):e2935. https://doi.org/10.1371/journal.pone.0002935

Rodriguez JJ, Terzieva S, Olabarria M, Lanza RG, Verkhratsky A (2013) Enriched environment and physical activity reverse astrogliodegeneration in the hippocampus of AD transgenic mice. Cell Death Dis 4:e678. https://doi.org/10.1038/cddis.2013.194

Rodriguez JJ, Yeh CY, Terzieva S, Olabarria M, Kulijewicz-Nawrot M, Verkhratsky A (2014) Complex and region-specific changes in astroglial markers in the aging brain. Neurobiol Aging 35(1):15–23. https://doi.org/10.1016/j.neurobiolaging.2013.07.002

Rose CR, Verkhratsky A (2016) Principles of sodium homeostasis and sodium signalling in astroglia. Glia 64(10):1611–1627. https://doi.org/10.1002/glia.22964

Salminen LE, Conturo TE, Laidlaw DH, Cabeen RP, Akbudak E, Lane EM, Heaps JM, Bolzenius JD, Baker LM, Cooley S, Scott S, Cagle LM, Phillips S, Paul RH (2016) Regional age

differences in gray matter diffusivity among healthy older adults. Brain Imaging Behav 10(1): 203–211. https://doi.org/10.1007/s11682-015-9383-7

Sampedro-Piquero P, De Bartolo P, Petrosini L, Zancada-Menendez C, Arias JL, Begega A (2014) Astrocytic plasticity as a possible mediator of the cognitive improvements after environmental enrichment in aged rats. Neurobiol Learn Mem 114:16–25. https://doi.org/10.1016/j.nlm.2014.04.002

Semyanov A, Verkhratsky A (2021) Astrocytic processes: from tripartite synapses to the active milieu. Trends Neurosci 44(10):781–792. https://doi.org/10.1016/j.tins.2021.07.006

Semyanov A, Verkhratsky A (2022) Inclusive brain: from neuronal doctrine to the active milieu. Function 3(2):zqab069. https://doi.org/10.1093/function/zqab069

Shapson-Coe A, Januszewski M, Berger DR, Pope A, Wu Y, Blakely T, Schalek RL, Li P, Wang S, Maitin-Shepard J, Karlupia N, Dorkenwald S, Sjostedt E, Leavitt L, Lee D, Bailey L, Fitzmaurice A, Kar R, Field B, Wu H, Wagner-Carena J, Aley D, Lau J, Lin Z, Wei D, Pfister H, Peleg A, Jain V, Lichtman JW (2021) A connectomic study of a petascale fragment ofhuman cerebral cortex. BioRxiv Preprint. https://doi.org/10.1101/2021.05.29.446289

Shih PY, Savtchenko LP, Kamasawa N, Dembitskaya Y, McHugh TJ, Rusakov DA, Shigemoto R, Semyanov A (2013) Retrograde synaptic signaling mediated by K+ efflux through postsynaptic NMDA receptors. Cell Rep 5(4):941–951. https://doi.org/10.1016/j.celrep.2013.10.026

Song S, Stern Y, Gu Y (2022) Modifiable lifestyle factors and cognitive reserve: a systematic review of current evidence. Ageing Res Rev 74:101551. https://doi.org/10.1016/j.arr.2021.101551

Stern Y (2009) Cognitive reserve. Neuropsychologia 47(10):2015–2028. https://doi.org/10.1016/j.neuropsychologia.2009.03.004

Streit WJ, Sammons NW, Kuhns AJ, Sparks DL (2004) Dystrophic microglia in the aging human brain. Glia 45(2):208–212. https://doi.org/10.1002/glia.10319

Streit WJ, Braak H, Xue QS, Bechmann I (2009) Dystrophic (senescent) rather than activated microglial cells are associated with tau pathology and likely precede neurodegeneration in Alzheimer's disease. Acta Neuropathol 118(4):475–485. https://doi.org/10.1007/s00401-009-0556-6

Streit WJ, Xue QS, Tischer J, Bechmann I (2014) Microglial pathology. Acta Neuropathol Commun 2:142. https://doi.org/10.1186/s40478-014-0142-6

Streit WJ, Khoshbouei H, Bechmann I (2020) Dystrophic microglia in late-onset Alzheimer's disease. Glia 68(4):845–854. https://doi.org/10.1002/glia.23782

Sweeney MD, Zhao Z, Montagne A, Nelson AR, Zlokovic BV (2019) Blood-brain barrier: from physiology to disease and back. Physiol Rev 99(1):21–78. https://doi.org/10.1152/physrev.00050.2017

Tarantini S, Tran CHT, Gordon GR, Ungvari Z, Csiszar A (2017) Impaired neurovascular coupling in aging and Alzheimer's disease: contribution of astrocyte dysfunction and endothelial impairment to cognitive decline. Exp Gerontol 94:52–58. https://doi.org/10.1016/j.exger.2016.11.004

Tyurikova O, Shih PY, Dembitskaya Y, Savtchenko LP, McHugh TJ, Rusakov DA, Semyanov A (2022) K^+ efflux through postsynaptic NMDA receptors suppresses local astrocytic glutamate uptake. Glia 70(5):961–974. https://doi.org/10.1002/glia.24150

Vanzuli I, Rivera A, Arellano JJ, Butt A (2015) Decreased regenerative capacity of oligodendrocyte progenitor cells (NG2-glia) in the ageing brain: a vicious cycle of synaptic dysfunction, myelin loss and neuronal disruption? Curr Alzheimer Res 13(4):413–418

Vaughan DW, Peters A (1974) Neuroglial cells in the cerebral cortex of rats from young adulthood to old age: an electron microscope study. J Neurocytol 3:405–429

Ventura R, Harris KM (1999) Three-dimensional relationships between hippocampal synapses and astrocytes. J Neurosci 19(16):6897–6906

Verkhratsky A, Butt AM (2013) Glial physiology and pathophysiology. Wiley-Blackwell, Chichester

Verkhratsky A, Nedergaard M (2016) The homeostatic astroglia emerges from evolutionary specialization of neural cells. Philos Trans R Soc Lond Ser B Biol Sci 371(1700):20150428. https://doi.org/10.1098/rstb.2015.0428

Verkhratsky A, Nedergaard M (2018) Physiology of astroglia. Physiol Rev 98(1):239–389. https://doi.org/10.1152/physrev.00042.2016

Verkhratsky A, Rose CR (2020) Na^+-dependent transporters: the backbone of astroglial homeostatic function. Cell Calcium 85:102136. https://doi.org/10.1016/j.ceca.2019.102136

Verkhratsky A, Orkand RK, Kettenmann H (1998) Glial calcium: homeostasis and signaling function. Physiol Rev 78(1):99–141. https://doi.org/10.1152/physrev.1998.78.1.99

Verkhratsky A, Marutle A, Rodriguez-Arellano JJ, Nordberg A (2015) Glial asthenia and functional paralysis: a new perspective on neurodegeneration and Alzheimer's disease. Neuroscientist 21(5):552–568. https://doi.org/10.1177/1073858414547132

Verkhratsky A, Ho MS, Parpura V (2019) Evolution of neuroglia. Adv Exp Med Biol 1175:15–44. https://doi.org/10.1007/978-981-13-9913-8_2

Verkhratsky A, Semyanov A, Zorec R (2020a) Physiology of astroglial excitability. Function 1(2): zqaa016. https://doi.org/10.1093/function/zqaa016

Verkhratsky A, Untiet V, Rose CR (2020b) Ionic signalling in astroglia beyond calcium. J Physiol 598(9):1655–1670. https://doi.org/10.1113/JP277478

Verkhratsky A, Augusto-Oliveira M, Pivoriunas A, Popov A, Brazhe A, Semyanov A (2021) Astroglial asthenia and loss of function, rather than reactivity, contribute to the ageing of the brain. Pflugers Archiv 473(5):753–774. https://doi.org/10.1007/s00424-020-02465-3

Westlund KN, Denney RM, Rose RM, Abell CW (1988) Localization of distinct monoamine oxidase A and monoamine oxidase B cell populations in human brainstem. Neuroscience 25(2):439–456. https://doi.org/10.1016/0306-4522(88)90250-3

Yang Z, Gong M, Jian T, Li J, Yang C, Ma Q, Deng P, Wang Y, Huang M, Wang H, Yang S, Chen X, Yu Z, Wang M, Chen C, Zhang K (2022) Engrafted glial progenitor cells yield long-term integration and sensory improvement in aged mice. Stem Cell Res Ther 13(1):285. https://doi.org/10.1186/s13287-022-02959-0

Zorec R, Parpura V, Verkhratsky A (2018) Preventing neurodegeneration by adrenergic astroglial excitation. FEBS J 285(19):3645–3656. https://doi.org/10.1111/febs.14456

Chapter 12
Hearing and Ageing

Mariapia Guerrieri, Roberta Di Mauro, Stefano Di Girolamo, and Arianna Di Stadio

Abstract Age-related hearing loss (ARHL), or presbycusis, occurs in most mammals, humans included, with a different age of onset and magnitude of loss. It is associated with two major symptoms: loss of sensitivity to sound, especially for high pitches, and a reduced ability to understand speech in background noise. This phenomenon involves both the peripheral structures of the inner ear and the central acoustic pathways. Several mechanisms have been identified as pro-ageing in the human cochlea. The main one is the oxidative stress. The inner ear physiological degeneration can be affected by both intrinsic conditions, such as genetic predisposition, and extrinsic ones, such as noise exposure. The magnitude of neuronal loss precedes and exceeds that of inner hair cell loss, which is also less important than the loss of outer hair cells. Patients with HL often develop atrophy of the temporal lobe (auditory cortex) and brain gliosis can contribute to the development of a central hearing loss. The presence of white matter hyperintensities (WMHs) on the MRI, which is radiologic representation of brain gliosis, can justify a central HL due to demyelination in the superior auditory pathways. Recently, the presence of WMHs has been correlated with the inability to correctly understand words in elderly with normal auditory thresholds.

Keywords Hearing loss · Ageing · Hair cells · Inner ear · Spiral ganglions · ROS · Mitochondria

M. Guerrieri · S. Di Girolamo
Otolaryngology Department, Tor Vergata University, Rome, Italy
e-mail: stefano.digirolamo@uniroma2.it

R. Di Mauro
ENT Department, MVZ Dr. Roser und Kollegen, Remchingen, Baden-Württemberg, Germany

A. Di Stadio (✉)
GF Ingrassia Department, University of Catania, Catania, Italy

Rome, Italy
e-mail: ariannadistadio@hotmail.com

J. R. Harris, V. I. Korolchuk (eds.), *Biochemistry and Cell Biology of Ageing: Part IV, Clinical Science*, Subcellular Biochemistry 103,
https://doi.org/10.1007/978-3-031-26576-1_12

Introduction

Age-related hearing loss (ARHL), or presbycusis, occurs in most mammals, humans included, with a different age of onset and magnitude of loss (Keithley 2020). It is associated with two major symptoms: loss of sensitivity to sound, especially for high pitches, and a reduced ability to understand speech in background noise (Frisina 2001). It is usually evaluated with pure-tone audiometry (PTA), which shows a peculiar descending pattern with the major threshold shift on the high frequencies.

This phenomenon involves both the peripheral structures of the inner ear and the central acoustic pathways, with a wide interplay of different factors that contribute to various degrees to the onset and evolution of this condition.

Among these factors, long-term noise exposure is certainly one of the most studied, as evidence shows it causes cell degeneration in the cochlea, namely on the outer hair cells, but it is not the only element that acts on the inner ear. Genetic conditions, ototoxic drugs, and systemic pathologies which usually afflict the elderlies play, in fact, an important role in the ageing ear, even if not yet thoroughly understood.

On the other hand, hearing ageing is also the result of central nervous system rearrangements that physiologically occur during the years, such as brain atrophy, vascular impairment, and neurodegeneration, that eventually affect also the auditory pathways.

Peripheral Hearing Loss

Every ageing process results from a combination of biological degeneration, extrinsic and intrinsic damage. It occurs in many mammals, yet it does not affect individuals uniformly, nor does it appear to be uniform even within a single person (Wang and Puel 2020).

ARHL starts in the high-frequency region of the auditory spectrum and proceeds towards the low-frequency region with age, causing a deterioration in threshold sensitivity, difficulty in speech discrimination, sound detection and localization, especially in noisy environments.

Some authors analysed human temporal tones and classified ARHL into three major forms:

1. Sensory presbycusis: characterized by a rough pure-tone threshold elevation at high frequencies, associated with the loss of hair cells at the basal end of the cochlea.
2. Strial presbycusis: it shows a flat or gently descending pattern at the pure-tone audiogram, correlated with atrophy of the stria vascularis.
3. Neural presbycusis: caused by the loss of cochlear neurons throughout the whole cochlea (Schuknecht and Gacek 1993).

ARHL is a progressive, irreversible, symmetrical, and bilateral neuro-sensory hearing loss resulting from the degeneration of the inner ear structures such as the stria vascularis, the hair cells, mostly the outer ones, and the spiral ganglion cells.

The magnitude of neuronal loss precedes and exceeds that of inner hair cell loss, which is also less important than the loss of outer hair cells. OHCs loss, in fact, affects both the basal and the apical end of the human cochlea, causing a loss of amplification that is more evident for high frequencies, thus leading to the typical descending pattern at the pure-tone audiogram (Keithley 2020).

Several mechanisms have been identified as pro-ageing in the human cochlea. The main one is the oxidative stress. The cochlea is in fact a high energy-demanding tissue, with a high number of mitochondria, which inevitably produce reactive oxygen species (ROS). Despite intrinsic protecting mechanisms, ROS-induced DNA damage involves both nuclear and mitochondrial DNA (mtDNA), which is responsible for cochlear cell senescence (Benkafadar et al. 2019). The incidence and frequency of mtDNA point mutations and deletions increase exponentially with age and the accumulation of these mutations and deletions may promote mitochondrial dysfunction and mitochondrial redox imbalance leading to cochlear cell ageing.

Other factors have shown to have a role in the physiological functioning of the cochlea. Insulin-like growth factor 1 (IGF-1), for example, has been proven as otoprotective, as it has been shown to maintain the cochlear ribbon synapse ex vivo and it modulates neuroinflammation. IGF-1 age-related downregulation is a pro-inflammatory condition that contributes to the pathogenesis of ARHL (Celaya et al. 2021).

The inner ear physiological degeneration can be affected by both intrinsic conditions, such as genetic predisposition, and extrinsic ones, such as noise exposure.

Genetics

Presbycusis has a clear familial association. There are likely plenty of genes and combinations of genes with subtle variants that make an individual more, or less, susceptible to ARHL, including genes that relate to susceptibility to acoustic trauma (Keithley 2020).

The search for specific genes linked to presbycusis is still a work in progress, complicated by the numerous interactions between genetic variants that lead to different phenotypes; nevertheless, polymorphisms in some monogenic deafness-causing genes, neurotransmitter-related genes, and genes involved in detoxification of oxidative stress and mitochondrial function have been clearly associated with ARHL (Wang and Puel 2020).

Mendelian genetics does not explain alone the genetic expression, as other epigenetic mechanisms are involved, such as DNA methylation and histone modification. Several studies have in fact demonstrated that aberrant DNA hypermethylation of specific genes, such as the promoter region of GJB2 protein,

solute carrier family 26 member 4 (SLC26A4), purinergic receptor P2X2, etc., is correlated with ARHL (Bouzid et al. 2018; Wu et al. 2014; Zhao et al. 2015).

Noise Exposure

It has been reported that in a cohort unscreened for noise exposure, ototoxic drug exposure, and otologic disease history, presbycusis develops earlier and to a greater extent than in a highly screened cohort (without history of significant noise exposure or diseases that affect the ear) (Guest et al. 2012).

Acoustic overexposure can cause synaptopathy between the auditory nerve fibres and the inner hair cells. This slowly leads to the loss of peripheral axons of bipolar sensory neurons, and subsequently to the degeneration of the cell body in the spiral ganglion. This partial deafferentation of the hair cells only affects the threshold when it exceeds the 80% of acoustic fibres involved. The loss of acoustic nerve fibres is responsible for the common poor speech discrimination that can be seen in ARHL (Wu et al. 2021). Noise damage is showed as a peculiar "notched" audiogram with the worst threshold shift ad 4 kHz (McBride 2001).

As the years of exposure pass, the 4 kHz damage spreads towards the higher frequencies, as the basal end of the cochlea is more vulnerable to mechanical insults. With ageing, though, the noise-induced hearing loss (NIHL) converges with the audiometric pattern and cochlear damage of the normal ARHL, suggesting that noise-induced damage occurs on the same inner ear structures that are vulnerable to ageing, namely OHCs located at the basal end of the cochlea. Perhaps, much of "normal" cochlear ageing in humans is the result of a slow accumulation of ear abuse (Wu et al. 2021).

Central Hearing Loss

Patients with HL often develop atrophy of the temporal lobe (auditory cortex) and brain gliosis, can contribute to the development of a central hearing loss (Gouw et al. 2008).

Brain atrophy and brain gliosis have common risk factors which include atherosclerosis, cardiovascular disease, smoking, and diabetes (Chang et al. 2016; Livingston et al. 2017; Lourenco et al. 2018; Uchida et al. 2010; Wolters et al. 2018). These conditions are associated, by a variety of mechanisms such as the reduction of the brain vascular flow at the base of brain, with brain atrophy (Lin et al. 2014; Qian et al. 2017) or the gliosis observed in the elderly (Di Stadio et al. 2020).

Magnetic resonance imaging (MRI) studies have shown that brain atrophy is focal, located in the temporal lobe in case of HL (Lin et al. 2014; Qian et al. 2017). The progressive extension of the atrophy from the temporal lobe to other areas of the brain can affect other areas, as for example the one of the memory (Di Stadio et al.

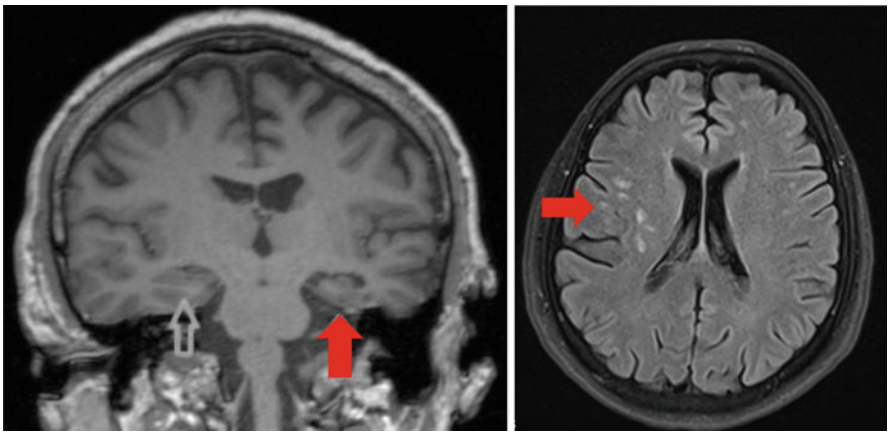


Fig. 12.1 *Left*: The red arrow shows the atrophy of right temporal lobe of the brain, compared with the normal left side (grey arrow). *Right*: the red arrow shows several white matter hyperintensities in the brain of a 60-year-old patient affected by severe ARHL

2020); an alteration in the signal transmission between the neurons in the area of the memory can result in the loss of the ability to identify words (recalling the word even without its entire understanding) that interfere with speech perception test results (Di Stadio et al. 2020).

The presence of white matter hyperintensities (WMHs) on the MRI, which is a radiologic representation of brain gliosis, can cause a central HL due to demyelination in the superior auditory pathways (Fusconi et al. 2019). Recently, the presence of WMHs has been correlated with the inability to correctly understand words in elderly with normal auditory thresholds (Di Stadio et al. 2020). The authors identified a negative correlation between the increasing number of WMHs and the worsening of speech understanding, suggesting that the spread of the lesions could cause difficulty in correctly identifying words. WMHs, depending on the site in which they are located, might be associated with both conditions; in the auditory cortex they can contribute to the speech discrimination difficulties, while in temporal area, they may contribute to the inability of correctly perceiving the sounds (Di Stadio et al. 2020; Gouw et al. 2008; Lin et al. 2014).

A possible explanation regarding the correlation between WMHs and hearing symptoms could be found in the study of Braffman and colleagues. The authors conducted a post-mortem study on 23 patients over 60 years of age and found a correlation between WMHs identified by MRI and the anatomopathological findings of the same patients (Braffman et al. 1988). According to these results, we speculate that gliosis altering the synaptic transmission (Brun et al. 1995) could be responsible for the central HL based on the site involved by the gliotic process (Bilello et al. 2015) (Fig. 12.1).

The presence of the apolipoprotein e4 (APOE4) allele (Kurniawan et al. 2012) has been correlated with a higher susceptibility to develop severe form of age-related HL.

The alteration of mitochondrial metabolism caused by Reactive Oxygen Species (ROS) induces mtDNA mutations or deletions (Yamasoba et al. 2013), reducing the production of ATP that is necessary for a correct function of the inner ear cells (Di Stadio et al. 2018a). These two concepts are supported by recent discoveries (Fetoni et al. 2019) which evidenced an increased level of ROS in patients suffering from HL; the researchers identified that patients with specific apolipoprotein E (APOE) genotype were the ones that more commonly had an increased level of circulating ROS (Guo et al. 2005). The excess of ROS, other than determining mitochondrial dysmetabolism, stimulates the activation of pro-inflammatory microglia (M1) in noise-induced HL (Fuentes-Santamaría et al. 2017). M1 microglia, once activated, produce ROS that consequently fuels additional M1 activation, (Di Stadio and Angelini 2019) enabling a perpetual inflammatory cycle. Moreover, oxidative stress can cause a direct damage to the inner ear destroying the inner ear cells and the spiral ganglions (Ralli et al. 2017). Recently, Di Stadio et al. (2018b) showed that microglia cells are active in the relapsing phase of neurodegenerative diseases and could be responsible of the HL observed in the patients affected by Multiple Sclerosis, maybe attacking the peripheral or the central auditory pathways (Di Mauro et al. 2019; Di Stadio and Ralli 2018).

Microglia has a relevant role in age-related hearing loss as shown by Tremblay and colleagues; in fact, with ageing processes these cells present an irregular distribution, a modified morphology and accumulate phagocytic inclusion and often displaying ultrastructural features of synaptic elements. Furthermore, these workers identified a series of myelinations defects, concluding that the age-related alteration of glial cells in sensory cortical areas can be accelerated by activity-driven central mechanisms that result from an age-related HL (Tremblay et al. 2012).

Another biomolecular explanation of central HL might be the action of NOD-like receptor protein 3 (NLRP3) inflammasomes that are responsible of ROS accumulation into the cells. Animal studies have shown that NLRP3 increases in the inner ear of ageing mice; NLRP3, as a sensor protein of ROS, might contribute to inflammasome assembly and subsequent inflammation in the cochleae and might play a role in age-related HL (presbycusis) (Nakanishi et al. 2018). These animal findings are supported by human evidence. In fact, Nakanishi et al. (2018) analysed a sample of patients with DFNA 34 HL identified in the missense substitution in the NLRP3 gene as responsible of progressive HL. The genetic alteration determined production of a modified protein, which made NLPR3-inflammasome responsible of the auto-inflammatory cascade, thus destroying the inner ear cells (Jin et al. 2019; Nakanishi et al. 2018) (Fig. 12.2).

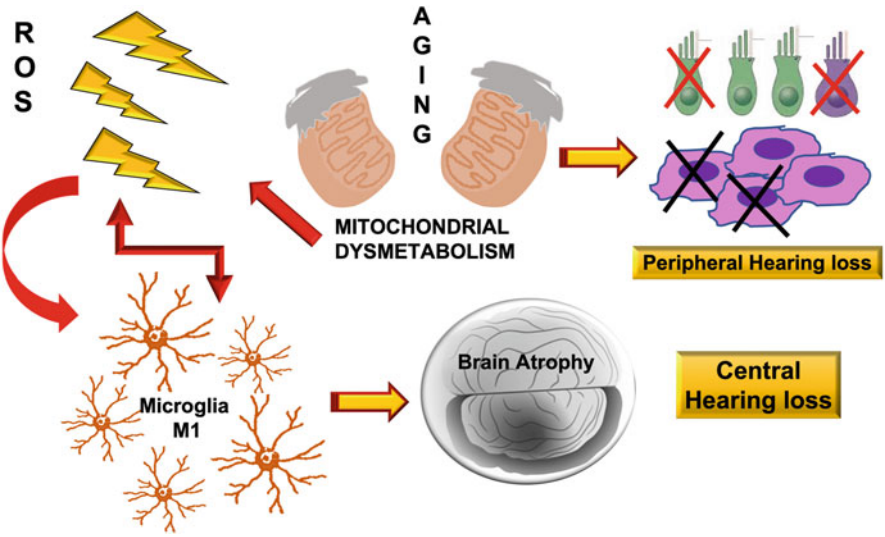


Fig. 12.2 The vignette summarizes the metabolic alterations of mitochondria and how these alterations cause both peripheral and central hearing loss. Regarding peripheral hearing loss, both spiral ganglion loss (pink egg fried images) and hair cell death (green→outer; violet→inner) can cause ARHL

The Effect of Hearing Rehabilitation on Brain and Cellular Functions

Functional MRI (fMRI) studies have shown that both congenital deafness (pre-lingual) (Wolak et al. 2019) and acquired sensorineural hearing loss (SNHL) (post-lingual) (Ghiselli et al. 2020) can alter the normal brain connectivity independently of age. Patients with acquired SNHL showed better connectivity between different areas of brain (cortex, parietal, and hippocampus) compared to the subjects with pre-lingual deafness; however, the use of hearing aids (HAs) was extremely useful to restore the normal brain connectivity (Patel et al. 2007; Pereira-Jorge et al. 2018). In particular, comparing the subjects' brain area before and after use of a HA, the researchers noted that cortical thickness increased in multimodal integration regions, particularly the very caudal end of the superior temporal sulcus, the angular gyrus, and the inferior parietal gyrus/superior temporal gyrus/insula (Pereira-Jorge et al. 2018).

HAs can also have a biomolecular effect, as the use of HA stimulates the astrocyte function and the activation of M2 microglia (Rosskothen-Kuhl et al. 2018). Researchers studied a sample of animals with deafness comparing the brain of the ones treated with cochlear implant (CI) with the ones without treatment and evaluated the effect on astrocytes and microglia. The hearing recovery may activate the anti-inflammatory microglia (M2) in the temporal lobe and, thanks to the capacity of

these cells of repairing gliosis, the brain connection might be restored and functions recovered (Fuentes-Santamaría et al. 2012).

Histopathology of the Ageing Ear

According to the classification made by Schuknecht and Gacek (1993), sensory cell losses are the least important type of loss in the aged ear (Fig. 12.3 right side); neural losses are constant and predictable expressions of ageing (Fig. 12.4) and atrophy of

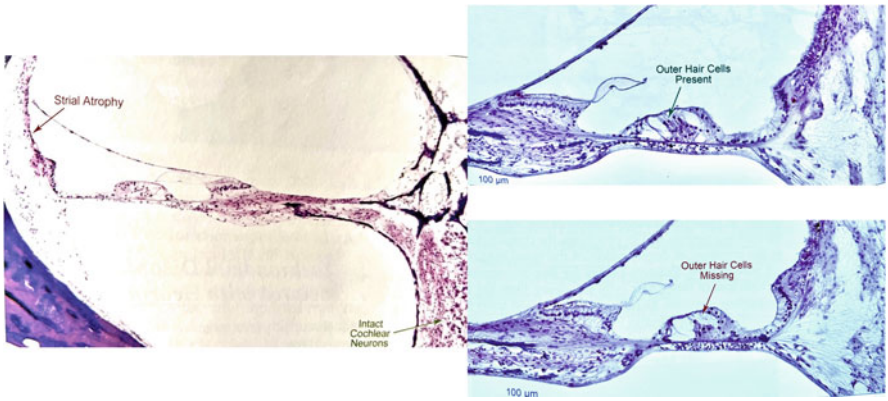


Fig. 12.3 Cochlea affected by ageing process. Vascular stria atrophy (left) and loss of the outer cells in the organ of Corti (right) (normal on the top RHS)

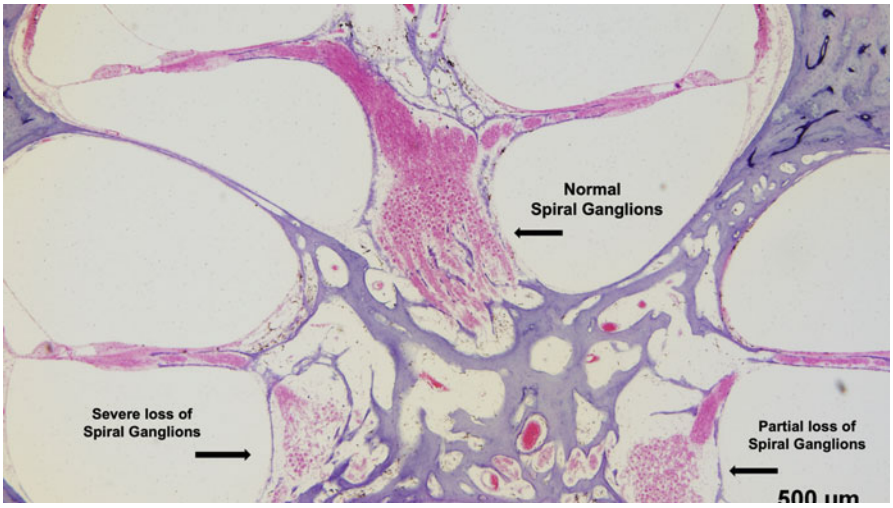


Fig. 12.4 Cochlea affected by ageing process. The spiral ganglions are preserved in middle turn of the cochlea and lost in the basal portion

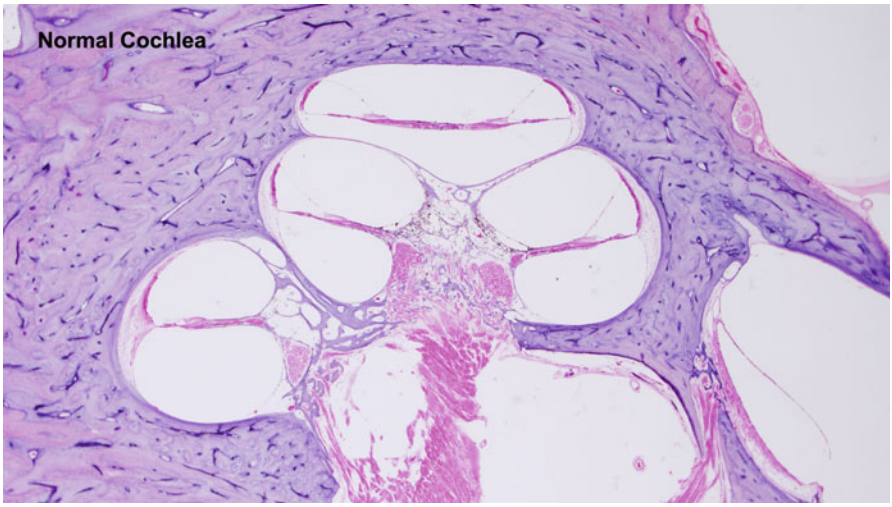


Fig. 12.5 Normal anatomy of human cochlea

the stria vascularis (Fig. 12.3 left side) is the predominant lesion of the ageing ear. On the contrary, all structures are normally represented in the young ear (Fig. 12.5).

Conclusions

Ageing can cause both peripheral and central hearing loss. The alteration of cellular metabolisms caused by age progression and some risk factors such as hypercholesterol, hypertension, and cardiac diseases can cause the death of the hair cells and spiral ganglions in the inner ear, degeneration of the cochlear nerves, and demyelination in the superior auditory pathways as temporal lobe and cortex. The use of hearing aids can invert the process thanks to their effect at a cellular level.

References

Benkafadar N, François F, Affortit C, Casas F, Ceccato J-C, Menardo J, Venail F, Malfroy-Camine B, Puel J-L, Wang J (2019) ROS-induced activation of DNA damage responses drives senescence-like state in postmitotic cochlear cells: implication for hearing preservation. Mol Neurobiol 56:5950–5969. https://doi.org/10.1007/s12035-019-1493-6

Bilello M, Doshi J, Nabavizadeh SA, Toledo JB, Erus G, Xie SX, Trojanowski JQ, Han X, Davatzikos C (2015) Correlating cognitive decline with white matter lesion and brain atrophy magnetic resonance imaging measurements in Alzheimer's disease. J Alzheimers Dis 48:987–994. https://doi.org/10.3233/JAD-150400

Bouzid A, Smeti I, Dhouib L, Roche M, Achour I, Khalfallah A, Gibriel AA, Charfeddine I, Ayadi H, Lachuer J, Ghorbel A, Petit C, Masmoudi S (2018) Down-expression of P2RX2,

KCNQ5, ERBB3 and SOCS3 through DNA hypermethylation in elderly women with presbycusis. Biomarkers 23:347–356. https://doi.org/10.1080/1354750X.2018.1427795

Braffman BH, Zimmerman RA, Trojanowski JQ, Gonatas NK, Hickey WF, Schlaepfer WW (1988) Brain MR: pathologic correlation with gross and histopathology. 2. Hyperintense white-matter foci in the elderly. AJR Am J Roentgenol 151:559–566. https://doi.org/10.2214/ajr.151.3.559

Brun A, Liu X, Erikson C (1995) Synapse loss and gliosis in the molecular layer of the cerebral cortex in Alzheimer's disease and in frontal lobe degeneration. Neurodegeneration 4:171–177. https://doi.org/10.1006/neur.1995.0021

Celaya AM, Rodríguez-de la Rosa L, Bermúdez-Muñoz JM, Zubeldia JM, Romá-Mateo C, Avendaño C, Pallardó FV, Varela-Nieto I (2021) IGF-1 haploinsufficiency causes age-related chronic cochlear inflammation and increases noise-induced hearing loss. Cell 10:1686. https://doi.org/10.3390/cells10071686

Chang J, Ryou N, Jun HJ, Hwang SY, Song J-J, Chae SW (2016) Effect of cigarette smoking and passive smoking on hearing impairment: data from a population–based study. PLoS One 11: e0146608. https://doi.org/10.1371/journal.pone.0146608

Di Mauro R, Di Girolamo S, Ralli M, de Vincentiis M, Mercuri N, Albanese M (2019) Subclinical cochlear dysfunction in newly diagnosed relapsing-remitting multiple sclerosis. Mult Scler Relat Disord 33:55–60. https://doi.org/10.1016/j.msard.2019.05.020

Di Stadio A, Angelini C (2019) Microglia polarization by mitochondrial metabolism modulation: a therapeutic opportunity in neurodegenerative diseases. Mitochondrion 46:334–336. https://doi.org/10.1016/j.mito.2018.09.003

Di Stadio A, Dipietro L, Ralli M, Meneghello F, Minni A, Greco A, Stabile MR, Bernitsas E (2018a) Sudden hearing loss as an early detector of multiple sclerosis: a systematic review. Eur Rev Med Pharmacol Sci 22:4611–4624. https://doi.org/10.26355/eurrev_201807_15520

Di Stadio A, Messineo D, Ralli M, Roccamatisi D, Musacchio A, Ricci G, Greco A (2020) The impact of white matter hyperintensities on speech perception. Neurol Sci 41:1891–1898. https://doi.org/10.1007/s10072-020-04295-8

Di Stadio A, Pegoraro V, Giaretta L, Dipietro L, Marozzo R, Angelini C (2018b) Hearing impairment in MELAS: new prospective in clinical use of microRNA, a systematic review. Orphanet J Rare Dis 13:35. https://doi.org/10.1186/s13023-018-0770-1

Di Stadio A, Ralli M (2018) Inner ear involvement in multiple sclerosis: an underestimated condition? Mult Scler 24:1264–1265. https://doi.org/10.1177/1352458517750010

Fetoni AR, Paciello F, Rolesi R, Paludetti G, Troiani D (2019) Targeting dysregulation of redox homeostasis in noise-induced hearing loss: oxidative stress and ROS signaling. Free Radic Biol Med 135:46–59. https://doi.org/10.1016/j.freeradbiomed.2019.02.022

Frisina RD (2001) 37 – Anatomical and neurochemical bases of presbycusis. In: Hof PR, Mobbs CV (eds) Functional neurobiology of ageing. Academic Press, San Diego, pp 531–547. https://doi.org/10.1016/B978-012351830-9/50039-1

Fuentes-Santamaría V, Alvarado JC, Juiz JM (2012) Long-term interaction between microglial cells and cochlear nucleus neurons after bilateral cochlear ablation. J Comp Neurol 520:2974–2990. https://doi.org/10.1002/cne.23088

Fuentes-Santamaría V, Alvarado JC, Melgar-Rojas P, Gabaldón-Ull MC, Miller JM, Juiz JM (2017) The role of glia in the peripheral and central auditory system following noise overexposure: contribution of TNF-α and IL-1β to the pathogenesis of hearing loss. Front Neuroanat 11: 9. https://doi.org/10.3389/fnana.2017.00009

Fusconi M, Attanasio G, Capitani F, Di Porto E, Diacinti D, Musy I, Ralli M, Ralli G, Greco A, de Vincentiis M, Colonnese C (2019) Correction to: is there a relation between sudden sensorineural hearing loss and white matter lesions? Eur Arch Otorhinolaryngol 276:3521. https://doi.org/10.1007/s00405-019-05622-2

Ghiselli S, Gheller F, Trevisi P, Favaro E, Martini A, Ermani M (2020) Restoration of auditory network after cochlear implant in prelingual deafness: a P300 study using LORETA. Acta Otorhinolaryngol Ital 40:64–71. https://doi.org/10.14639/0392-100X-2316

Gouw AA, Seewann A, Vrenken H, van der Flier WM, Rozemuller JM, Barkhof F, Scheltens P, Geurts JJG (2008) Heterogeneity of white matter hyperintensities in Alzheimer's disease: post-mortem quantitative MRI and neuropathology. Brain 131:3286–3298. https://doi.org/10.1093/brain/awn265

Guest M, Boggess M, Attia J, SHOAMP study team and Scientific Advisory Committee (2012) Relative risk of elevated hearing threshold compared to ISO1999 normative populations for Royal Australian Air Force male personnel. Hear Res 285:65–76. https://doi.org/10.1016/j.heares.2012.01.007

Guo Y, Zhang C, Du X, Nair U, Yoo T-J (2005) Morphological and functional alterations of the cochlea in apolipoprotein E gene deficient mice. Hear Res 208:54–67. https://doi.org/10.1016/j.heares.2005.05.010

Jin X, Liu M-Y, Zhang D-F, Zhong X, Du K, Qian P, Yao W-F, Gao H, Wei M-J (2019) Baicalin mitigates cognitive impairment and protects neurons from microglia-mediated neuroinflammation via suppressing NLRP3 inflammasomes and TLR4/NF-κB signaling pathway. CNS Neurosci Ther 25:575–590. https://doi.org/10.1111/cns.13086

Keithley EM (2020) Pathology and mechanisms of cochlear ageing. J Neurosci Res 98:1674–1684. https://doi.org/10.1002/jnr.24439

Kurniawan C, Westendorp RGJ, de Craen AJM, Gussekloo J, de Laat J, van Exel E (2012) Gene dose of apolipoprotein E and age-related hearing loss. Neurobiol Ageing 33:2230.e7–2230.e12. https://doi.org/10.1016/j.neurobiolageing.2012.04.001

Lin FR, Ferrucci L, An Y, Goh JO, Doshi J, Metter EJ, Davatzikos C, Kraut MA, Resnick SM (2014) Association of hearing impairment with brain volume changes in older adults. NeuroImage 90:84–92. https://doi.org/10.1016/j.neuroimage.2013.12.059

Livingston G, Sommerlad A, Orgeta V, Costafreda SG, Huntley J, Ames D, Ballard C, Banerjee S, Burns A, Cohen-Mansfield J, Cooper C, Fox N, Gitlin LN, Howard R, Kales HC, Larson EB, Ritchie K, Rockwood K, Sampson EL, Samus Q, Schneider LS, Selbæk G, Teri L, Mukadam N (2017) Dementia prevention, intervention, and care. Lancet 390:2673–2734. https://doi.org/10.1016/S0140-6736(17)31363-6

Lourenco J, Serrano A, Santos-Silva A, Gomes M, Afonso C, Freitas P, Paul C, Costa E (2018) Cardiovascular risk factors are correlated with low cognitive function among older adults across Europe based on the SHARE database. Ageing Dis 9:90–101. https://doi.org/10.14336/AD.2017.0128

McBride DI (2001) Audiometric notch as a sign of noise induced hearing loss. Occup Environ Med 58:46–51. https://doi.org/10.1136/oem.58.1.46

Nakanishi H, Kawashima Y, Kurima K, Muskett JA, Kim HJ, Brewer CC, Griffith AJ (2018) Gradual symmetric progression of DFNA34 hearing loss caused by an NLRP3 mutation and cochlear autoinflammation. Otol Neurotol 39:e181–e185. https://doi.org/10.1097/MAO.0000000000001715

Patel AM, Cahill LD, Ret J, Schmithorst V, Choo D, Holland S (2007) Functional magnetic resonance imageing of hearing-impaired children under sedation before cochlear implantation. Arch Otolaryngol Head Neck Surg 133:677–683. https://doi.org/10.1001/archotol.133.7.677

Pereira-Jorge MR, Andrade KC, Palhano-Fontes FX, Diniz PRB, Sturzbecher M, Santos AC, Araujo DB (2018) Anatomical and functional MRI changes after one year of auditory rehabilitation with hearing aids. Neural Plast 2018:9303674. https://doi.org/10.1155/2018/9303674

Qian ZJ, Chang PD, Moonis G, Lalwani AK (2017) A novel method of quantifying brain atrophy associated with age-related hearing loss. Neuroimage Clin 16:205–209. https://doi.org/10.1016/j.nicl.2017.07.021

Ralli M, Rolesi R, Anzivino R, Turchetta R, Fetoni AR (2017) Acquired sensorineural hearing loss in children: current research and therapeutic perspectives. Acta Otorhinolaryngol Ital 37:500–508. https://doi.org/10.14639/0392-100X-1574

Rosskothen-Kuhl N, Hildebrandt H, Birkenhäger R, Illing R-B (2018) Astrocyte hypertrophy and microglia activation in the rat auditory midbrain is induced by electrical intracochlear stimulation. Front Cell Neurosci 12:43

Schuknecht HF, Gacek MR (1993) Cochlear pathology in presbycusis. Ann Otol Rhinol Laryngol 102:1–16. https://doi.org/10.1177/00034894931020S101

Tremblay M-È, Zettel ML, Ison JR, Allen PD, Majewska AK (2012) Effects of ageing and sensory loss on glial cells in mouse visual and auditory cortices. Glia 60:541–558. https://doi.org/10.1002/glia.22287

Uchida Y, Sugiura S, Ando F, Nakashima T, Shimokata H (2010) Diabetes reduces auditory sensitivity in middle-aged listeners more than in elderly listeners: a population-based study of age-related hearing loss. Med Sci Monit 16:PH63-68

Wang J, Puel J-L (2020) Presbycusis: an update on cochlear mechanisms and therapies. J Clin Med 9:218. https://doi.org/10.3390/jcm9010218

Wolak T, Cieśla K, Pluta A, Włodarczyk E, Biswal B, Skarżyński H (2019) Altered functional connectivity in patients with sloping sensorineural hearing loss. Front Hum Neurosci 13:284

Wolters FJ, Segufa RA, Darweesh SKL, Bos D, Ikram MA, Sabayan B, Hofman A, Sedaghat S (2018) Coronary heart disease, heart failure, and the risk of dementia: a systematic review and meta-analysis. Alzheimers Dement 14:1493–1504. https://doi.org/10.1016/j.jalz.2018.01.007

Wu P-Z, O'Malley J, Gruttola V, Liberman M (2021) Primary neural degeneration in noise-exposed human cochleas: correlations with outer hair cell loss and word-discrimination scores. J Neurosci 41:4439. https://doi.org/10.1523/JNEUROSCI.3238-20.2021

Wu X, Wang Y, Sun Y, Chen S, Zhang S, Shen L, Huang X, Lin X, Kong W (2014) Reduced expression of Connexin26 and its DNA promoter hypermethylation in the inner ear of mimetic ageing rats induced by d-galactose. Biochem Biophys Res Commun 452:340–346. https://doi.org/10.1016/j.bbrc.2014.08.063

Yamasoba T, Lin FR, Someya S, Kashio A, Sakamoto T, Kondo K (2013) Current concepts in age-related hearing loss: epidemiology and mechanistic pathways. Hear Res 303:30–38. https://doi.org/10.1016/j.heares.2013.01.021

Zhao W, Liu J, Xu J, Wang H (2015) Duplicated posterior belly of digastric muscle and absence of omohyoid muscle: a case report and review of literature. Surg Radiol Anat 37:547–550. https://doi.org/10.1007/s00276-014-1374-7

Chapter 13
Melatonin and Aging

Stephen C. Bondy

Abstract The health problems associated with the aging process are becoming increasingly widespread due to the increase in mean life expectancy taking place globally. While decline of many organ functions is an unavoidable concomitant of senescence, these can be delayed or moderated by a range of factors. Among these are dietary changes and weight control, taking sufficient exercise, and the utilization of various micronutrients. The utility of incurring appropriate changes in lifestyle is generally not confined to a single organ system but has a broadly positive systemic effect.

Among one of the most potent means of slowing down age-related changes is the use of melatonin, a widely distributed biological indole. While melatonin is well known as a treatment for insomnia, it has a wide range of beneficial qualities many of which are relevant. This overview describes how several of the properties of melatonin are especially relevant to many of the changes associated with senescence. Changes in functioning of the immune system are particularly marked in the aged, combining diminishing effectiveness with increasing ineffective and harmful activity. Melatonin treatment appears able to moderate and partially reverse this detrimental drift toward immune incompetence.

Keywords Melatonin · Cancer · Cardiac disease · Cerebrovascular disease · Diabetes · Frailty · Immune competence · Receptor activation · SARS-CoV-2

S. C. Bondy (✉)
Center for Occupational and Environmental Health, University of California, Irvine, CA, USA
e-mail: scbondy@uci.edu

J. R. Harris, V. I. Korolchuk (eds.), *Biochemistry and Cell Biology of Ageing: Part IV, Clinical Science*, Subcellular Biochemistry 103,
https://doi.org/10.1007/978-3-031-26576-1_13

Introduction

Melatonin is synthesized from tryptophan and is widely distributed among both plant, animal and bacterial phyla. This breadth of dispersal suggests an ancient origin and its current retention in its original form suggests that it must play one or more important biological roles.

From the earliest times to the present, it seems as though melatonin has acted continuously in a distinct pattern:

1. Melatonin is a light-sensitive factor. It may have originated as preventing cell damage due to sunlight (Roopin et al. 2013). However, in mammals it fluxes in a circadian manner, release being depressed by light and elevated during darkness. This may have its origins in the earliest role of melatonin in protecting organisms from the oxidative stress during hours of sunlight, and in consequence, being degraded at that time (Bonmati-Carrion and Tomas-Loba 2021).
2. In the Vertebrata, a subphylum of Chordata, melatonin is able to act on a large-range metabolic events in a broadly protective manner. In plants and unicellular organisms, melatonin has been reported as preventing cell damage caused by a host of stress factors including drought, excessive salinity, oxidative damage, extreme heat or cold, overcrowding, predators, and the presence of toxic heavy metals (Park et al. 2020; Sun et al. 2020; Schwarzenberger et al. 2014).
3. While melatonin may have originated as a direct antioxidant, its low intracellular concentration and multitude of effects imply an ability to influence gene expression. This is evidenced by the existence of several receptor species whose activity is modulated by melatonin. There is good evidence for a role for melatonin in many signal transduction pathways, despite limited knowledge of the exact mechanism involved.

Given the major extension of longevity of mankind during the last century, and the many deleterious consequences of aging, it is critical to find means of reducing the effects of both harmful exogenous factors to which the aged are exposed for a longer duration. In addition, it would be useful to sustain the efficiency of intrinsic metabolic events which are inevitably in decline.

Unsurprisingly, senescence is characterized by increased incidence of most of the key causes of mortality. Aging is unquestionably the greatest risk factor for incurring many chronic diseases. In those over 65 years of age, the five leading causes of death are cancer, Alzheimer's disease, diabetes, heart disease, and cerebrovascular disease (stroke) (Diaconu et al. 2020). Most of these are to some degree underlain by slow developing changes which gradually convert to a visibly pathological state over an extended period. The effect of melatonin on each of these is discussed. While melatonin may address specific deficits associated with each disease, some of its utility may reside in its ability to slow the overall non-pathological aging processes. This alone would serve to reduce the incidence of these disorders all of which are increased with age.

Melatonin and Diseases Where Aging Is a Potential Comorbidity

Cancer

Age is one of the main risk factors of incurring cancer and many of the changes associated with aging can account for this. Decline of competent immune function is likely a major contributor. The connection between cancer and the immune system is binary. An efficacious immune response can lead to invasion of and destruction of abnormal cells even prior to completion of their progression toward a transformed phenotype (Cham and Chase 2012). This preventive mechanism is likely to occur daily in normal individuals. On the other hand, persistent unresolved inflammatory activity typifies both the pre-cancerous and cancer condition. Superimposed on this is the close relation between inflammatory activity and the presence of oxidative stress. Both of these can create an environment that facilitates the trajectory of initiation and promotion of carcinogenesis (Murata 2018). The continued presence of inflammatory cytokines such as TNF-α, IL-6, TGF-β, and IL-10 therefore advances the progression of cancer (Landskron et al. 2014). However, reactive macrophages can also have antitumor effects by promotion of apoptosis. They can exist in either a pro-inflammatory M1 state, enhancing immune responses and thereby suppressing cancer, or in an immunosuppressive M2 state which protects cancer cells from immune attack (den Breems and Eftimie 2016). The classical M2/M1 distinction is overly simplistic as M1-induced inflammation can also promote tumor metastasis (Cho et al. 2018) and M2 macrophages are also present in tumors (Na et al. 2018). Despite the ability of melatonin to promote the conversion of M2 to M1 macrophages and thereby reduce stress-induced inflammation consequent to injury, melatonin has never been reported as carcinogenic (Yi and Kim 2017; Zhang et al. 2019).

We have reported treatment of very old mice (over 20 months of age at the start of treatment) with 40 ppm dietary melatonin for 3 months, to lead to a 60% reduction of incidence of tumors (Sharman et al. 2011). In addition, the mean size of any size of tumors in aged mice treated in this manner was considerably less than in tumors found in control mice. Finally, the total death rate of mice during the course of the study was lower in mice receiving melatonin. At the end of the study, when mice were 26 months old there was 50% reduction of mortality in treated mice from 18% in untreated, to 9%.

There are several reports of a reduced incidence of tumors and their slower progression, in animals subjected to carcinogens or tumor cells when receiving melatonin (Samec et al. 2021). These findings are likely to be relevant to humans, as age-related changes in expression of specific genes have clear parallels between humans and mice (Sharman et al. 2004). The addition of melatonin to clinical therapeutic protocols appears to enhance the effectiveness of chemotherapeutic agents and radiation procedures and diminishes the severity of undesirable side effects (González et al. 2021; Sezen et al. 2021). The large number of actions of

melatonin that may potentially function in retardation of tumor initiation and development have recently been summarized (Bonmati-Carrion and Tomas-Loba 2021). These include regulation of clock genes, inhibition of angiogenesis, activation of apoptotic events, and protection of cell–cell anchoring proteins, thus retarding metastasis.

This is superimposed upon the upsurge of inflammation found in the brain with normal aging. Such exacerbation of neuroinflammation which is found in both human AD and animal models of this disease (Liu et al. 2015; Hu et al. 2019) may reflect impaired glial function. The properties of melatonin in enabling the modulation of immune function imply that it may be useful in the treatment of AD. Melatonin has also been reported to enhance α-secretase activity (which facilitates non-pathogenic processing of amyloid precursor protein), APP while inhibiting the transcription of β- and γ-secretases (which further the conversion of APP into a more pathogenic form), amyloid-β, capable of forming amyloid plaques (Li et al. 2020; Bondy and Campbell 2018). Overall, melatonin usage in a clinical setting increases the likelihood of substantial reductions in cancer incidence at little cost and with minimal risk of adverse side effects.

The restraining influence of melatonin on cancer progression may be mediated by increased activity of the microRNA, miR-152-3p (Marques et al. 2018), elevation of sirtuin-1, SIRT1, or modulation of various signaling pathways (Hardeland 2019). It has been claimed that "melatonin represents the only molecule existing in nature that is potentially capable of suppressing the overall phases of cancer development, progression, invasion, and neoangiogenesis" (Giudice et al. 2018).

Alzheimer's Disease (AD)

Alzheimer's disease (AD) is broadly prevalent among the elderly and is a major cause of disability and eventual death. AD is associated with excessive immune activity within the brain, which seems to be caused by the deviant processing of amyloid precursor protein. Both the innate and adaptive immune system contribute to this extended and unproductive neuroinflammation (Lutshumba et al. 2021). This leads to formation of abnormal amyloid peptides which can coalesce and form the senile plaques characteristic of AD pathology (Forloni and Balducci 2018). Interestingly, people suffering from systemic inflammation or various autoimmune disorders are more prone to develop dementia (Atzeni et al. 2017). The levels of several markers of inflammation are elevated in the brains of Alzheimer patients (Calsolaro and Edison 2016).

In a genetic mouse model of AD, melatonin both restored functioning of the mitochondrial electron transport chain while enhancing mitophagy of aberrant mitochondria (Chen et al. 2021). Nevertheless, in a clinical setting the use of melatonin or its analogs has not shown really pronounced effects on AD progression. Positive effects are largely confined to improved sleep patterns resulting in less delirium which are often disrupted in AD. In some studies, this has resulted in

reduced delirium (Hatta et al. 2014) and mental status (Sumsuzzman et al. 2021), but these findings remain controversial. Regrettably, a large number of phytochemicals that have also been found beneficial in cell culture of animal models of many neurological disorders have never been firmly established as of value in human disease (Soo et al. 2020).

Poor sleep quality has long been associated with both pre-clinical and clinical AD. The intensity of sleeping pattern disruption in AD has been related to the degree of the amyloid burden, and to the extent of cognitive and memory deficits. The ability of melatonin to restore a more regular sleeping profile may account for its positive effects on both insomnia and cardiovascular disease (Zisapel 2018).

Diabetes

The risk for Type-2 diabetes (T2D) is associated with a non-coding single nucleotide polymorphism (SNP) of the gene MTNR1B, encoding melatonin receptor 2 (MT2) (Bonnefond and Froguel 2017). However, since loss of function of other MT2 receptor variants is associated with elevated risk for T2D, the role of melatonin signaling in insulin secretion remains obscure (Karamitri and Jockers 2019). Reconciliation of conflicting results has been suggested to involve the relative timing between peak melatonin concentrations and a glycemic challenge (Garaulet et al. 2020). The disruption of circadian cycles found with chronic obesity may increase the risk of developing diabetes (Otamas et al. 2020) and the restoration of normal circadian cycling may be a means by which melatonin can improve diabetes.

Excessive weight gain is associated with increased mortality in the elderly, afflicted with several disease states in addition to diabetes (hypertension, coronary disease, several types of cancer) (Cheng et al. 2015). However, it is discussed in this section as metabolic syndrome has a strong relation to diabetes. Melatonin supplementation has been found to moderate weight gain in both rodent models and clinical obesity trials (Genario et al. 2021; de Farias et al. 2019).

Diabetic neuropathy is a common and serious complication of T2D. The utility of melatonin treatment to ameliorate such retinal changes has been suggested using several experimental models (Mehrzadi et al. 2018; Tu et al. 2021).

It has also been suggested that reduction in melatonin production in the aged may contribute to obesity by promotion of insulin resistance and disruption of circadian metabolic fluxes (Cipolla-Neto et al. 2014). These findings imply that melatonin complementary therapy can play a vital role in reestablishing better health in individuals with chronic metabolic diseases.

Cardiac Disease, Cerebrovascular Disease

The prevalence of hypertension increases with aging. As a result of increasing stiffness of the arterial wall, a large proportion of the elderly have isolated systolic hypertension (Rubio-Guerra et al. 2015). The evidence strongly supports that hypertension in the elderly is associated with an increase in stroke risk and cardiovascular mortality and morbidity. The risk of cerebrovascular disease is higher in patients with autoimmune diseases including systemic lupus erythematosus and arthritis (Wiseman et al. 2016). Recruitment of immune active cells to the arterial wall is characteristic of the atherosclerotic artery (Tellides and Pober 2015) and seems to be related to plaque deposition. The antigens likely to be involved in the development of atherosclerosis include oxidized low density lipoprotein (LDL) and apolipoprotein H (Tabas and Lichtman 2017). A similar penetration of immune cells into the vascular wall is found in hypertension. Macrophages are able to activate inflammatory events even in the apparent absence of a stimulating antigen. Genetically deficient mice lacking macrophages have a reduced hypertensive response to agents that raise blood pressure (Luft et al. 2012). In a mouse model of atherosclerosis, melatonin therapy, by activation of the Sirt3/FOXO3a/Parkin signaling pathway, blocked the activity of the NLRP3 inflammasome and thus inhibited progression of atherosclerotic plaque formation (Ma et al. 2018). Although no causal relation has been clearly established, nocturnal melatonin secretion is severely in reduced in patients with coronary heart disease (Yaprak et al. 2003). Unfortunately, most of the reports of the utility of melatonin in arterial plaque formation come from experiments using animals modeling atherosclerosis (Ding et al. 2019; Li et al. 2019a, b). Success with such models has often not translated to success in the clinic. Since the toxicity of melatonin has repeatedly been found to be very low, more clinical study is urgently needed. The lack of significant commercial viability of melatonin has resulted in very few major clinical trials as to its beneficial potential.

Melatonin has been reported to have value in a clinical setting in the treatment of patients with hypertension (Koziróg et al. 2011; Gubin et al. 2016) but this has recently not been confirmed (Rahbari-Oskoui et al. 2019). Since patients not expressing reduced blood pressure at night ("non-dippers") do not respond to exogenous melatonin (Rechciński et al. 2010), the circadian features of melatonin levels must be taken into account. Beneficial effects of melatonin administration have been reported in several rodent models of hypertension (Simko et al. 2018; Zuo and Jiang 2020), despite a clear reduction of blood pressure by melatonin in a spontaneously hypertensive rat strain (Tain et al. 2010). The issues of time of melatonin administration and type of hypertension (nocturnal "dippers" vs. "non-dippers") are probably critical and may account for some apparent discrepancies in the literature. Some of the conflicting data published concerning blood pressure and melatonin is also likely to result from great variance in the dose administered. For example, while a dose of 2 mg nightly had no effect on diastolic or systolic blood pressure (Kim et al. 2021), the use of 6 or 250 mg melatonin per night reduced both of these parameters (Bahrami et al. 2020; Bazyar et al. 2021).

Frailty

General muscle atrophy characterizes senescence and this cannot be completely attributed to lack of exercise. Melatonin, perhaps by scavenging of free radicals, may help to maintain mitochondrial effectiveness with aging and has been shown to be protective against sarcopenia in aged animals (Stacchiotti et al. 2020). Such protective changes may be mediated by activation of sirtuin-1 (Cristòfol et al. 2012) and modulation of the actions of the NLRP3 inflammasome (Sayed et al. 2021). Among the aged, levels of the microRNA miR-223 are associated with increased activation of NF-kB based inflammatory pathway. The increased expression miRNA-483 with aging leads to a decrease in melatonin secretion and this play a role in the onset of sarcopenia in the elderly (Jin et al. 2021). Age-related increases of the related miR-21 were especially pronounced among the subgroup of the aged experiencing frailty (Rusanova et al. 2018). The plasma content of miRNA-21 is higher in cardiovascular patients with high levels of inflammation than in age-matched controls, as judged by C-reactive protein level (Olivieri et al. 2012). The expression of all of these miRNAs can be reduced in aged animals by administration of exogenous melatonin (Sayed et al. 2021).

SARS-CoV-2

COVID-19 is especially severe among the elderly and the violence of the ensuing inflammatory response ("cytokine storm") can be deadly. This may be due to two key immune failures associated with senescence. These features make the aged more vulnerable than younger cohorts to any bacterial or viral attack. Firstly, the precision of immune responses becomes increasingly compromised with age. Such immunosenescence involves decline of both innate and adaptive components of the immune system (Nikolich-Žugich 2018). This means that there is less protection against viral incursion and dissemination throughout the body. Second, the failure to respond effectively to vaccines is paralleled by an increasing degree of fruitless systemic inflammatory activity without a distinct goal (Pereira et al. 2020). This can be very damaging to tissues and accounts for most of the lethality of COVID-19. Melatonin is known to address both of these age-associated shortcomings of immune function, so it has been suggested as of use in treatment of COVID-19 (Cardinali 2021), but no clinical studies have been reported to date. However, the cytokine storm that can result from COVID-19 infection bears a close resemblance to that associated with sepsis (Root-Bernstein 2021) and there is extensive evidence concerning the value of melatonin in the treatment of sepsis (Sharman et al. 2014).

Pathways Contributing to the Multiplicity of Melatonin Actions

The Centrality of Receptor Activation

Since the intracellular concentration of melatonin is very low and it is thus unable to play a major direct role as a significant antioxidant, despite reports to the contrary (Tan et al. 2015). Its effectiveness stems from its binding to a series of receptor sites, many of which remain uncharacterized. Through activation of specific receptors, the upregulation of crucial transcription factors can be initiated, resulting into major changes in gene expression. For example, triggering of Sirt1 results in activation of the transcription factor, nuclear factor erythroid 2-related factor 2 (Nrf2). This in turn promotes expression of central antioxidant genes (Ma 2013; Ali et al. 2018). It is likely that sirtuin-1 mediates the anti-inflammatory effects of melatonin since these are blocked by inhibitors of sirtuin-1 (Hardeland 2019). In mice, SIRT6 overexpression can extend the length of the health lifespan. Melatonin activates SIRT6 and this leads to amelioration of symptoms in a rodent model of cardiomyopathy (Yu et al. 2021).

The binding of melatonin to the G-protein-coupled membrane-bound melatonin receptors MT1/MT2 can alter the activity of key transcription factors, either by means of a GTPase pathway or by phospholipase C (Luo et al. 2017). The epigenetic regulation of MT1/MT2 receptor expression could be a therapeutic direction for improvement of age-related events and disorders, where these receptors are reduced in number (Bahna and Niles 2018). Melatonin also acts on the nuclear orphan receptor (ROR) (Farez et al. 2015; Xu et al. 2019), but the sequence of events associated with this is uncertain. Nevertheless, ROR acts in an anti-inflammatory manner by inhibition of NF-kB (García et al. 2015). Another means of gene regulation by melatonin may be by its regulation of histone acetyl transferases which vary with both tissue and maturation (Bonmati-Carrion and Tomas-Loba 2021).

Selectivity of Melatonin Engagement

Melatonin is not an unfocussed antioxidant or anti-inflammatory hormone. Its actions are very much determined by the nature of the target cell. Thus, melatonin can promote pro-oxidant events in tumor cells, in contrast to its broadly protective effect in normal non-transformed cells (Lu et al. 2016; Xu et al. 2019). Melatonin generally acts in an anti-apoptotic manner and stimulates neurogenesis (Aranarochana et al. 2019). However, in tumor cells, melatonin can depress Sirt1 levels and foster apoptosis (Bizzarri et al. 2013). Melatonin is able to stimulate the immune system. It can behave in a pro- or anti-inflammatory manner depending upon the physiological milieu. Thus, while melatonin can both effect the release of

pro-inflammatory cytokines, but also, in other circumstances, it can inhibit inflammation-enhancing events (Hardeland 2018). In the spleen, melatonin given during the day heightens the inflammatory response to a lipopolysaccharide challenge but an elevated level of melatonin at night has the opposite effect and diminishes the reaction to an inflammogen (Naidu et al. 2010). NK cells are components of the innate immune system important in defense against aberrant tumor cells. Melatonin promotes their action by enhancing their ability to secrete the inflammatory cytokine IL-2 (Bonmati-Carrion and Tomas-Loba 2021). Generally, melatonin may act as an immune stimulant in basal or immunosuppressed states, while it behaves in an anti-inflammatory manner when immune responses are exaggerated, such as acute inflammation. The remarkable context specificity of melatonin means that it can enhance immune function under immunosuppressive conditions (such as aging) but inhibit excessive immune reactions (Ren et al. 2017). This may be accomplished by regulation of the balance between inflammatory T helper 17 cells and naive regulatory T (Treg) cells (Ma et al. 2020). These latter cells can mount a response to novel antigens but decline with age (Shintouo et al. 2021). Melatonin effects both proliferation and activation of Treg cells (Yoo et al. 2016) and thus may retard age-related immunosenescence.

In summary, melatonin is able to regulate a series of aspects of cellular metabolism by maintenance of a precise and selective targeting quality in comparison with broad spectrum antioxidant and anti-inflammatory agents.

Discussion and Conclusion

Normal aging is typified by an elevated basal level of inflammation but this is not accompanied by increased immune competency. In fact, aging involves a gradual diminution of ever-decreasing efficiency of immune responses (Molony et al. 2018). This decline in immunocompetence combined with excess unproductive inflammation leads to manifestation of several undesirable conditions associated with age-related disease. Reduced effectiveness of immune function can also explain the continuously growing onset of cancer incidence with aging (Nolen et al. 2017). Efficient immune monitoring leads to the identification and destruction of damaged senescent cells and this allows effective tissue regeneration. As aging progresses, the precision of this process is attenuated, and senescent cells can then accumulate (Ogrodnik et al. 2019). Senescent cells can attract immune activity which if unsuccessful in removing abnormal cells may cause to a state of persistent ineffectual inflammation, leading to tissue fibrosis and serving as a focal point for further pathological events (Wynn and Ramalingam 2012).

The gene expression profile of the brains of aged mice is different to that of younger mice. An increasing expression of many genes related to induction of inflammation typifies the aged brain. Since the central nervous system appears to remember events challenging the immune system for an extended period (Qin et al. 2007) such an increase may reflect the additive effect of a succession of

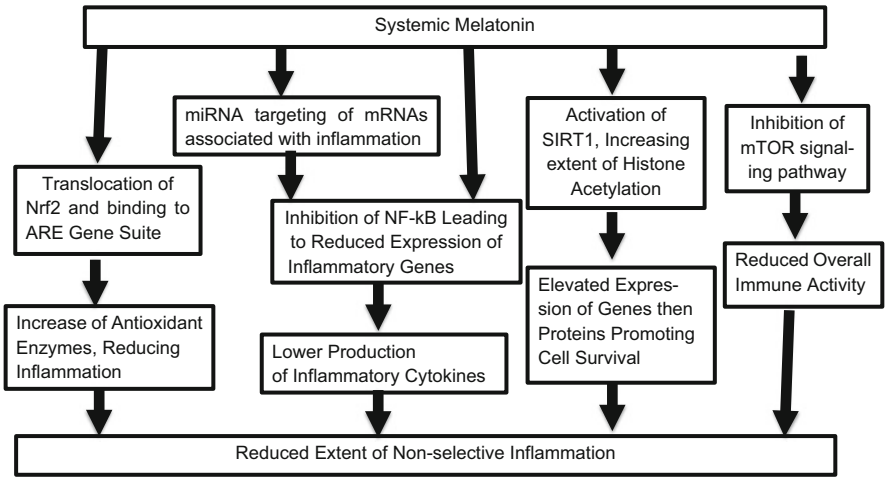


Fig. 13.1 Means by which melatonin may dampen age-related increases in nontargeted inflammation

inflammatory events taking place over the lifespan. The treatment of aged mice with dietary melatonin alters their pattern of mRNA expression profile in a direction so that it to more closely matches that of younger mice (Sharman et al. 2004). This restoration of a more youthful gene expression profile is likely to contribute to optimally healthy aging. This broad effect of melatonin may at least in part account for its widespread reported utility in many apparently chronic disease states predominating in the elderly. Many types of evidence ranging from the mRNA expression profile, to the lifespan of experimental animals, suggest that the aging process can be slowed by melatonin.

Melatonin is not a modern rapid-acting drug with a limited site of action, but rather an ancient endocrine material that is likely to have several key sites of action which may collaborate to produce an overall desirable outcome. Thus, it is less likely to have value in an acute health crisis than in a more drawn-out decline of function. This tilts its special value more to the preventive aspect of disease. The elderly are prone to a host of processes leading to deteriorating health, often related to each other. In addition to the single cause listed on a death certificate, many accompanying disorders are commonly found as contributing factors. These accumulated multimorbidities have the underlying commonality of excessive inflammation (Fabbri et al. 2015). Some of the mechanisms by which these may be attenuated by melatonin are summarized in Fig. 13.1. Melatonin can retard the advance of a series of changes that characterize the aging process (Bondy 2018). Routine consumption of melatonin by the elderly is therefore recommended without consideration of the presence of any specific age-related disorder. Based largely on animal studies, the optimal therapeutic dose of melatonin that may be needed by humans to obtain the optimal results in metabolic and neurodegenerative diseases of aging has been described as being in the 100 mg per diem range (Stacchiotti et al. 2020; Cardinali

2021). However, few studies with such amounts of melatonin have been conducted. Melatonin offers an inexpensive and safe means of slowing down the appearance of some of the undesirable attributes of age. By concurrent physical and nutritional strategies pursuing the same goal, such a combination will permit entering old age in a well-prepared manner, extending the useful and gratifying period of life.

References

Ali T, Rehman SU, Shah FA, Kim MO (2018) Acute dose of melatonin via Nrf2 dependently prevents acute ethanol-induced neurotoxicity in the developing rodent brain. J Neuroinflammation 15(1):119. https://doi.org/10.1186/s12974-018-1157-x

Aranarochana A, Chaisawang P, Sirichoat A, Pannangrong W, Wigmore P, Welbat JU (2019) Protective effects of melatonin against valproic acid-induced memory impairments and reductions in adult rat hippocampal neurogenesis. Neuroscience 406:580–593. https://doi.org/10.1016/j.neuroscience.2019.02.022

Atzeni F, Pipitone N, Iaccarino L, Masala IF, Weiss R, Alciati A, Doria A, Chapmanand J, Sarzi-Puttini P (2017) Rheumatic diseases and autoimmune vascular dementia. Autoimmun Rev 16(12):1265–1269. https://doi.org/10.1016/j.autrev.2017.10.011

Bahrami M, Cheraghpour M, Jafarirad S, Alavinejad P, Asadi F, Hekmatdoost A, Mohammadi M, Yari Z (2020) The effect of melatonin on treatment of patients with non-alcoholic fatty liver disease: a randomized double blind clinical trial. Complement Ther Med 52:102452. https://doi.org/10.1016/j.ctim.2020.102452

Bazyar H, Zare Javid A, Bavi Behbahani H, Moradi F, Moradi Poode B, Amiri P (2021) Consumption of melatonin supplement improves cardiovascular disease risk factors and anthropometric indices in type 2 diabetes mellitus patients: a double-blind, randomized, placebo-controlled trial. Trials 22(1):231. https://doi.org/10.1186/s13063-021-05174-z

Bizzarri M, Proietti S, Cucina A, Reiter RJ (2013) Molecular mechanisms of the pro-apoptotic actions of melatonin in cancer: a review. Expert Opin Ther Targets 17(12):1483–1496. https://doi.org/10.1517/14728222.2013.834890

Bondy SC (2018) Melatonin: beneficial aspects and underlying mechanisms. In: Correia L, Meyers G (eds) Melatonin: medical uses and role in health and disease. Nova Press, Hauppauge, pp 277–294

Bondy SC, Campbell A (2018) Mechanisms underlying tumor suppressive properties of melatonin. Int J Mol Sci 19(8):2205. https://doi.org/10.3390/ijms19082205

Bonmati-Carrion MA, Tomas-Loba A (2021) Melatonin and cancer: a polyhedral network where the source matters. Antioxidants 10:210. https://doi.org/10.3390/antiox10020210

Bonnefond A, Froguel P (2017) Disentangling the role of melatonin and its receptor MTNR1B in type 2 diabetes: still a long way to go? Curr Diab Rep 17(12):122. https://doi.org/10.1007/s11892-017-0957-1. PMID: 29063374

Calsolaro V, Edison P (2016) Neuroinflammation in Alzheimer's disease: current evidence and future directions. Alzheimers Dement 12(6):719–732. https://doi.org/10.1016/j.jalz.2016.02.010

Cardinali DP (2021) Melatonin and healthy aging. Vitam Horm 115:67–88. https://doi.org/10.1016/bs.vh.2020.12.004

Cham BE, Chase TR (2012) Solasodine rhamnosyl glycosides cause apoptosis in cancer cells. Do they also prime the immune system resulting in long-term protection against cancer? Planta Med 78(4):349–353. https://doi.org/10.1055/s-0031-1298149

Chen C, Yang C, Wang J, Huang X, Yu H, Li S, Shupeng L, Zhang Z, Liu J, Yang X, Liu GP (2021) Melatonin ameliorates cognitive deficits through improving mitophagy in a mouse model of Alzheimer's disease. J Pineal Res 71:e12774. https://doi.org/10.1111/jpi.12774

Cheng FW, Gao X, Jensen GL (2015) Weight change and all-cause mortality in older adults: a meta-analysis. J Nutr Gerontol Geriatr 34(4):343–368. https://doi.org/10.1080/21551197.2015.1090362

Cho U, Kim B, Kim S, Han Y, Song YS (2018) Pro-inflammatory M1 macrophage enhances metastatic potential of ovarian cancer cells through NF-κB activation. Mol Carcinog 57(2):235–242. https://doi.org/10.1002/mc.22750

Cipolla-Neto J, Amaral FG, Afeche SC, Tan DX, Reiter RJ (2014) Melatonin, energy metabolism, and obesity: a review. J Pineal Res 56(4):371–381. https://doi.org/10.1111/jpi.12137

Cristòfol R, Porquet D, Corpas R, Coto-Montes A, Serret J, Camins A, Pallàs M, Sanfeliu C (2012) Neurons from senescence-accelerated SAMP8 mice are protected against frailty by the sirtuin 1 promoting agents melatonin and resveratrol. J Pineal Res 52(3):271–281. https://doi.org/10.1111/j.1600-079X.2011.00939.x

de Farias TDSM, Cruz MM, de Sa RCDC, Severi I, Perugini J, Senzacqua M, Cerutti SM, Giordano A, Cinti S, Alonso-Vale MIC (2019) Melatonin supplementation decreases hypertrophic obesity and inflammation induced by high-fat diet in mice. Front Endocrinol (Lausanne) 10:750. https://doi.org/10.3389/fendo.2019.00750

den Breems NY, Eftimie R (2016) The re-polarisation of M2 and M1 macrophages and its role on cancer outcomes. J Theor Biol 390:23–39. https://doi.org/10.1016/j.jtbi.2015.10.03

Diaconu V, Ouellette N, Bourbeau R (2020) Modal lifespan and disparity at older ages by leading causes of death: a Canada-U.S. comparison. J Popul Res 37(4):323–344. https://doi.org/10.1007/s12546-020-09247-9

Ding S, Lin N, Sheng X, Zhao Y, Su Y, Xu L, Tong R, Yan Y, Fu Y, He J, Gao Y, Yuan A, Ye L, Reiter RJ, Pu J (2019) Melatonin stabilizes rupture-prone vulnerable plaques via regulating macrophage polarization in a nuclear circadian receptor RORα-dependent manner. J Pineal Res 67(2):e12581. https://doi.org/10.1111/jpi.12581

Fabbri E, An Y, Zoli M, Simonsick EM, Guralnik JM, Bandinelli S, Boyd CM, Ferrucci L (2015) Aging and the burden of multimorbidity: associations with inflammatory and anabolic hormonal biomarkers. J Gerontol A Biol Sci Med Sci 70(1):63–70. https://doi.org/10.1093/gerona/glu127

Farez MF, Mascanfroni ID, Méndez-Huergo SP, Yeste A, Murugaiyan G, Garo LP, Balbuena Aguirre ME, Patel B, Ysrraelit MC, Zhu C, Kuchroo VK, Rabinovich GA, Quintana FJ, Correale J (2015) Melatonin contributes to the seasonality of multiple sclerosis relapses. Cell 162(6):1338–1352. https://doi.org/10.1016/j.cell.2015.08.025

Forloni G, Balducci C (2018) Alzheimer's disease, oligomers, and inflammation. J Alzheimers Dis 62(3):1261–1276. https://doi.org/10.3233/JAD-170819

Garaulet M, Qian J, Florez JC, Arendt J, Saxena R, Scheer FAJL (2020) Melatonin effects on glucose metabolism: time to unlock the controversy. Trends Endocrinol Metab 31(3):192–204. https://doi.org/10.1016/j.tem.2019.11.011

García JA, Volt H, Venegas C, Doerrier C, Escames G, López LC, Acuña-Castroviejo D (2015) Disruption of the NF-κB/NLRP3 connection by melatonin requires retinoid-related orphan receptor-α and blocks the septic response in mice. FASEB J 29(9):3863–3875. https://doi.org/10.1096/fj.15-273656

Genario R, Cipolla-Neto J, Bueno AA, Santos HO (2021) Melatonin supplementation in the management of obesity and obesity-associated disorders: a review of physiological mechanisms and clinical applications. Pharmacol Res 163:105254. https://doi.org/10.1016/j.phrs.2020.105254

Giudice A, Crispo A, Grimaldi M, Giudice A, Crispo A, Grimaldi M, Polo A, Bimonte S, Capunzo M, Amore A, D'Arena G, Cerino P, Budillon A, Botti G, Costantini S, Montella M (2018) The effect of light exposure at night (LAN) on carcinogenesis via decreased nocturnal melatonin synthesis. Molecules 23(6):1308. https://doi.org/10.3390/molecules23061308

González A, Alonso-González C, González-González A, Menéndez-Menéndez J, Cos S, Martínez-Campa C (2021) Melatonin as an adjuvant to antiangiogenic cancer treatments. Cancers 13(13):3263. https://doi.org/10.3390/cancers13133263

Gubin DG, Gubin GD, Gapon LI, Weinert D (2016) Daily melatonin administration attenuates age-dependent disturbances of cardiovascular rhythms. Curr Aging Sci 9(1):5–13. https://doi.org/10.2174/1874609809666151130220011

Hardeland R (2018) Melatonin and inflammation-story of a double-edged blade. J Pineal Res 65(4): e12525. https://doi.org/10.1111/jpi.12525

Hardeland R (2019) Aging, melatonin, and the pro- and anti-inflammatory networks. Int J Mol Sci 20(5):1223. https://doi.org/10.3390/ijms20051223

Hatta K, Kishi Y, Wada K, Takeuchi T, Odawara T, Usui C, Nakamura H, DELIRIA-J Group (2014) Preventive effects of ramelteon on delirium: a randomized placebo-controlled trial. JAMA. Psychiatry 71(4):397–403. https://doi.org/10.1001/jamapsychiatry.2013.3320

Hu WT, Howell JC, Ozturk T, Gangishetti U, Kollhoff AL, Hatcher-Martin JM, Anderson AM, Tyor WR (2019) CSF cytokines in aging, multiple sclerosis, and dementia. Front Immunol 10: 480. https://doi.org/10.3389/fimmu.2019.00480

Jin H, Xie W, Hu P, Tang K, Wang X, Wu Y, He M, Yu D, Li Y (2021) The role of melatonin in sarcopenia: advances and application prospects. Exp Gerontol 149:111319. https://doi.org/10.1016/j.exger.2021.111319

Karamitri A, Jockers R (2019) Melatonin in type 2 diabetes mellitus and obesity. Nat Rev Endocrinol 15(2):105–125. https://doi.org/10.1038/s41574-018-0130-1

Kim Y, Kang HT, Lee DC (2021) Melatonin supplementation for six weeks had no effect on arterial stiffness and mitochondrial DNA in women aged 55 years and older with insomnia: a double-blind randomized controlled study. Int J Environ Res Public Health 18(5):2561. https://doi.org/10.3390/ijerph18052561

Koziróg M, Poliwczak AR, Duchnowicz P, Koter-Michalak M, Sikora J, Broncel M (2011) Melatonin treatment improves blood pressure, lipid profile, and parameters of oxidative stress in patients with metabolic syndrome. J Pineal Res 50(3):261–266. https://doi.org/10.1111/j.1600-079X.2010.00835.x

Landskron G, De la Fuente M, Thuwajit P, Thuwajit C, Hermoso MA (2014) Chronic inflammation and cytokines in the tumor microenvironment. J Immunol Res 2014:149185. https://doi.org/10.1155/2014/149185

Li T, Jiang S, Lu C, Yang W, Yang Z, Hu W, Xin Z, Yang Y (2019a) Melatonin: another avenue for treating osteoporosis? J Pineal Res 66(2):e12548. https://doi.org/10.1111/jpi.12548

Li H, Li J, Jiang X, Liu S, Liu Y, Chen W, Yang J, Zhang C, Zhang W (2019b) Melatonin enhances atherosclerotic plaque stability by inducing prolyl-4-hydroxylase α1 expression. J Hypertens 37: 964–971

Li Y, Zhang J, Wan J, Liu A, Sun J (2020) Melatonin regulates Aβ production/clearance balance and Aβ neurotoxicity: a potential therapeutic molecule for Alzheimer's disease. Biomed Pharmacother 132:110887. https://doi.org/10.1016/j.biopha.2020.110887

Liu B, Le KX, Park MA, Wang S, Belanger AP, Dubey S, Frost JL, Holton P, Reiser V, Jones PA, Trigg W, Di Carli MF, Lemere CA (2015) In vivo detection of age- and disease-related increases in neuroinflammation by 18F-GE180 TSPO microPET imaging in wild-type and Alzheimer's transgenic mice. J Neurosci 35(47):15716–15730. https://doi.org/10.1523/JNEUROSCI.0996-15.2015

Lu YX, Chen DL, Wang DS, Chen LZ, Mo HY, Sheng H, Bai L, Wu QN, Yu HE, Xie D, Yun JP, Zeng ZL, Wang F, Ju HQ, Xu RH (2016) Melatonin enhances sensitivity to fluorouracil in oesophageal squamous cell carcinoma through inhibition of Erk and Akt pathway. Cell Death Dis 7(10):e2432. https://doi.org/10.1038/cddis.2016.330

Luft FC, Dechend R, Müller DN (2012) Immune mechanisms in angiotensin II-induced target-organ damage. Ann Med 44(Suppl 1):S49–S54. https://doi.org/10.3109/07853890.2011.653396

Luo Y, Peng M, Wei H (2017) Melatonin promotes brain-derived neurotrophic factor (BDNF) expression and anti-apoptotic effects in neonatal hemolytic hyperbilirubinemia via a phospholipase (PLC)-mediated mechanism. Med Sci Monit 23:5951–5959. https://doi.org/10.12659/msm.907592

Lutshumba J, Nikolajczyk BS, Bachstetter AD (2021) Dysregulation of systemic immunity in aging and dementia. Front Cell Neurosci 15:652111. https://doi.org/10.3389/fncel.2021.652111

Ma Q (2013) Role of nrf2 in oxidative stress and toxicity. Annu Rev Pharmacol Toxicol 53:401–426. https://doi.org/10.1146/annurev-pharmtox-011112-140320

Ma S, Chen J, Feng J, Zhang R, Fan M, Han D, Li X, Li C, Ren J, Wang Y, Cao F (2018) Melatonin ameliorates the progression of atherosclerosis via mitophagy activation and NLRP3 inflammasome inhibition. Oxidative Med Cell Longev 2018:9286458. https://doi.org/10.1155/2018/9286458

Ma F, Hao H, Gao X, Cai Y, Zhou J, Liang P, Lv J, He Q, Shi C, Hu D, Chen B, Zhu L, Xiao X, Li S (2020) Melatonin ameliorates necrotizing enterocolitis by preventing Th17/Treg imbalance through activation of the AMPK/SIRT1 pathway. Theranostics 10(17):7730–7746. https://doi.org/10.7150/thno.45862

Marques JHM, Mota AL, Oliveira JG, Lacerda JZ, Stefani JP, Ferreira LC, Castro TB, Aristizábal-Pachón AF, Zuccari DAPC (2018) Melatonin restrains angiogenic factors in triple-negative breast cancer by targeting miR-152-3p: in vivo and in vitro studies. Life Sci 208:131–138. https://doi.org/10.1016/j.lfs.2018.07.012

Mehrzadi S, Motevalian M, Rezaei Kanavi M, Fatemi I, Ghaznavi H, Shahriari M (2018) Protective effect of melatonin in the diabetic rat retina. Fundam Clin Pharmacol 32(4):414–421. https://doi.org/10.1111/fcp.12361

Molony RD, Malawista A, Montgomery RR (2018) Reduced dynamic range of antiviral innate immune responses in aging. Exp Gerontol 107:130–135. https://doi.org/10.1016/j.exger.2017.08.019

Murata M (2018) Inflammation and cancer. Environ Health Prev Med 23(1):50. https://doi.org/10.1186/s12199-018-0740-1

Na YR, Je S, Seok SH (2018) Metabolic features of macrophages in inflammatory diseases and cancer. Cancer Lett 413:46–58. https://doi.org/10.1016/j.canlet.2017.10.044

Naidu KS, Morgan LW, Bailey MJ (2010) Inflammation in the avian spleen: timing is everything. BMC Mol Biol 11:104. https://doi.org/10.1186/1471-2199-11-104

Nikolich-Žugich J (2018) The twilight of immunity: emerging concepts in aging of the immune system. Nat Immunol 19(1):10–19. https://doi.org/10.1038/s41590-017-0006-x. Erratum in: Nat Immunol. 2018;19(10):1146

Nolen SC, Evans MA, Fischer A, Corrada MM, Kawas CH, Bota DA (2017) Cancer-incidence, prevalence and mortality in the oldest-old. A comprehensive review. Mech Ageing Dev 164:113–126. https://doi.org/10.1016/j.mad.2017.05.002

Ogrodnik M, Salmonowicz H, Gladyshev VN (2019) Integrating cellular senescence with the concept of damage accumulation in aging: relevance for clearance of senescent cells. Aging Cell 18(1):e12841. https://doi.org/10.1111/acel.12841. Erratum in: Aging Cell. 201918(2):e12942

Olivieri F, Spazzafumo L, Santini G, Lazzarini R, Albertini MC, Rippo MR, Galeazzi R, Abbatecola AM, Marcheselli F, Monti D, Ostan R, Cevenini E, Antonicelli R, Franceschi C, Procopio AD (2012) Age-related differences in the expression of circulating microRNAs: miR-21 as a new circulating marker of inflammaging. Mech Ageing Dev 133(11–12):675–685. https://doi.org/10.1016/j.mad.2012.09.004

Otamas A, Grant PJ, Ajjan RA (2020) Diabetes and atherothrombosis: the circadian rhythm and role of melatonin in vascular protection. Diab Vasc Dis Res 17(3):1479164120920582. https://doi.org/10.1177/1479164120920582

Park YS, Kim SH, Park JW, Kho Y, Seok PR, Shin JH, Choi YJ, Jun JH, Jung HC, Kim EK (2020) Melatonin in the colon modulates intestinal microbiota in response to stress and sleep deprivation. Intest Res 18(3):325–336. https://doi.org/10.5217/ir.2019.00093

Pereira B, Xu XN, Akbar AN (2020) Targeting inflammation and immunosenescence to improve vaccine responses in the elderly. Front Immunol 11:583019. https://doi.org/10.3389/fimmu.2020.583019

Qin L, Wu X, Block ML, Liu Y, Breese GR, Hong JS, Knapp DJ, Crews FT (2007) Systemic LPS causes chronic neuroinflammation and progressive neurodegeneration. Glia 55(5):453–462. https://doi.org/10.1002/glia.20467

Rahbari-Oskoui FF, Abramson JL, Bruckman AM, Chapman AB, Cotsonis GA, Johnson SA, Bliwise DL (2019) Nighttime administration of high-dose, sustained-release melatonin does not decrease nocturnal blood pressure in African-American patients: results from a preliminary randomized, crossover trial. Complement Ther Med 43:157–164. https://doi.org/10.1016/j.ctim.2019.01.026

Rechciński T, Trzos E, Wierzbowska-Drabik K, Krzemińska-Pakuła M, Kurpesa M (2010) Melatonin for nondippers with coronary artery disease: assessment of blood pressure profile and heart rate variability. Hypertens Res 33(1):56–61. https://doi.org/10.1038/hr.2009.174

Ren W, Liu G, Chen S, Yin J, Wang J, Tan B, Wu G, Bazer FW, Peng Y, Li T, Reiter RJ, Yin Y (2017) Melatonin signaling in T cells: functions and applications. J Pineal Res 62(3):e12394. https://doi.org/10.1111/jpi.12394

Roopin M, Yacobi YZ, Levy O (2013) Occurrence, diel patterns, and the influence of melatonin on the photosynthetic performance of cultured Symbiodinium. J Pineal Res 55(1):89–100. https://doi.org/10.1111/jpi.12046

Root-Bernstein R (2021) Innate receptor activation patterns involving TLR and NLR synergisms in COVID-19, ALI/ARDS and sepsis cytokine storms: a review and model making novel predictions and therapeutic suggestions. Int J Mol Sci 22(4):2108. https://doi.org/10.3390/ijms22042108

Rubio-Guerra AF, Duran-Salgado MB (2015) Recommendations for the treatment of hypertension in elderly people. Cardiovasc Hematol Agents Med Chem 12(3):146–151. https://doi.org/10.2174/1871525713666150310110357

Rusanova I, Diaz-Casado ME, Fernández-Ortiz M, Aranda-Martínez P, Guerra-Librero A, García-García FJ, Escames G, Mañas L, Acuña-Castroviejo D (2018) Analysis of plasma microRNAs as predictors and biomarkers of aging and frailty in humans. Oxidative Med Cell Longev 2018:7671850. https://doi.org/10.1155/2018/7671850

Samec M, Liskova A, Koklesova L, Zhai K, Varghese E, Samuel SM, Šudomová M, Lucansky V, Kassayova M, Pec M, Biringer K, Brockmueller A, Kajo K, Hassan STS, Shakibaei M, Golubnitschaja O, Büsselberg D, Kubatka P (2021) Metabolic anti-cancer effects of melatonin: clinically relevant prospects. Cancers 13(12):3018. https://doi.org/10.3390/cancers13123018

Sayed RK, Fernández-Ortiz M, Fernández-Martínez J, Aranda Martínez P, Guerra-Librero A, Rodríguez-Santana C, de Haro T, Escames G, Acuña-Castroviejo D, Rusanova I (2021) The impact of melatonin and NLRP3 inflammasome on the expression of microRNAs in aged muscle. Antioxidants 10(4):524. https://doi.org/10.3390/antiox10040524

Schwarzenberger A, Christjani M, Wacker A (2014) Longevity of Daphnia and the attenuation of stress responses by melatonin. BMC Physiol 14:8. https://doi.org/10.1186/s12899-014-0008-y

Sezen O, Erdemci B, Calik M, Koc M (2021) The role of melatonin in preventing radiation-induced intestinal injury. J BUON 26(2):626–633

Sharman EH, Sharman KG, Ge YW, Lahiri DK, Bondy SC (2004) Age-related changes in murine CNS mRNA gene expression are modulated by dietary melatonin. J Pineal Res 36(3):165–170. https://doi.org/10.1046/j.1600-079x.2003.00112.x

Sharman EH, Sharman KG, Bondy SC (2011) Extended exposure to dietary melatonin reduces tumor number and size in aged male mice. Exp Gerontol 46(1):18–22. https://doi.org/10.1016/j.exger.2010.09.004

Sharman EH, Bondy SC. Melatonin as a therapeutic agent for sepsis. In: Melatonin, therapeutic value and neuroprotection, Srinivasan Gobbi GV, Shillcutt SD, Suzen S (eds). CRC Press, Boca Raton, pp. 71–88, 2014

Shintouo CM, Shey RA, Mets T, Vanhamme L, Souopgui J, Ghogomu SM, Njemini R (2021) Onchocerciasis fingerprints in the geriatric population: does host immunity play a role? Trop Med Infect Dis 6(3):153. https://doi.org/10.3390/tropicalmed6030153

Simko F, Baka T, Krajcirovicova K, Repova K, Aziriova S, Zorad S, Poglitsch M, Adamcova M, Reiter RJ, Paulis L (2018) Effect of melatonin on the renin-angiotensin-aldosterone system in l-NAME-induced hypertension. Molecules 23(2):265. https://doi.org/10.3390/molecules23020265

Soo SK, Rudich PD, Traa A, Harris-Gauthier N, Shields HJ, Van Raamsdonk JM (2020) Compounds that extend longevity are protective in neurodegenerative diseases and provide a novel treatment strategy for these devastating disorders. Mech Ageing Dev 190:111297. https://doi.org/10.1016/j.mad.2020.111297

Stacchiotti A, Favero G, Rodella LF (2020) Impact of melatonin on skeletal muscle and exercise. Cell 9(2):288. https://doi.org/10.3390/cells9020288

Sumsuzzman DM, Choi J, Jin Y, Hong Y (2021) Neurocognitive effects of melatonin treatment in healthy adults and individuals with Alzheimer's disease and insomnia: a systematic review and meta-analysis of randomized controlled trials. Neurosci Biobehav Rev 127:459–473. https://doi.org/10.1016/j.neubiorev.2021.04.034

Sun C, Lv T, Huang L, Liu X, Jin C, Lin X (2020) Melatonin ameliorates aluminum toxicity through enhancing aluminum exclusion and reestablishing redox homeostasis in roots of wheat. J Pineal Res 68(4):e12642. https://doi.org/10.1111/jpi.12642

Tabas I, Lichtman AH (2017) Monocyte-macrophages and T cells in atherosclerosis. Immunity 47(4):621–634. https://doi.org/10.1016/j.immuni.2017.09.008

Tain YL, Huang LT, Lin IC, Lau YT, Lin CY (2010) Melatonin prevents hypertension and increased asymmetric dimethylarginine in young spontaneous hypertensive rats. J Pineal Res 49(4):390–398. https://doi.org/10.1111/j.1600-079X.2010.00806.x

Tan DX, Manchester LC, Esteban-Zubero E, Zhou Z, Reiter RJ (2015) Melatonin as a potent and inducible endogenous antioxidant: synthesis and metabolism. Molecules 20(10):18886–18906. https://doi.org/10.3390/molecules201018886

Tellides G, Pober JS (2015) Inflammatory and immune responses in the arterial media. Circ Res 116(2):312–322. https://doi.org/10.1161/CIRCRESAHA.116.301312

Tu Y, Song E, Wang Z, Ji N, Zhu L, Wang K, Sun H, Zhang Y, Zhu Q, Liu X, Zhu M (2021) Melatonin attenuates oxidative stress and inflammation of Müller cells in diabetic retinopathy via activating the Sirt1 pathway. Biomed Pharmacother 137:111274. https://doi.org/10.1016/j.biopha.2021.111274

Wiseman SJ, Ralston SH, Wardlaw JM (2016) Cerebrovascular disease in rheumatic diseases: a systematic review and meta-analysis. Stroke 47(4):943–950. https://doi.org/10.1161/STROKEAHA.115.012052

Wynn TA, Ramalingam TR (2012) Mechanisms of fibrosis: therapeutic translation for fibrotic disease. Nat Med 18(7):1028–1040. https://doi.org/10.1038/nm.2807

Xu L, Su Y, Zhao Y, Sheng X, Tong R, Ying X, Gao L, Ji Q, Gao Y, Yan Y, Yuan A, Wu F, Lan F, Pu J (2019) Melatonin differentially regulates pathological and physiological cardiac hypertrophy: crucial role of circadian nuclear receptor RORα signaling. J Pineal Res 67(2):e12579. https://doi.org/10.1111/jpi.12579

Yaprak M, Altun A, Vardar A, Aktoz M, Ciftci S, Ozbay G (2003) Decreased nocturnal synthesis of melatonin in patients with coronary artery disease. Int J Cardiol 89(1):103–107. https://doi.org/10.1016/s0167-5273(02)00461-8

Yi WJ, Kim TS (2017) Melatonin protects mice against stress-induced inflammation through enhancement of M2 macrophage polarization. Int Immunopharmacol 48:146–158. https://doi.org/10.1016/j.intimp.2017.05.006

Yoo YM, Jang SK, Kim GH, Park JY, Joo SS (2016) Pharmacological advantages of melatonin in immunosenescence by improving activity of T lymphocytes. J Biomed Res 30(4):314–321. https://doi.org/10.7555/JBR.30.2016K0010

Yu LM, Dong X, Xue XD, Xu S, Zhang X, Xu YL, Wang ZS, Wang Y, Gao H, Liang YX, Yang Y, Wang HS (2021) Melatonin attenuates diabetic cardiomyopathy and reduces myocardial vulnerability to ischemia-reperfusion injury by improving mitochondrial quality control: role of SIRT6. J Pineal Res 70(1):e12698. https://doi.org/10.1111/jpi.12698

Zhang Y, Liu Z, Zhang W, Wu Q, Zhang Y, Liu Y, Guan Y, Chen X (2019) Melatonin improves functional recovery in female rats after acute spinal cord injury by modulating polarization of spinal microglial/macrophages. J Neurosci Res 97(7):733–743. https://doi.org/10.1002/jnr.24409

Zisapel N (2018) New perspectives on the role of melatonin in human sleep, circadian rhythms and their regulation. Br J Pharmacol 175(16):3190–3199. https://doi.org/10.1111/bph.14116

Zuo J, Jiang Z (2020) Melatonin attenuates hypertension and oxidative stress in a rat model of L-NAME-induced gestational hypertension. Vasc Med 25(4):295–301. https://doi.org/10.1177/1358863X20919798

Chapter 14
Protein and Energy Supplements for the Elderly

Novi Silvia Hardiany, Istiqomah Agusta, Syarifah Dewi, Febriana Catur Iswanti, and Reni Paramita

Abstract The proportion of elderly individuals is rising globally, and data have shown that as high as 8% of the elderly community suffer from malnutrition. Protein energy malnutrition has shown to elevate morbidity and mortality risk in the elderly; therefore, protein and energy supplement are needed for the elderly populations to create healthy conditions. This chapter describes about general structure of protein, protein turnover, amino acid metabolism including metabolism in the elderly, protein change in aging, supplementation of amino acid as well as vitamin and mineral for the elderly. The discussion in this section aims to provide a general description of protein, amino acids, changes in amino acid metabolism in the elderly, and the benefits of supplementing amino acids as well as vitamins and minerals for the elderly.

Keywords Elderly · Protein · Metabolism · Amino acid · Supplementation · Vitamin · Mineral

N. S. Hardiany (✉) · F. C. Iswanti
Department of Biochemistry and Molecular Biology, Faculty of Medicine Universitas Indonesia, Jakarta, Indonesia

Center of Hypoxia and Oxidative Stress Studies, Faculty of Medicine Universitas Indonesia, Jakarta, Indonesia

Molecular Biology and Proteomic Core Facilities, Indonesia Medical Education and Research Institute, Faculty of Medicine Universitas Indonesia, Jakarta, Indonesia
e-mail: novi.silvia@ui.ac.id; febriana.iswanti@ui.ac.id

I. Agusta · S. Dewi · R. Paramita
Department of Biochemistry and Molecular Biology, Faculty of Medicine Universitas Indonesia, Jakarta, Indonesia

Center of Hypoxia and Oxidative Stress Studies, Faculty of Medicine Universitas Indonesia, Jakarta, Indonesia
e-mail: istiqomah.agusta@ui.ac.id; syarifah.dewi@ui.ac.id; reni.paramita01@ui.ac.id

J. R. Harris, V. I. Korolchuk (eds.), *Biochemistry and Cell Biology of Ageing: Part IV, Clinical Science*, Subcellular Biochemistry 103,
https://doi.org/10.1007/978-3-031-26576-1_14

Introduction

Proteins are one of the essential macromolecules required to create living organisms. In humans, protein makes up roughly 15% of the body mass, 40% of which is found within skeletal muscles, more than 25% is found within organs, and the remaining make up components of the skin and blood. A single living cell may consist of thousands of protein types, with each type performing a specific function. In general, the cellular roles of proteins include structural, regulatory, contractile, and protective functions. Proteins are made up of chains of amino acids that are bound together by peptide bonds. The amino acids required by the human body can be either synthesized within the body or obtained from dietary sources. Dietary sources of amino acids are transported by the intestinal epithelium toward the bloodstream. Various cells use these amino acids to synthesize proteins and other nitrogen-rich compounds or are oxidized to produce energy. Among geriatric populations, adequate protein intake is required according to age needs in order to keep the balance of protein levels in the body and lower the risk of sarcopenia.

General Structure of Proteins

Proteins are polymer that comprises building blocks in the form of amino acids. Every amino acid composed of a carbon atom at the center (known as α-carbon or Cα) which form bonds with a carboxyl group, amine group, a hydrogen atom, and a variable R group (Fig. 14.1). The bonds formed by the α-carbon with the carboxyl group, amine group, and hydrogen atom are known as the main branch, whereas the R group is known as the side branch. The R groups consist of variable physicochemical structures and behaviors and are used to identify the types of amino acid. R groups may be in the form of singular atoms, such as the hydrogen atom in Glycine, or a methyl group such as in Alanine. R groups in amino acids may be categorized based on behaviors such as acidity, polarity, and charge. The structure of R groups may be aliphatic, aromatic or consist of a phenol center. The diversity of behaviors among R groups determines the characteristics of the protein that is formed (Nelson and Cox 2005). Based on the ability of the living organism to produce them, amino acids can be grouped as essential and non-essential. Essential amino acids cannot be produced by the living organism and hence are a need sourced from the food intake. There are nine essential amino acids required by humans including isoleucine, leucine, lysine, valine, threonine, tryptophan, tyrosine, methionine, and phenylalanine. A single complete protein must consist of all nine essential amino acids. Complete proteins are usually obtained from animal sources (Wu 2009; Lopez and Mohiuddin 2022). Non-essential amino acids, such as arginine, alanine, aspartate, asparagine, cysteine, glycine, glutamate, serine, glutamine, tyrosine, and proline are synthesized by the body (Nelson and Cox 2005; Lopez and Mohiuddin 2022). Protein structures consist of multiple levels, including primary, secondary, tertiary,

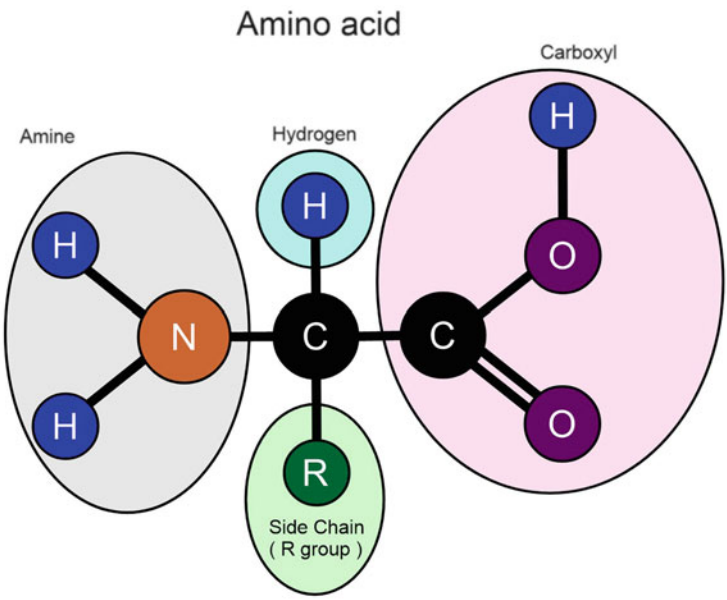


Fig. 14.1 Amino acid structure

and quaternary. Every protein will have a primary, secondary, and tertiary structure, however, not all proteins have a quaternary structure (Lopez and Mohiuddin 2022). Proteins can be identified based on the differences in every level of structure. The complexity of protein structures is responsible for the variation in the types and functions of proteins.

The primary structure of proteins is defined as the linear sequence of amino acids within a single protein. Amino acids at the primary level are covalently bonded to each other via peptide bonds, forming polymers known as polypeptides (Sanvictores and Farci 2022). Peptide bonds are formed between amine groups of amino acid with the carboxyl group of another amino acid next in the sequence. Each polypeptide contains a free carboxyl group at one end (C-terminus) and a free amine group at the other end (N-terminus) (Fig. 14.2). A protein is formed from a minimum one polypeptide chains.

Secondary structures of proteins involve three-dimensional patterns that are formed locally due to hydrogen bonds between the atoms of the main branches of polypeptides. There are two most common forms of secondary structures that are α-helix and β-sheets (Fig. 14.3). The α-helix structures are formed when oxygen atoms of carboxyl groups in amino acids form bonds with hydrogen atoms of amine groups in amino acids three peptides apart within one linear polypeptide chain. These hydrogen bonds constantly form coiling patterns that coil around a vertical axis. The polypeptide helix formed from chiral amino acids will display chirality, which determines either clockwise or counter-clockwise rotation of the polypeptide (Murray 2009). Formation of α-helix structures requires very little energy and hence

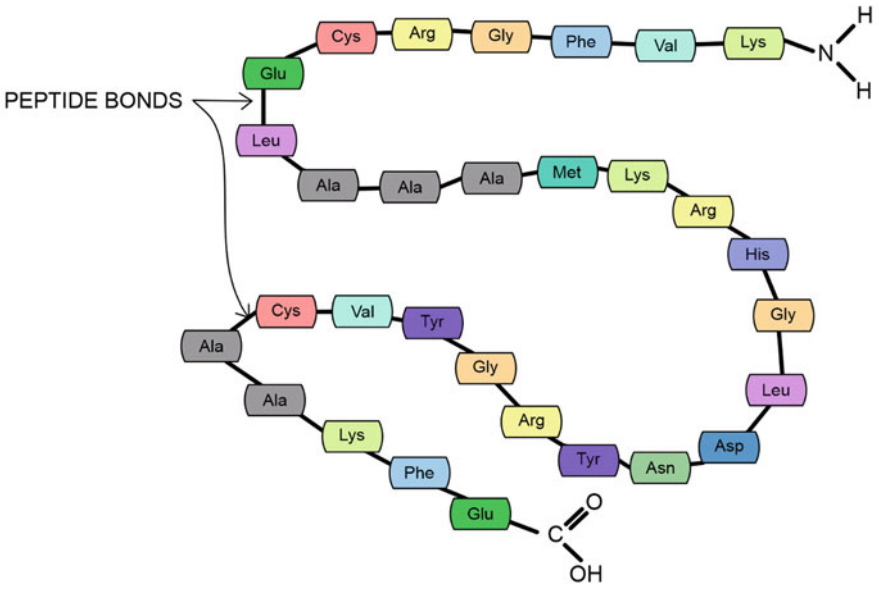
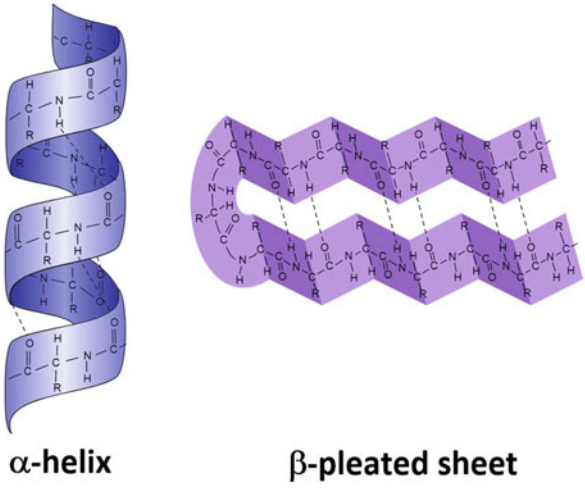


Fig. 14.2 Primary structure of protein

Fig. 14.3 Secondary structure of protein



forms spontaneously. The stability of the α-helix structure is directly correlated to the number of hydrogen bonds formed within the α-helix structure. Furthermore, atoms that are in the center of the α-helix structure will form Van Der Waals interactions that further provide stability. The α-helix structure is usually located on the surfaces of proteins but may also be partially or completely submerged within the interior part of the protein. Proteins with α-helix structure that exhibit amphipathic behavior are commonly present in venomous compounds, antibiotics, and

Fig. 14.4 Tertiary structure of proliferating cell nuclear antigen (PCNA) protein subunit. From: "*Analisis Bioinformatika Mutasi S2281 Protein PCNA dan pengaruhnya pada Struktur 3 Dimensi Protein*" by Syarifah Dewi, 2017, Indonesian Journal of Biotechnology and Biodiversity, 1(1), p. 20–27

glycoproteins of the HIV virus. Secondary structures in the form of β-sheets are formed via hydrogen bonds between carboxyl and amine groups of amino acids on different polypeptide chains (interstrand or intermolecular bonds). These chains are stacked on top of each other, forming the folding structure of the protein. The shape of β-sheets is considered parallel if the forming polypeptides line up in the same order (N-terminus of one polypeptide aligns with the N-terminus of another), whereas antiparallel β-sheets are formed when polypeptides are layered in opposite directions (N-terminus of one polypeptide aligns with the C-terminus of another). Antiparallel β-sheets are generally more stable due to more optimal hydrogen bonds between the polypeptides (Murray 2009).

Tertiary structures of proteins are three-dimensional structures formed due to the interactions between the atoms of the R groups (Fig. 14.4). Hydrogen bonds, ionic bonds, dipole interactions, and hydrophobic and disulfide interactions are some of the interactions that are involved in the formation of tertiary structures of proteins. Hydrophobic interactions result in the grouping of hydrophobic amino acids toward the internal surface of the protein molecule, while hydrophilic amino acids are located on the exterior of the protein and interact with the surrounding water molecules. Disulfide bonds are the covalent bonds formed between the sulfur atoms in the R group of the amino acid, cysteine. These disulfide bonds are the strongest bonds formed within the tertiary structure of proteins, behaving as "safety

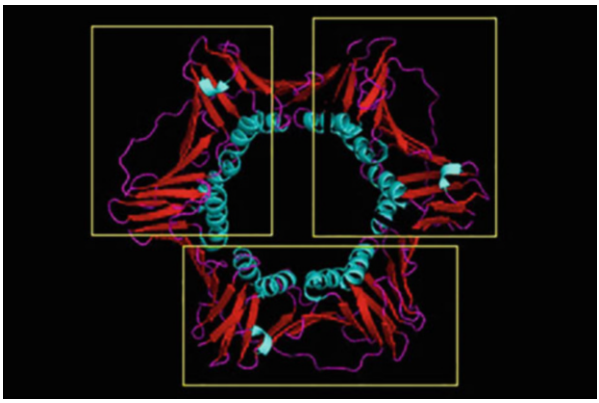


Fig. 14.5 Quaternary structure of proliferating cell nuclear antigen (PCNA) protein. The protein consists of three subunits (yellow square). From: "*Analisis Bioinformatika Mutasi S2281 Protein PCNA dan pengaruhnya pada Struktur 3 Dimensi Protein*" by Syarifah Dewi, 2017, Indonesian Journal of Biotechnology and Biodiversity, 1(1), p. 20–27

pins" to maintain the attachment between polypeptides (Murray 2009; Sanvictores and Farci 2022).

Quaternary structures of proteins are formed in proteins which are made of more than one polypeptide (Fig. 14.5). Every single polypeptide will conform to its structure until a stable tertiary structure is formed (henceforth called a subunit or protomer). Multiple subunits will join together to form the quaternary structures of proteins (Sanvictores and Farci 2022). Examples of proteins that have a quaternary structure include hemoglobin. Hemoglobin consists of 4 subunits (2 α and 2 β subunits). Interactions that have a role in the formation of most of quaternary structures include non-covalent bonds and are generally weak, such as hydrogen bonds, electrostatic and hydrophobic interactions (Nelson and Cox 2005; Murray 2009).

Every protein will have a final shape which is unique and specific toward its surrounding optimal environments, such as temperature and pH. Changes in temperature, pH, and interactions with specific chemicals will result in the loss of a protein's three-dimensional structure via a process known as denaturation (Nelson and Cox 2005). Proteins that are denatured still retain its primary structure, i.e. sequence of amino acids. However, all functions are lost. Most proteins that undergo denaturation may return to their original shape, if placed in an optimal environment. This renaturation process may occur independently or with the aid of proteins which are known as *chaperones*.

Protein Turnover

Within the body of an organism, proteins will undergo turnover, which is a process where a fraction of proteins degrade into amino acids, of which some are used again to synthesize new proteins. The body's reservoir of amino acids is maintained via amino acid absorption from food and degradation of intracellular proteins. All proteins have a specific half-life, which is the period of time required to degrade

proteins to half the original amount. Some proteins have a very short half-life, ranging between 5 and 20 min, whereas some proteins have half-lives that last hours and even days. As a result, proteins must be continuously synthesized and degraded inside the body. Proteins with sequences rich in proline (P), glutamate (E), serine (S), and threonine (T) amino acid, also known as PEST sequences, are known to have short half-lives (Lieberman and Peet 2018; Rodwell et al. 2018). Proteins that act as regulatory molecules like transcription factors will degrade quickly. High turnover rate of these proteins is essential for responding external stimuli. These proteins degrade quickly as a response toward specific signaling to regulate intracellular enzyme activity. Furthermore, damaged or incorrect protein structures can be easily recognized and degraded rapidly within the cell, resulting in fewer protein errors.

Within eukaryotic cells, there are two major protein degradation pathways: the ubiquitin–proteasome and the lysosomal proteolysis (Cooper 2000). The ubiquitin–proteasome pathway is the primary pathway for protein degradation that occurs selectively within eukaryotic cells, through protein targets marked with ubiquitin molecules for rapid proteolysis. Ubiquitin is a highly conserved protein in eukaryotes (including yeast, animals, and plants) and consists of 76 amino acids in length. Proteins that are bound with ubiquitin to its lysine residues will undergo degradation. Around four or more ubiquitin molecules further bind to the primary ubiquitin molecule to form multiubiquitin (or polyubiquitin) chains. After undergoing polyubiquitination, proteins will undergo degradation by the large multisubunit protease complex which is known as the proteosome. Ubiquitin is released after the protein is degraded, so that it can be reused for another degradation cycle. Amino acids that are released by this process enter the intracellular free amino acid pool.

The majority of proteins that consist of PEST sequences will be degraded by the ubiquitin–proteasome system (Ciechanover and Schwartz 1998; Spencer et al. 2004). Ubiquitination is a multistep process that begins with the activation of ubiquitin molecules by ubiquitin activating enzymes (E1). Next, the ubiquitin is transferred to another enzyme, known as ubiquitin conjugating enzyme (E2). Finally, the enzyme ubiquitin ligase (E3) will catalyze the binding of ubiquitin to the target protein for the selective identification of target protein. The E2 and E3 family of enzymes consist of different types of enzymes that recognize multiple protein substrates, and this specificity enables the selective targeting of cellular protein degradation through the ubiquitin–proteasome pathway.

The attachment of ubiquitin molecule to target protein requires ATP. This pathway is used to degrade proteins with a short half-life (Ciechanover and Schwartz 1998). Another primary pathway to degrade intracellular protein is the digestion of protein in lysosomes. Lysosomes are organelles containing the digestive enzymes including protease. These digestive enzymes have a role in the cell metabolism, extracellular protein degradation obtained by endocytosis, organelles, and intracellular protein turnover. Protein degradation via lysosomal proteolysis occurs when cellular proteins are ingested by the lysosomes. A major process of ingesting cellular proteins is autophagy, where vesicles (autophagosomes) are formed around small areas of cytoplasm or cytoplasmic organelles enclosed within a membrane formed

by the endoplasmic reticulum and sent to the lysosome (Ciechanover 2005; Eldeeb et al. 2020). Within the lysosome, the proteins will be degraded by cathepsin family protease into free amino acids and contribute to amino acid pool in cytosol. The absorption of proteins into the autophagosome is non-selective, and this pathway of protein degradation aims to degrade cytoplasmic proteins that have a long half-life. Multiple studies have shown that cellular hunger is a trigger to induce the autophagy process. Through autophagy, older proteins are recycled, and the resulting amino acids are used to synthesize new proteins. This occurs to ensure that the cell remains in a hunger state. One of the molecules essential for regulating autophagy is the mTOR kinase (Ciechanover 2005; Eldeeb et al. 2020). The role of mTOR kinase is to integrate and regulate various stimuli and signals for inducing protein, lipid, and nucleotide synthesis and inhibiting catabolic processes such as autophagy that occurs post-transcription and translation (Kim and Guan 2015).

The rate of protein degradation is influenced by multiple factors, including the requirement of specific amino acids for protein synthesis and the dietary protein intake. Amino acid requirement is determined by the differences in amino acid composition of various proteins that are synthesized, protein turnover rate, and amino acid recycling rate. Multiple conditions, including fasting or fed state, will induce the synthesis of some enzymes and degrade them when are no longer required. Furthermore, conditions that result in protein damage include oxidation and modifications that limit protein function. Protein degradation generated from either the ubiquitin–proteasome or the lysosomal pathway will produce free amino acids that can be utilized to synthesize new proteins or may be oxidized to produce energy. Every day, around 1–2% of total protein in the body is degraded, especially muscle protein. High degrees of degradation occur in tissues experiencing significant structural changes, such as in uterine muscles during pregnancy or structural muscles during starvation. Meanwhile, 75% of amino acids that are released by protein degradation will be reused, the remaining are not stored for future use. Amino acids that are not instantly used to form new proteins will be rapidly metabolized. Most of the carbon framework will be converted into amphibolic intermediates, whereas nitrogen released from the amino acids will be converted into urea and excreted via urine (Lieberman and Peet 2018).

Protein Turnover in Aging

The aging process can be triggered by decreased lysosomal function. The lysosomal degradation is closely related to autophagy, which destroys unwanted or damaged molecules that accumulate during aging. Lysosomes contribute to signaling level of the autophagic process, such as controlling the activity of mTORC1 kinase complex, an autophagy negative regulator that exerts the activity on lysosomal surfaces. Transcription factor EB (TFEB), which organizes both autophagy activation and lysosomal biogenesis, also located on the lysosomal surfaces. The interaction between lysosome and mTORC1 is facilitated by ATP-sensitive Na+ channel,

which directly participated in nutrient sensing. mTORC1 will be released from the lysosome under starvation condition, stimulates the activation to control pH stability and homeostasis of amino acid in response to low ATP levels. mTORC1 will inhibit nuclear translocation of TFEB and could not bind to its promoter, so some genes cannot be expressed (Carmona-Gutierrez et al. 2016).

Proteasome plays a role in clearance of damaged or abnormal proteins as well as for the degradation of short-lived proteins. Although the exact underlying mechanism is unclear, decreasing of proteasome activity suppress cellular capacity to remove damaged proteins and contribute to the development of age-related diseases. Several studies reported that declining of proteasome function was observed in aged mammalian tissues. Senescent human fibroblasts show reduction levels of proteasome activities. Replicative lifespan of fibroblasts treated by proteasome inhibitors is shortened and accompanied by a senescent-like phenotype. A study using a transgenic mouse with declined proteasomal activity shows a reduced lifespan and premature age-related phenotypes (Saez and Vilchez 2014).

Amino Acid Metabolism

Regulation of individual amino acid synthesis is a complex process; however, in general, it is driven by negative feedback signaling. An increase in the concentration of free amino acids will cause the inhibition of biosynthetic enzymes via allosteric regulation or gene expression. Levels of amino acids are constantly maintained at a certain value, to ensure that protein synthesis occurs continuously through the aminoacyl tRNA synthetase activity. Amino acid degradation pathways are generally different from biosynthetic pathways, which allows for regulatory separation for the anabolic and catabolic pathways. Since proteins can be used to generate energy, almost every amino acid degradation pathway will produce NADH molecules and transfer its electron in mitochondrial oxidative phosphorylation. Besides that, amino acids can be used for energy production directly via oxidation, undergoing conversion into an intermediate in the TCA cycle, which is converted into glucose or ketone bodies that can be oxidized eventually. The fate of amino acids depends on the individual physiological condition, for example in the fasting state, the liver will convert the carbon structure of amino acids to glucose, ketone bodies, and CO_2. However, in the fed state, the liver will convert the metabolites of amino acids into glycogen and triacylglycerol (Lieberman and Peet 2018; Rodwell et al. 2018).

Eleven of the twenty amino acids can be synthesized by the body and the remaining “essential” amino acids must be obtained through dietary sources. Nine of the eleven “non-essential” amino acids are produced from glucose and nitrogen sources (from other amino acids or ammonia). The remaining two non-essential amino acids require other essential amino acid for their synthesis, such as tyrosine requires phenylalanine and cysteine requires methionine. The carbon backbone of serine, glycine, cysteine, and alanine is synthesized using the metabolites of glycolysis, while remaining non-essential amino acids require metabolites of the TCA

cycle. Glutamate, glutamine, proline, and arginine could be synthesized from α-ketoglutaric acid, whereas aspartate and asparagine could be synthesized from oxaloacetate (Lieberman and Peet 2018).

Metabolism of amino acids is dependent on the fate of the two atoms that form the structure of amino acids, carbon and nitrogen atoms. Nitrogen atoms when released from carbon structure will be converted into urea in the liver, and carbon atoms will be oxidized into CO_2 and H_2O in some tissues. The concentration of plasma amino acids that are circulating between meals will depend on the equilibrium between the output of proteins from endogenous sources and its use in several tissues. Muscles produce more than half of the total free amino acids in the body and the primary site for removal of nitrogen atoms via the urea cycle is the liver. As a result, muscles and liver play a primary role in maintaining amino acid levels in the blood (Lieberman and Peet 2018).

Several enzymes are essential in interconverting amino acids and removing nitrogen, including transaminases, glutamate dehydrogenase, glutaminase, and deaminase. The transaminase reaction plays a role in the biosynthesis of almost all amino acids, except threonine, lysine, proline, and hydroxyproline. This reaction is a reversible reaction between amino acids and α-keto acids. Alanine transaminase or glutamate-pyruvate transaminase catalyzes the transfer of amino groups from alanine to α-ketoglutarate forming pyruvate and glutamate. Alpha-amino nitrogen from all amino acids will undergo transamination and is concentrated in glutamate. The L-glutamate molecule becomes essential since it is the only amino acid that undergoes oxidative deamination at a large scale within mammalian tissues via the activation of the L-glutamate dehydrogenase (GDH) enzyme, a reaction that uses NAD+ and NADP+ to release nitrogen into the form of ammonia. The conversion of α-amino nitrogen into ammonia via glutamate aminotransferase and GDH is also known as "transdeamination" (Lieberman and Peet 2018; Rodwell et al. 2018).

Glutamine formation from glutamate is catalyzed by mitochondrial glutamine synthetase. Glutamine synthesis occurs due to the attachment of NH_3 groups to glutamate, requiring ATP. Conversely, the hydrolytic cleaving of NH_3 groups from glutamine is catalyzed by glutaminase, producing glutamate. Glutamine synthetase also functions to provide glutamine as nitrogen carrier between organs, detoxify ammonia, and maintain acid–base homeostasis. The integrated activity of glutamine synthetase and glutaminase aids in the interconversion between free ammonium ions and glutamine (Rodwell et al. 2018).

The end product of nitrogen catabolism in humans is urea. Liver is the primary organ to convert the nitrogen of amino acids into urea. The urea cycle produces urea using the substrates NH_4+ ions, bicarbonate ions, and nitrogen from aspartate. The cycle begins with a reaction of NH_4^+ ions, bicarbonate ions, and ATP to produce carbamoyl phosphate, which further reacts with ornithine to produce citrulline. Citrulline will react with aspartate to produce argininosuccinate, which will later release fumarate and forming arginine. The final reaction involves the cleaving of arginine by arginase to release urea and generate ornithine, after which the cycle repeats itself. The urea cycle is regulated by a feed-forward mechanism, the rate of urea cycle will increase when amino acid degradation occurs. Other than excreted

via urea, the nitrogen atom is also excreted in other forms, such as uric acid, creatinine, and ammonia. Uric acid is the degradation product of purine bases, creatinine is produced from creatinine phosphate, and ammonia (NH_3) is produced from releasing amino group glutamine in the kidneys, which will react with protons to form ammonium ions (NH_4^+) in urine. After nitrogen is removed from amino acids, the carbon structure undergoes oxidation to produce pyruvic acid or acetyl coenzyme A (Acetyl-CoA) (Lieberman and Peet 2018; Rodwell et al. 2018).

In a fasting or hunger state, muscle proteins are broken down into amino acids, and a portion of which is oxidized to produce energy, the remaining is converted into alanine, glutamine, and other amino acids that are released into the bloodstream. Alanine is mostly extracted by the liver, and glutamine is extracted by the intestines and kidneys. A majority of the glutamine will be converted into alanine. Alanine and other amino acids enter the liver, the nitrogen atom will form urea, and the carbon atoms will convert into glucose and ketone bodies. Glucose which is produced via gluconeogenesis is further oxidized into CO_2 and H_2O in various tissues in the body. Ketone bodies could be utilized as an energy source in brain, muscle, and kidneys. Muscle can store branch chain amino acids as its energy source. These branched-chain amino acids (valine) could be released by the muscle and used by the brain for energy (Howarth et al. 2010; Lieberman and Peet 2018; Rodwell et al. 2018; Anthony et al. 2000).

Amino Acid Metabolism in the Elderly

Basal metabolism of amino acids is not affected by age, however, aging leads to a reduced ability to respond toward anabolic stimuli such as insulin. Studies have shown a reduction in muscle protein synthesis in geriatric subjects when compared to young subjects, due to insulin resistance, which is believed to cause sarcopenia in geriatric subjects (Wilkes et al. 2009). Prior studies have concluded that changes in amino acid metabolism due to aging may be controlled by supplementation of leucine, changes in protein intake, or physical activity, all of which increase muscle protein synthesis (Anthony et al. 2000).

Imbalances between muscle protein degradation and synthesis in elderly subjects are caused by insulin resistance that affects muscle protein metabolism. Insulin could inhibit muscle protein breakdown, which is mediated by nutrients and slows down proteolysis without the presence of hyper-aminoacidemia, hence increasing muscle protein equilibrium (Churchward-Venne et al. 2014). Insulin resistance is caused by a reduction in vasodilation that involves endothelium (Rasmussen et al. 2006). Previous studies have shown a relationship between endothelial dysfunction and anabolic resistance of skeletal muscles, showing that impairment of insulin-mediated vasodilation causes a reduction in capillary recruitment and microvascular perfusion of skeletal muscle, hence reducing the availability of amino acids. Studies have also demonstrated a reduction in muscle protein synthesis among the elderly upon administration of insulin, compared to young subjects. However, when

microvascular perfusion and blood flow are pharmacologically stimulated (via administration of nitroprusside) to maximum capacity, muscle protein synthesis can be increased to levels comparable to young subjects after insulin administration (Timmerman et al. 2010). Hence, insulin can increase muscle protein synthesis; however, this ability must be accompanied with an enhancement in amino acid supply and muscle microvascular perfusion. This shows that vasodilation and adequate nutrition to the muscle are essential regulatory factors of anabolic responses of a muscle stimulated by insulin (Churchward-Venne et al. 2014).

Protein Changes in Aging

Aging is a process of accelerated cellular degeneration and tissue homeostasis that can cause dysfunction, morbidity, and mortality. The life span of proteins is generally shorter than that of a cell and the organism. As a result, an improvement in protein activity underlies the improvement of an organism's quality of life. The complexity of aging and associated diseases reflects the overall complexity of healthy organisms. The root cause of the aging process is oxidative damage to proteins. Cellular aging is caused by missense errors in the structure and functions of proteins. In the process of protein biosynthesis, errors in protein folding or misfolding can cause the induction of chaperone molecules to correct them. Proteins that are not misfolded are generally more stable and functional. However, misfolded proteins are more easily identified by reactive oxygen species (ROS) that cause oxidative stress and damage. Damaged proteins due to oxidative stress will undergo proteolysis or aggregation, leading to dysfunction. Oxidative damage of proteins is a predictor of cell death (Krisko and Radman 2019). Aging causes multiple changes in proteins, such as lack of proteostasis, loss of muscle protein (sarcopenia), and changes in structural proteins such as elastin and collagen.

Loss of Proteostasis

Dyshomeostasis of proteins is one of the symptoms of aging. The proteostasis network is a multicompartmental system. This system coordinates synthesis, folding, aggregation, and disaggregation. Besides the common factors required to synthesize and maintain proteins, the expression of various components of proteostasis is affected by specific proteomic demands from cells and various tissues. Proteostasis can be achieved through coordinated action from various proteins that are collectively known as the proteostasis network. A proteostasis network is defined as a network of proteins that play a direct role in the synthesis, degradation, disaggregation, or folding of proteins. This definition includes the translation engine, chaperone molecules and co-chaperones, the ubiquitin–proteosome system, and autophagy (Labbadia and Morimoto 2015). In addition, the unfolded protein response (UPR)

is also included in that system. Chaperone molecules known as heat-shock proteins can recognize polypeptides that undergo folding errors and eventually correct their structure or send them to the ubiquitin–proteosome system for degeneration.

Autophagy consists of four different systems: macro-autophagy, micro-autophagy, selective autophagy, and chaperone-mediated autophagy (CMA) with different target substrates or complete supramolecular protein structures such as mitochondria or mechanism of function. The ubiquitin–proteosome degradation system targets polypeptides that are not required by labeling them with ubiquitin, for breakdown down by proteosomes. UPR is a stress reaction activated by an overabundance of unfolded proteins in cell organelles particularly in the endoplasmic reticulum. An unconstrained activation is related to multiple pathologies, including neurodegeneration, cancer, metabolic diseases, and inflammation. Recent studies have shown that in aging a loss of efficacy of the proteostasis system occurs. A change in the proteostasis capacity during early adulthood is marked by the reduced capacity of protein folding and formation of protein aggregates (Labbadia and Morimoto 2015).

Sarcopenia

The primary storage of amino acids in the body is found in skeletal muscles that contain 50–70% of all proteins in the human body. Skeletal muscles play a role in body movement and posture, as well as an essential amino acid storage system that can be used for energy by the immune system and brain during hunger or malnutrition. Aging in humans is marked by muscle mass reduction and function, named sarcopenia. This degenerative muscle reduction amounts to 3–8% per decade after the age of 30 and is accelerated by aging.

Loss of muscle mass is generally affected by two factors which are atrophy and cell death, which can cause loss of muscle strength and mass. Sarcopenia is associated with a deceleration of metabolism, loss of muscle strength, increased fall risk and fracture, increased morbidity, and lack of independence (Timmerman and Volpi 2008).

The mechanism of sarcopenia is most likely attributed to the increased rate of muscle protein degradation rather than muscle synthesis. This disproportion between synthesis and breakdown can occur slightly in fasting or hunger states, infection, and injury. However, if not treated, it can lead to significant and steady muscle loss. Numerous research studies have shown that muscle protein degeneration does not change with aging; however, reduced protein synthesis is observed with age (Timmerman and Volpi 2008; Churchward-Venne et al. 2014). At a molecular level, changes of important factors for synthesis and degradation of protein occur with age. Sarcopenia is a multifactorial process, including a neurological process associated with a loss of motor neurons, endocrine change caused by reduced or loss of hormone expression, loss of motor units of muscles and changes in nutrition and a lifestyle that is sedentary. Low activity is usually associated with a diet rich in

saturated fats, causing an increase in fat deposits within adipose tissues, liver, and muscles. Hence, a high-fat diet can affect the composition and structure of skeletal muscles. There also exist changes in transcription regulation at the level of myocytes that induce proteolytic activity (Choi 2016; Pascual-Fernandez et al. 2020).

Changes in Collagen Fibers

Collagen fibers are a primary component of the extracellular matrix of the skin's dermis layer. Around 75% of the skin's dry mass comprises collagen that primarily functions to maintain tightness and skin elasticity. There are multiple types of collagen in the skin, including type I, which is composed of 80–90% of total collagen in the body, type III is around 8–12%, and the remaining type V that is <5%. Quantitative and structural alteration in collagen fibers are the primary changes formed in aging. Collagen fibers in the aging skin are fragmented and distributed randomly. There is an increase in collagen degradation and reduction in collagen biosynthesis resulting in disruption of collagen homeostasis leading to collagen deficiency. This condition induces clinical alteration, including wrinkling and reduction of skin elasticity (Shin et al. 2019).

Changes in Elastin Fibers

In addition to collagen fibers are elastin macromolecule components that are durable in high-order vertebrates and function to provide elasticity and recoil ability to various tissues and organs as well as lungs, blood vessels, skin, and many more. Elastin is made of the monomer tropoelastin, which crosslinks with lysine residues. In the aging process, elastin fibers are primarily affected by two factors; intrinsic and extrinsic. Intrinsic factors include enzymatic degradation by serine proteases, glycation processes, and calcification that leads to calcium deposits in elastic tissues which is a primary factor in the formation of atherosclerosis. Furthermore, intrinsic factors that play a role also include oxidative damage that leads to structural and functional molecular changes and the progressivity of cardiovascular disease, racemization of aspartic acid from D-aspartate into L-aspartate, carbamylation of plasma proteins that increases the risk of type 2 diabetes mellitus, increase in cholesterol binding by elastin peptides that can cause an increased risk of atherosclerosis as well as mechanical fatigue of the elastin fibers that can lead to the clinical manifestation of angina pectoris.

Extrinsic factors that play a role in aging of elastin fibers among others include ultraviolet light that leads to wrinkling, loss of elasticity, and skin thickening that can be histologically proven by the presence of elastotic materials. Furthermore, smoking is also an essential external factor that causes premature skin aging, marked by skin wrinkling and loss of elasticity. The effect of aging on elastin fibers are

fragmentation and thinning of the elastic structure. This condition can cause dysfunction, loss of tissue elasticity, and even organ function (Heinz 2021).

Amino Acid Supplementation in the Elderly

Aging is an unavoidable process, and the proportion of elderly individuals is rising globally. Hence, intervention is required to create healthy conditions for the quality of life for elderly populations, which includes nutrition (Fukagawa 2013). Currently available data have shown that as high as 8% of the elderly community suffer from malnutrition, 35% are at risk of malnutrition, and 66% of the geriatric population in hospitals are at risk of malnutrition (Geurden et al. 2015). Protein energy malnutrition has shown to elevate morbidity and mortality risk in the elderly (Sullivan et al. 2002). This form of malnutrition can also impair the immune system since immune cell function relies on the availability of energy and amino acids, hence leading to an increase in viral infections with increasing age (Alam et al. 2019). Furthermore, aging is associated with sarcopenia that worsens with a lack of physical activity and malnutrition commonly found in the elderly population. The high prevalence of sarcopenia is found in the elderly populations living in nursing homes, geriatric populations with clinical conditions (including osteoporosis, obesity, and cancer), or elderly individuals recovering from injury and disease. Inactivity for a brief period due to hospital stay or injury can cause anabolic resistance and worsen the level of sarcopenia in the aging population. As a result, optimal energy and protein intake in the elderly population is essential for the management and prevention of sarcopenia (Ispoglou et al. 2021). Proteins and associated amino acids are primary components in food which play a role in maintaining a healthy life, especially when individuals experience stress due to injury or disease (Fukagawa 2013). Studies have shown that the supply of amino acids is essential in regulating muscle protein metabolism (Biolo et al. 1997). Increases in amino acid levels in the blood will stimulate muscle protein synthesis via the increase of the amino acid transport into muscle cells (Biolo et al. 1997). Studies have proven that essential amino acids are the most efficient nutrients to empower synthesis of muscular protein in aged subjects and young individuals, although the cellular mechanism responsible for this is still being studied (Paddon-Jones et al. 2004). Among essential amino acids, branched-chain amino acids (BCAA) are the main amino nitrogen transporter between visceral and peripheral tissues, notably skeletal muscles, and play a role in stimulating protein synthesis directly (Yoshizawa et al. 1998). Generally, the number of ribosomes present in a cell and each ribosome's translational efficiency affect how quickly protein synthesis can be accelerated. Leucine can activate multiple signaling pathways that are involved with the initiation of translation, signaling pathway mammalian target of rapamycin (mTOR), protein ribosomal S6 kinase 70-kDa (S6K1), and eukaryotic initiation factor 4E binding protein-1 (4E-BP1) (Anthony et al. 2000). The maintenance of skeletal muscle mass is dependent on the equilibrium of muscle proteins,

where the rate of degeneration must be balanced with the rate of muscle protein synthesis (Murton 2015).

Studies have shown that protein intake in the weak elderly is lower, and hence requires supplementation to achieve optimal health (Fujita and Volpi 2006). However, elderly individuals also suffer from a loss in appetite (Johnson et al. 2020), difficulty in chewing, dysphagia, and dysgeusia (Landi et al. 2016). Furthermore, aging is also associated with changes in the gastric emptying process and amino acid absorption (Ispoglou et al. 2021). The inability of amino acid use in the elderly population is caused by changes in the muscle fibers including a lack of amino acid transmembrane transporters, lack of substrates to synthesize proteins due to changes in protein turnover in the body, and changes in muscle responses toward hormonal stimuli after feeding (Johnson et al. 2020). As a result, optimal strategies are required in the form of nutritional intervention for the elderly, to achieve protein energy equilibrium (Ispoglou et al. 2021). High-quality protein consisting of complete essential amino acids must be consumed by elderly individuals to maintain the health of skeletal muscles. Some points that must be considered to optimize protein intake include: (a) daily consumption of protein, (b) consumption of protein in every meal, and (c) protein quality assessed by presence of essential amino acids (Ispoglou et al. 2021). The role of essential amino acids as primary regulators of protein synthesis has been studied by comparing muscle protein synthesis responses between low-dose amino acid combinations (3.2 g essential amino acids; 2.4 g protein), high-dose amino acid combinations (6.4 g essential amino acids; 4.4 g proteins), and protein supplementations consisting of 17.9 g of whey (Park et al. 2020). Consumption of a combination of whey protein and essential amino acids stimulated a greater anabolic response compared to consumption of whey protein only. An increase in serum amino acids occurred rapidly after consumption of high-dose essential amino acids. This showed that the presence of enough essential amino acids and leucine in the diet is required to promote a good skeletal muscle anabolic response in the elderly.

The composition of amino acids in protein sources can greatly affect protein synthesis postprandial (Traylor et al. 2018). Leucine content in protein sources is essential in decelerating muscle mass loss when consumed with other essential amino acids. As a result, elderly populations require higher levels of leucine compared to other essential amino acids to stimulate synthesis of muscular protein (Devries et al. 2018). Multiple researches have exhibited that synthesis of muscle protein in aging mice does not undergo changes or resistance after supplementations of leucine in physiological concentrations (Dardevet et al. 2000). This resistance may result from a problem with leucine's ability to stimulate S6K1 activity. A study has shown that myofibrillar and sarcoplasmic protein synthesis response and sensitivity is lower in elderly populations after the supplementation with essential amino acids in divided doses, which is attributed to a decreased mTOR signaling activity (include S6K1 and mTOR) (Cuthbertson et al. 2005). Despite that, in aging mice, inadequate induction of synthesis of muscle protein can be reversed by increasing leucine concentrations (Dardevet et al. 2000). Arnal et al. (1999) show that the anabolic response from protein turnover is in normal ranges among elderly subjects

if 80% of daily protein requirements are given at once compared to protein intake divided equally in the food consumed throughout the day, hence an abundance of amino acids is required by the aged population to achieve an anabolic effect comparable to young adults. In addition, another study showed that in elderly subjects who consume limited amounts of essential amino acids (6.7 g) with a high proportion of leucine (2.8 g), the synthesis of muscle protein can be significantly increased. However, supplementing with low doses of amino acids with a lower proportion of leucine has no discernible benefit (1.7 g) (Katsanos et al. 2005). This shows that skeletal muscles in elderly populations are insensitive to the stimulatory effects of amino acids, especially low concentration leucine, however this can be managed by supplementations of high-dose leucine.

Houston et al. (2008) reported that healthy elderly who consume higher than the recommended daily allowance (RDA) of proteins is associated with lower muscle mass loss throughout a 3-year follow-up. However, elderly individuals that consume only slightly higher than the RDA of protein (that is up to 1.1 g/kg/per day) still experience a loss in muscle mass. Research by Mitchell et al. (2017) proved that an increase in protein consumption roughly twice the RDA (that is 1.6 g/kg/day) resulted in an increase in lean muscle mass in healthy elderly males. Elderly individuals require higher protein intakes in every meal due to the reduced sensitivity toward anabolic stimuli such as proteins, also known as "anabolic resistance" (Breen and Phillips 2011). The lack of physical activity or muscle use in a specific time period (immobilization of limbs due to injury and disease) can stimulate anabolic resistance in the elderly, which is shown by the reduced expression of amino acid transporters in skeletal muscles, signaling errors of mTORC1 and decrease the synthesis of protein in response to essential amino acids (Breen et al. 2013). Elderly individuals require a protein dose of 0.4 g/kg body weight (that is roughly 30 g of protein for a normal elderly man) to stimulate optimum postprandial protein synthesis, or roughly 67% greater dose compared to healthy adults (Moore et al. 2015). As a result, a renowned international association, that is the Society for Clinical Nutrition and Metabolism, recommends an increased protein intake for elderly individuals (Deutz et al. 2014) which is a recommendation of: 25–50% more for healthy elderly adults, 50–90% for geriatric population with acute or chronic illness, and a rise of more than 50% over the RDA for elderly individuals suffering from severe illness or injury.

Acute impact of essential amino acids supplementation toward muscle protein turnover has been widely studied and shown to act as a regulator of muscle protein synthesis (Wilkinson et al. 2018; Park et al. 2020). However, long-term effects of amino acid supplementation on the modulation of muscle mass, strength, and function of muscles in the elderly have not yet been studied. The anabolic response of leucine is only noticed with the existence of amino acids, especially essential amino acids, since the increase in muscle protein synthesis requires the availability of all essential amino acids (Moberg et al. 2016). Therefore, essential amino acids composition must also be considered, since a high relative ratio of leucine is needed to induce maximum synthesis of muscle protein in the elderly (Katsanos et al. 2005). Intervention, in the form of essential amino acid supplementation, has shown

Table 14.1 Dietary source of protein contains 8 g of essential amino acid

Food type	Amount (g)
Chicken (breast)	74
Canned tuna fish	74
Ham	79
Lean beef meat	97
Codfish	97
Cheese	105
Mortadella	131
Eggs	138
Trout	153
Whole milk	480

numerous beneficial effects on the functions and mass of muscles, in patients with obesity or sarcopenia (Kim et al. 2012; Zhou et al. 2018), with other studies performed on male and female elderly subjects with glucose intolerance (Borsheim et al. 2008). In this study, a majority of essential amino acid supplementation was given between meals. Supplementation of essential amino acids given twice a day as much as 4 g (total 8 g) given between meals was shown to increase the life quality, physical fitness, and dietary habits (Rondanelli et al. 2011). In concordance with these findings, lean muscle mass throughout the body increased after 1.5 years with daily consumption of 16 g of essential amino acids (8 g given twice daily in the form of snacks between meals) to elderly subjects with sarcopenia (Solerte et al. 2008), as well as an improvement in insulin sensitivity and a significant increase in insulin-like growth factor 1 (IGF-1). Even though muscle protein synthesis was not analyzed in this research (Solerte et al. 2008), the IGF-1 levels rising after consumption of essential amino acids is related with the higher stimulus protein synthesis in muscle. These results are supported by Dillon et al. (2009), whose findings showed that there was an increase in muscle protein synthesis among subjects of elderly obese females who were given essential amino acid supplementation, as well as an increase in lean body mass and IGF-1 protein expression in the muscle cells. Numerous dietary sources of proteins that contain 8 g of essential amino acids are seen in Table 14.1 (Aquilani et al. 2014).

Supplementation of 7.5 g of essential amino acids given twice a day (15 g daily) that consist of 18.6% of L-leucine results in significant enhance lean body mass (approximately 3.9%) after 90 days consumption (Aquilani et al. 2014), albeit a lack of increase in muscle power. Studies conducted in women with sarcopenia (Kim and Guan 2015) showed that a supplementations regimen of 3 g of leucine-rich essential amino acids given twice a day (6 g) for 90 days did not significantly increase functional performance or muscle power. However, it improved walking pace, compared to initial conditions or the control group. In a related study, consumption of 10 g of essential amino acids in the form of light meals twice a day (total 20 g daily) for 28 months in addition to strict diet control showed an increase in index of appendicular skeletal muscle in sarcopenic and obese subjects (Zhou et al. 2018). Research showed that supplementing 11 g of essential amino acids include

L-arginine (22 g per day) twice a day between meals for 16 weeks period increase physical and performance function of elderly subjects with hyperglycemia (Borsheim et al. 2008). Lean body mass in this research raised by roughly 1 kg around week 12 compared with baseline, but did not rise around week 16 postintervention (Borsheim et al. 2008). Despite the positive effect of essential amino acid supplementation on muscle weight among patients with sarcopenia, obesity, or altered health condition, the positive effect in functional performance and muscle strength is not clear. Variation in methods used (such as consumption between meals), amount and ratio of amino acids consumed, and lack of adjunctive workout intervention along with amino acid supplementations are plausible causes for the different results obtained. Despite that, early evidence shows that essential amino acid supplementation can contribute to the increase in muscle function and strength, with a low level of evidence showing an enhanced muscle mass (Ispoglou et al. 2021).

Markofski et al. (2018) gave 15 g of essential amino acid to two healthy elderly adults over 24 weeks, showing no overall increase in lean body mass. In this study, although the amino acid supplementation was strategically given between meals, it is not known whether the total daily protein intake reached the recommended daily allowance since this was not measured. Conversely, when dietary intake of protein in elderly individuals undergoing complete bed rest for 10 days was raised by 1–4 g/kg/day (3 × 15 g essential amino acids), muscle activity was preserved. However, overall muscle mass had decreased even with enhanced dietary protein and muscle protein synthesis. This occurrence supports the assertion that muscle function is preserved due to the recruitment of more functional fibers while inactive. A study showed that among healthy elderly individuals, an increase in functional performance and lean muscle mass was observed after 3 months of essential amino acids supplementation with a dose of around 7.5 g of essential amino acids rich in leucine (40%) twice a day (total 0.21 g/kg/day or 15 g/day) (Ispoglou et al. 2016).

Anabolic responses from essential amino acid supplementations increased with exercise, a majority of which occurred through the synergistic effects of mTOR (Dickinson et al. 2011). Early researchers showed that acute muscle protein synthesis response in elderlies who consume 20 g of essential amino acids 1 h after exercise in the form of bilateral leg extensions is higher, compared to a young adult (Drummond et al. 2008). However, the phosphorylation status of the primary intramyocellular signaling target within the mTORC1 pathway (i.e., mTOR, 4E-BP1, eEF2 and S6K1) was not different between different age ranges. Hence, it is recommended to optimize both the anabolic stimuli (that is essential amino acids/proteins and resistance exercise) which can effectively overcome anabolic resistance in the elderly as well as increase anabolic response and adaptation (Moro et al. 2018). Combinations of resistance training and essential amino acid or protein supplementation that are applied to an individual suffering from sarcopenia and or weakness can be more benefit, compared to healthy elderly adults. Kim et al. (2012) reported an increase in the strength of knee extension (9.3%) among elderly Japanese females living in communities with sarcopenia, after consumption of essential amino acid supplementations rich in leucine twice a day (6 g

Table 14.2 Composition of 8 g essential amino acid for chronic heart failure and chronic obstruction pulmonary disease patient (Aquilani et al. 2014)

Composition of amino acid	Dose (mg)
L-tryptophan	20
L-tyrosine	30
L-methionine	50
L-phenylalanine	100
L-histidine	150
L-cysteine	150
L-threonine	350
L-valine	625
L-isoleucine	625
L-lysine	650
L-leucine	1250

daily; 42% leucine) along with comprehensive physical training twice a week (resistance type activity) for 3 months. The leg muscle mass had increased in groups receiving both supplementation and exercise and those receiving exercise only. Other studies have proven that resistance training, when combined with essential amino acid supplementation provides benefits for sarcopenic elderly people healing from trauma. In a randomized control trial, subjects with pelvic fracture due to osteoporosis were recruited, and daily essential amino acid supplementation was provided to the treatment arm for 2 months (total 8 g daily; around 30% leucine) which was combined with a physical training program including resistance type training. Results showed that the intervention significantly increased total grip strength with optimum time and performance (Park et al. 2020). As a result, evidence shows that the elderly recovering from injury, such as pelvic fractures, may be able to increase muscle strength and function with the consumption of essential amino acids in addition to resistance type training activity (Drummond et al. 2008).

Research has consistently shown that supplementation of essential amino acids (8 g per day) composed of ingredients listed in Table 14.2, to geriatric subjects with chronic obstructive pulmonary disease (COPD) and chronic heart failure (CHF) can increase their exercise tolerance after 1–3 months of supplementation (8 g per day). In elderly with chronic heart failure, the exercise capacity increased from 8.7% to 23% (watt; cycle test), and increased from 12% to 22% (meters) in the 6 min' walk test. In addition, plasma lactate levels of patients decreased during rest (as much as 25%) and sensitivity of tissue insulin increased by roughly 16%. Geriatric patients with COPD received similar benefits as elderly subjects experiencing CHF. Physical activity increased as much as 78.6% of steps per day and plasma lactate concentration reduced while resting by as much as 23%. It can be concluded from this research that those essential amino acids can increase the aerobic metabolism of muscles, function and mass of muscles, and tissue insulin sensitivity (only among geriatric subjects with chronic heart failure). Physiological activity increased with essential amino acid supplementation due to the formation of mitochondria and myofibrils of skeletal and cardiac muscles, including control of blood glucose (Aquilani et al. 2014).

Supplementation of essential amino acids both directly and indirectly increases the capacity of muscle cells to process high energy compounds which are essential to increase the performance and muscle strength in elderly people. This occurs in all elderly individuals both healthy and those suffering from CHF or COPD (Aquilani et al. 2014). Essential amino acid supplementation can cause more efficient aerobic metabolism due to the reduction in insulin resistance (Solerte et al. 2008). Essential amino acids can directly increase their synthesis of insulin receptors and its autophosphorylation process (Solerte et al. 2008). The reduction in insulin resistance reduces the blocking of the cellular pyruvate dehydrogenase enzyme complex by inflammatory cytokines and insulin resistance that circulate in both COPD and CHF (Aquilani et al. 2008). Activity of this enzyme complex increases the production of energy from glucose oxidation. In hearts of mice induced with diabetes, long-term oral essential amino acid supplementation increases cytochrome C mitochondrial activity and NADH activity, as well as significantly shifting the heavy chain of ventricular myosin toward a quicker phenotype (Pellegrino et al. 2008). Both in healthy and diabetic rats, essential amino acid supplementation modulates skeletal muscle redox status via the increase in antioxidant defense systems, which is shown by an increase in superoxide dismutase (SOD) enzyme expression and simultaneously reduces heat-shock protein levels (Brocca et al. 2008). Essential amino acids may decrease resistance of insulin by reducing circulating tumor necrosis factor alpha (TNF-α) cytokines. It has been shown to occur in geriatric patients with sarcopenia (Solerte et al. 2008).

Supplementations of leucine-rich branched-chain amino acids increase protein synthesis in adipose tissues, liver, and skeletal muscles by increasing the action of protein synthesis engaged in translation of mRNA (Anthony et al. 1999). In geriatric populations with COPD/CHF, leucine (as well as other essential amino acids) acts as nutritional signaling molecules that do not depend on insulin (Anthony et al. 1999). However, insulin signaling is regulated by amino acids through nutritional signaling mTOR and the availability of amino acids is essential for the anabolic activity of insulin (Dickinson et al. 2011). Conversely, the lack of amino acids can lower mTOR activation despite increased insulin signaling (Dickinson et al. 2011). Supplementation with exogenous essential amino acids, even in small doses, can induce protein metabolism in muscle (Volpi et al. 2003). Another pathway where essential amino acids induce protein synthesis includes the increase in anabolic hormone production in the liver, which is the insulin-like growth factor 1 (IGF-1). This hormone depends on the presence of essential amino acids in the blood and is active only when the amino acids are in optimum quantity. A study has reported that 7.5 g of amino acids increases levels of IGF-1 (Dillon et al. 2009). Essential amino acids can stimulate protein synthesis by decreasing breakdown of muscular protein. In a study with human subjects, protein utilization efficiency was more dependent on its low sensitivity toward proteolysis instead of a change in protein synthesis (Kadowaki and Kanazawa 2003). Leucine is an amino acid that can prevent protein breakdown in the heart, and its suppression action is facilitated by extracellular leucine (Chua 1994). Conversely, transamination of branched-chain amino acids in the heart occurs three times more compared to skeletal muscles. The effect of

essential amino acids toward the myocardium is proven by the presence of left ventricular dysfunction improvement among healthy elderly adults and a rapid improvement of postexercise VO_2 among geriatric subjects with CHF (Aquilani et al. 2008).

Creatine Supplementation in the Elderly

Creatine is a nonprotein compound containing nitrogen which can be obtained primarily from meat, poultry, and fish. It is also synthesized from glycine, arginine, and methionine in the kidney and liver. In the kidney, glycine and arginine react to produce guanidinoacetate. Methylation of guanidinoacetate using S-adenosyl methionine (SAM) occurs in the liver to produce creatine. One produced, creatine is transported to tissue, in which approximately 95% is located in muscle and the rest is located in brain and kidney. About 1–2% of creatine in muscle is spontaneously cyclized to form creatinine which excreted as a waste product in urine (Gropper and Smith 2013). In order to create normal creatine level, 1000–3000 mg/day of creatine is needed. This requires that daily about 50% can be obtained from food sources, the rest contribution derived from endogenous synthesis of creatine (Brosnan and Brosnan 2016). The important role of creatine in muscle is to provide an energy reserve due to the ability of creatine to react with inorganic phosphate to produce phosphocreatine which is catalyzed by creatine kinase. Hydrolysis of phosphocreatine provides free energy (inorganic phosphate) which is used to regenerate Adenosine Triphosphate (ATP) (Gropper and Smith 2013).

Creatine supplementation in elderly has the potential benefit to enhance muscle mass and force, diminish the risk fall as well as reduce mineral bone loss (Candow et al. 2019). Stout et al. (2007) concluded that supplementation of 20 g/day creatine for 1 week, continued with 10 g/day for 1 week improve the strength of hand-grip in aging subjects. Another researcher found that 30 days of creatine supplementation with two different doses, 10 g/day for the first 10 days and 4 g/day for the remaining 20 days alleviated muscle exhaustion in the lower body of elderly men aged 60–82 years (Rawson et al. 1999). Moreover, Gotshalk et al. (2002) showed that 0.3 g/kg/day of creatine supplementation for 7 days raised muscle power and function in aging subjects. In ten elderly women, 0.3 g/kg/day of creatine supplementation for 1 week ameliorated physical performance of lower extremity. In addition, creatine supplementation stimulates expression of regulatory factors for muscular protein synthesis such as myogenin, Myo-D, IGF-1, Myf5, and MFR-4, as well as enhance the number of satellite cells (Willoughby and Rosene 2003). However, a controversial result from Chami and Candow (2019) showed that 0.1–0.3 g/kg of creatine supplementation for 10 days did not improve physical performance, resistance, and muscle power in aging subjects. In addition, 1 g/day of creatine supplementation for long-term period (1 year) did not provide advantage for muscle of post menopause old female (Lobo et al. 2015). Beside the function of creatine supplementation toward muscle, many researches also clarified the function

of creatine supplementation toward the brain, especially to remedy cognitive function. However, most of this study was conducted in healthy young subject and limited in old subjects (Roschel et al. 2001). One study by McMorris and colleagues proved that 20 g/day of creatine supplementation for 1 week in 15 subjects of elderly aged 76.4 ± 8.48 years old (8 men and 7 women) could increase cognitive performance, using a memory test (Terry et al. 2007). Clearly, more studies are needed to elaborate the benefit effect of creatine supplementation to brain in elderly.

Vitamin and Mineral Supplementation in the Elderly

Young and old people have different nutritional demands. Energy requirements decrease proportionally with age as physical activity level, muscle mass, and basal metabolic rate decrease (Wurtman et al. 1988; Ahmed and Haboubi 2010). Aging, on the other hand, can result in a decreased ability to absorb and metabolize certain nutrients (Amarya et al. 2015). As a result, it is critical for elderly people to consume more nutritious foods in order to meet their nutritional needs. Most of vitamin serves as an antioxidant for reducing free radical and preventing oxidative stress. Multivitamin and mineral supplementation in elderly plays a role in enhancing immune system and maintaining healthy condition (High 1999).

Vitamin Supplementation

The concentration of retinol (pre-vitamin A) in the plasma is reduced in older mice, whereas liver retinol concentrations increase in aging mice. Liver vitamin concentrations in humans also increase proportionally with age (Basu and Doeve 2011). Early study by Van Der Loo in 2004 showed that the hepatic concentrations of vitamin A are increased with increasing age (Van Der Loo et al. 2004). Clinical study regarding supplementation of vitamin A is limited to be conducted. Two studies showed no clinical effect in elderly who consumed vitamin A supplementation (Fortes et al. 1998; Murphy et al. 1992). One study was conducted in 118 elderly subjects given 800 mg of retinol palmitate for 3 months (Fortes et al. 1998) and the other study was conducted in 53 elderly subjects given 200,000 IU of single dose vitamin A (Murphy et al. 1992). In fact, the supplementation of 800 mg retinol palmitate had an unexpected effect shown by a decreasing the number of immune cells (Fortes et al. 1998).

Vitamin B1 (thiamin) plays an important role in the metabolism of carbohydrates and food energy (EFSA Panel on Dietetic Products Nutrition and Allergies (NDA) 2010). Vitamin B1 includes the active form of thiamine, thiamine pyrophosphate (TPP), a cofactor for various enzymes participated in the carbohydrate's metabolism and branched-chain amino acids (Depeint et al. 2006; EVM 2002; Tardy et al. 2020). Vitamin B1 does not change with age, but elderly can have vitamin B1 deficiency

when they consume diuretics because of congestive heart failure (Skully 2014). Vitamin B2 (riboflavin) is a coenzyme for flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD) (Suwannasom et al. 2020). FMN and FAD act as proton carriers in the redox reactions that are essential for carbohydrate, fat, and protein metabolism. In the electron transport chain, FADH functions as an electron donor (Tardy et al. 2020). Daily requirements of vitamin B2 do not diminish with age although elderly have lower energy expenditure. Meanwhile, there are other studies that recommended higher intake of vitamin B2 supplements because of biochemical evidence of vitamin B2 deficiencies in elderly (Skully 2014). Vitamin B3 (niacin) refers to two different molecules, which are nicotinic acid (NA) and nicotinamide (NAM) (Denu 2007). Niacin is a precursor of nicotinamide adenine dinucleotide (NAD^+) and nicotinamide adenine dinucleotide phosphate ($NADP^+$). Throughout glycolysis, NAD^+ (which releases NADH) loses an electron from multiple carbon atoms of glucose. Oxidative phosphorylation in mitochondria begins at complex I of the electron transport chain, where NADH is oxidized and an electron is released to start the transport chain (Tardy et al. 2020). A person older than 51 years is recommended to consume lesser thiamine (vitamin B1), riboflavin (vitamin B2), niacin (vitamin B3), and iron than a younger adult since these vitamins are involved with protein and energy metabolism (Russell 2000). Elderly people from Spain consume Mediterranean diets. Study done by Vaquero et al. (2004) shows that elderly males displayed higher intakes of thiamin (vitamin B1), riboflavin (vitamin B2), niacin (vitamin B3), retinol (vitamin A), and iron (Vaquero et al. 2004).

Vitamin B5 (pantothenic acid) is an important precursor for the coenzyme A biosynthesis. Acetyl coenzyme A is an intermediate molecule that reacts with acyl group (Pietrocola et al. 2015; Tardy et al. 2020), to produce acetyl-CoA and succinyl-CoA, both of which are used in the citric acid cycle. Vitamin B6 (pyridoxine) active metabolic form functions as a cofactor for enzymes participated in metabolism of amino acid, gluconeogenesis and glycogenolysis, lipid metabolism, heme synthesis, and hormonal action. Pyridoxal phosphate (PLP) acts as a cofactor for glycogen phosphorylase, which breaking up glucose-1-phosphate from glycogen, to produce glucose (Tardy et al. 2020). Several studies show high prevalence of vitamin B6 deficiencies in elderly. (Skully 2014) Deficiency of vitamin B6 in the elderly can cause immune system dysfunction and increase infectious disease (Russell 2000). Vitamin B8 (biotin) acts as a cofactor in several enzymes required for fat synthesis, branched-chain amino acid catabolism, gluconeogenesis, and energy production within the cell. Besides that, biotin is a cofactor of enzyme that controls the fatty acids availability for mitochondrial oxidation or the amino acids availability for the Krebs cycle (Tardy et al. 2020).

Elderly people with atrophic gastritis frequently have vitamin B12 malabsorption. Deficiency in vitamin B12 causes high plasma concentrations of homocysteine, which results in a greater risk of vascular disease, neurological damage, and dysfunction of the brain (Russell 2000). Vitamin B12 (*Cobalamin, Cbl*) is not produced inside the human body and must be obtained from animal sources. Humans require vitamin B12 to support the function of two enzymes: cytosolic methionine synthase (MS, EC 2.1.1.13) and mitochondrial methylmalonyl-CoA mutase (MUT, EC

5.4.99.2) (Froese et al. 2019). Cobalamin is a cofactor for the enzyme methylmalonyl-CoA mutase which catalyzes the conversion of methylmalonyl-CoA into succinyl-CoA. This transformation occurs throughout the oxidation of odd chain fatty acids and the ketogenic catabolism of amino acids (Tardy et al. 2020). Elderly women showed higher intake of vitamin B12. Both genders displayed similar intakes of the other micronutrients. Their consumptions according to biochemical parameters are appropriate (Vaquero et al. 2004). Vitamin C is essential for the production of energy via beta oxidation (Tardy et al. 2020). Supplementation of vitamin C (500 mg/day) for 3 months remedy the immune cells function in human peripheral blood of elderly males and females, which disturbed by increasing age (De La Fuente et al. 2020).

Vitamin D is a prohormone that has to undergo a metabolic process to become active. Vitamin D is initially hydroxylated at the carbon-25 atom in the liver, converting it into 25OH-D, followed by further hydroxylation of carbon-1 in the kidney to become the active metabolite, which is $1,25(OH)_2$-D. The active form of vitamin D is useful in maintaining calcium and phosphate homeostasis and helps in bone mineralization (Basu and Doeve 2011). The active metabolite enters the cell and binds to vitamin D receptors, followed by the transcription and translation to produce calcium binding protein or osteocalcin. $1,25(OH)_2$-D binds to the vitamin D receptor inside enterocytes and synthesizes calcium binding proteins (Lips 2006). Elderly people have a risk of vitamin D deficiency caused by multiple factors. One of the factors is the inability of the kidneys of elderly individuals to hydroxylate 25 (OH)-D into the active form 1,25 $(OH)_2$-D, as a result vitamin D receptors in the intestinal mucosa reduce in number leading to malabsorption of calcium. The reduced absorption of calcium causes an increase in the parathyroid hormone and bone remodeling (Russell 2000).

Mineral Supplementation

Trace elements are chemical micronutrients that are needed in small amounts but play an important role in the human body's physiological and metabolic processes. Deficiency of trace elements can cause impairment of health since every trace element is associated with multiple enzymatic systems, and the intake of these elements is extremely important to maintain good health (Bhattacharya et al. 2016). Elderly usually have higher risk of osteoporosis which causes high incidence of fragility fractures. Since nearly 99% of the calcium of the human body is contained in the skeleton, so elderly should take calcium supplements. USA country recommends higher calcium supplements intake for women and person aged 70 years old. Many studies shows that taking calcium supplements can increase bone density thus prevent fractures. (Skully 2014).

Iron is present in the ring structure of porphyrin in hemoglobin and also in the cytochrome that acts as an electron carrier throughout ATP synthesis in the electron transport chain. The respiratory chain consists of 40 different proteins, including six

different heme ferritins and six iron-sulfur proteins located in the mitochondrial inner membrane complexes I, II, and III proteins (Tardy et al. 2020). Females aged 51 and above require lower iron levels due to menopause (Russell 2000). In the USA, around 11% of men and 10% of women aged above 65 are anemic; meanwhile, in England, around 5.2% men and women aged 65 are anemic. The cause of anemic in these men and women aged 65 is varied between individuals. Elderly usually has impaired absorption, reduced food intake, or changes in dietary pattern which usually have normalized hemoglobin response after given iron therapy. Otherwise, elderly who had malignancies, renal disease, or higher inflammatory marker did not respond to iron therapy (Fairweather-Tait et al. 2014).

Magnesium (Mg) plays a primary role in the production and use of adenosine triphosphate (ATP). The majority of ATP is in the form of the Mg-ATP complex within the cell. This complex is a cofactor for various kinases that are active in glycolysis. Magnesium also influences the activity of citric acid cycle enzymes such as isocitrate dehydrogenase and oxoglutarate dehydrogenase enzyme complexes. The Mg-ATP complex aids in the export of mitochondrial ATP into the cytoplasm to provide energy for intracellular functions (Tardy et al. 2020). Elderly often have Mg deficiency usually because of insufficient Mg intake, reduced Mg absorption, or increased Mg secretion related to kidney function (Barbagallo et al. 2021). Li et al. (2022) replaced iodized salt into non-iodized salt consumption in 61 elderly subjects for 6 months. This resulted 15.4% subjects had abnormal thyroid function, 7.7% subjects had first-onset new nodules or enlarged solid nodules and lower urine iodine concentration than other subjects (Li et al. 2022).

Conclusion

Protein is a macromolecule composed of amino acids and has a pivotal role in the body. Protein obtained from food serves as a source of amino acids for protein synthesis in tissues. In the aging process, various changes occur in amino acid metabolism resulting in an imparity between protein synthesis and degradation which causes sarcopenia and degenerative diseases. Low protein and energy intake in the elderly can increase the morbidity and mortality. Therefore, protein and energy supplementation is needed to create healthy aging. Several studies have shown that supplementation of amino acids, especially branched-chain amino acids, can increase muscle mass and function, thereby preventing sarcopenia. Creatine supplementation as a source of energy in muscles is also beneficial in increasing muscle mass and strength. The process of macromolecular catabolism to produce energy is highly dependent on the availability of vitamins as coenzymes and mineral as cofactors in various metabolic reactions. Thus, adequate intake of vitamins is beneficial in producing adequate energy. In addition, the administration of multivitamins and minerals has been shown to increase the immune system in the elderly.

References

Ahmed T, Haboubi N (2010) Assessment and management of nutrition in older people and its importance to health. Clin Interv Ageing 5:207–216
Alam I et al (2019) The immune-nutrition interplay in ageing – facts and controversies. Nutr Healthy Ageing 5(2):73–95
Amarya S et al (2015) Changes during ageing and their association with malnutrition. J Clin Gerontol Geriatr 6(3):78–84
Anthony JC et al (1999) Leucine supplementation enhances skeletal muscle recovery in rats following exercise. J Nutr 129(6):1102–1106
Anthony JC et al (2000) Leucine stimulates translation initiation in skeletal muscle of postabsorptive rats via a rapamycin-sensitive pathway. J Nutr 130(10):2413–2419
Aquilani R et al (2008) Oral amino acid supplements improve exercise capacities in elderly patients with chronic heart failure. Am J Cardiol 101(11):S104–S110
Aquilani R et al (2014) Essential amino acids and exercise tolerance in elderly muscle-depleted subjects with chronic diseases: a rehabilitation without rehabilitation? Biomed Res Int 2014: 341603
Arnal MA et al (1999) Protein pulse feeding improves protein retention in elderly women. Am J Clin Nutr 69:1202–1208
Barbagallo M, Veronese N, Dominguez LJ (2021) Magnesium in aging, health and diseases. Nutrients 13:463. https://doi.org/10.3390/nu13020463
Basu T, Doeve A (2011) Significance of vitamin a (retinol) in ageing. Open Nutraceuticals J 4:65–68
Bhattacharya PT et al (2016) Nutritional aspects of essential trace elements in oral health and disease: an extensive review. Scientifica 2016:5464373
Biolo G et al (1997) An abundant supply of amino acids enhances the metabolic effect of exercise on muscle protein. Am J Phys 273:E122–E129
Borsheim E et al (2008) Effect of amino acid supplementation on muscle mass, strength and physical function in elderly. Clin Nutr 27:189–195
Breen L, Phillips SM (2011) Skeletal muscle protein metabolism in the elderly: interventions to counteract the 'anabolic resistance' of ageing. Nutr Metab 8:68
Breen L et al (2013) Two weeks of reduced activity decreases leg lean mass and induces "anabolic resistance" of myofibrillar protein synthesis in healthy elderly. J Clin Endocrinol Metab 98(6): 2604–2612
Brocca L et al (2008) Amino acid supplements improve native antioxidant enzyme expression in the skeletal muscle of diabetic mice. Am J Cardiol 101(11):S57–S62
Brosnan ME, Brosnan JT (2016) The role of dietary creatinine. Amino Acids 48:1785–1791
Candow DG et al (2019) Effectiveness of creatine supplementation on aging muscle and bone: focus on falls prevention and inflammation. J Clin Med 8:488
Carmona-Gutierrez D, Hughes AL, Madeo F, Ruckenstuhl C (2016) The crucial impact of lysosome in aging and longevity. Ageing Res Rev 32:2–12
Chami J, Candow DG (2019) Effect of creatine supplementation dosing strategies on aging muscle performance. J Nutr Health Aging 23(3):281–285
Choi KM (2016) Sarcopenia and sarcopenic obesity. Korean J Intern Med 31(6):1054–1060
Chua BHL (1994) Specificity of leucine effect on protein degradation in perfused rat heart. J Mol Cell Cardiol 26(6):743–751
Churchward-Venne TA et al (2014) Alterations in human muscle protein metabolism with ageing: protein and exercise as countermeasures to offset sarcopenia. Biofactors 40(2):199–205
Ciechanover A (2005) Intracellular protein degradation: from a vague idea thru the lysosome and the ubiquitin-proteasome system and onto human diseases and drug targeting. Cell Death Differ 12(9):1178–1190
Ciechanover A, Schwartz AL (1998) The ubiquitin-proteasome pathway: the complexity and myriad functions of proteins death. Proc Natl Acad Sci 95(6):2727–2730

Cooper GM (2000) Protein degradation the cell: a molecular approach Sunderland. Sinauer Associates
Cuthbertson D et al (2005) Anabolic signaling deficits underlie amino acid resistance of wasting, ageing muscle. FASEB J 19:422–424
Dardevet D et al (2000) Stimulation of in vitro rat muscle protein synthesis by leucine decreases with age. J Nutr 130:2630–2635
De la Fuente M, Sáncheza C, Vallejoa C, Cerroa EDD, Arnalich F, Hernanz A (2020) Vitamin C and vitamin C plus E improve the immune function in the elderly. Exp Gerontol 142:111118
Denu JM (2007) Vitamins and ageing: pathways to NAD+ synthesis. Cell 129(3):453–454
Depeint F et al (2006) Mitochondrial function and toxicity: role of the B vitamin family on mitochondrial energy metabolism. Chem Biol Interact 163(1–2):94–112
Deutz NE et al (2014) Protein intake and exercise for optimal muscle function with ageing: recommendations from the ESPEN Expert Group. Clin Nutr 33(6):929–936
Devries MC et al (2018) Leucine, not total protein, content of a supplement is the primary determinant of muscle protein anabolic responses in healthy older women. J Nutr 148(7): 1088–1095
Dickinson JM et al (2011) Mammalian target of rapamycin complex 1 activation is required for the stimulation of human skeletal muscle protein synthesis by essential amino acids. J Nutr 141(5): 856–862
Dillon EL et al (2009) Amino acid supplementation increases lean body mass, basal muscle protein synthesis, and insulin-like growth factor-I expression in older women. J Clin Endocrinol Metab 94(5):1630–1637
Drummond MJ et al (2008) Skeletal muscle protein anabolic response to resistance exercise and essential amino acids is delayed with ageing. J Appl Physiol 104(5):1452–1461
EFSA Panel on Dietetic Products Nutrition and Allergies (NDA), E (2010) Scientific opinion on the substantiation of a health claim related to thiamine and carbohydrate and energy-yielding metabolism pursuant to Article 14 of Regulation (EC) no 1924/2006. EFSA J 8(7):1690
Eldeeb MA et al (2020) Extracellular protein degradation via the lysosome. Commun Chem 3:149. https://doi.org/10.1038/s42004-020-00397-8
EVM (Expert Group on Vitamins and Minerals) (2002) Revised review of thiamine. WHO/FAO (World Health Organization/Food and Agriculture Organization of the United Nations). Human vitamin and mineral requirements. Report of a joint FAO/WHO expert consultation. FAO/WHO, Bangkok
Fairweather-Tait SJ, Wawer AA, Gillings R, Jennings A, Myint PK (2014) Iron status in the elderly. Mech Ageing Dev 136-137:22–28
Fortes C, Forastiere F, Agabiti N et al (1998) The effect of zinc and vitamin A supplementation on immune response in an older population. J Am Geriatr Soc 46:19–26
Froese DS et al (2019) Vitamin B12, folate, and the methionine remethylation cycle-biochemistry, pathways, and regulation. J Inherit Metab Dis 42(4):673–685
Fujita S, Volpi E (2006) Amino acids and muscle loss with ageing. J Nutr 136(Suppl 1):277S–280S
Fukagawa NK (2013) Protein and amino acid supplementation in older humans. Amino Acids 44: 1493–1509
Geurden B et al (2015) The risk of malnutrition in community-living elderly on admission to hospital for major surgery. Acta Chir Belg 15:341–347
Gotshalk LA et al (2002) Creatine supplementation improves muscular performance in older men. Med Sci Sports Exerc 34(3):537–543
Gropper SS, Smith JL (2013) Advanced nutrition and human metabolism. Cengage Learning, Wadsworth
Heinz A (2021) Elastic fibers during ageing and disease. Ageing Res Rev 66:101255
High KP (1999) Micronutrient supplementation and immune function in the elderly. Clin Infect Dis 28(4):717–722. https://doi.org/10.1086/515208

Houston DK, Nicklas B, Ding J et al (2008) Dietary protein intake is associated with lean mass change in older, community-dwelling adults: the health, ageing, and body composition (health ABC) study. Am J Clin Nutr 87:150–155
Howarth KR, Phillips SM, MacDonald MJ, Richards D, Moreau NA, Gibala MJ (2010) Effect of glycogen availability on human skeletal muscle protein turnover during exercise and recovery. J Appl Physiol 109(2):431–438
Ispoglou T et al (2016) Double-blind, placebo-controlled pilot trial of L-Leucine-enriched amino-acid mixtures on body composition and physical performance in men and women aged 65-75 years. Eur J Clin Nutr 70(2):182–188
Ispoglou T et al (2021) The efficacy of essential amino acid supplementation for augmenting dietary protein intake in older adults: implications for skeletal muscle mass, strength and function. Proc Nutr Soc 80(2):230–242
Johnson KO et al (2020) Differences in circulating appetite-related hormone concentrations between younger and older adults: a systematic review and meta-analysis. Ageing Clin Exp Res 32(7):1233–1244
Kadowaki M, Kanazawa T (2003) Amino acids as regulators of proteolysis. J Nutr 133(6):2052S–2056S
Katsanos CS et al (2005) Effect of a leucine enriched amino acid drink on muscle protein synthesis in the elderly. FASEB J 19:A1573
Kim YC, Guan KL (2015) mTOR: a pharmacologic target for autophagy regulation. J Clin Invest 125(1):25–32
Kim HK et al (2012) Effects of exercise and amino acid supplementation on body composition and physical function in community-dwelling elderly Japanese sarcopenic women: a randomized controlled trial. J Am Geriatr Soc 60:16–23
Krisko A, Radman M (2019) Protein damage, ageing and age-related diseases. Open Biol 9(3): 180249
Labbadia J, Morimoto RI (2015) The biology of proteostasis in ageing and disease. Annu Rev Biochem 84:435–464
Landi F et al (2016) Anorexia of ageing: risk factors, consequences, and potential treatments. Nutrients 8(2):69
Li M, Wang R, Lu J et al (2022) Changes of iodine nutritional status in the elderly after replacing iodized salt with non-iodized salt for half a year. Biol Trace Elem Res. https://doi.org/10.1007/s12011-022-03216-4
Lieberman M, Peet A (2018) Nitrogen metabolism. Marks' basic medical biochemistry – a clinical approach. Lippincott Williams & Wilkins, Philadelphia, pp 1581–1684
Lips P (2006) Vitamin D physiology. Prog Biophys Mol Biol 92(1):4–8
Lobo DM et al (2015) Effects of long-term low-dose dietary creatine supplementation in older women. Exp Gerontol 70:97–104
Lopez MJ, Mohiuddin SS (2022) Biochemistry, essential amino acids. StatPearls Publishing, Treasure Island
Markofski MM et al (2018) Effect of aerobic exercise training and essential amino acid supplementation for 24 weeks on physical function, body composition, and muscle metabolism in healthy, independent older adults: a randomized clinical trial. J Gerontol A Biol Sci Med Sci 74(10):1598–1604
Mitchell CJ et al (2017) The effects of dietary protein intake on appendicular lean mass and muscle function in elderly men: a 10-wk randomized controlled trial. Am J Clin Nutr 106:1375–1383
Moberg M et al (2016) Activation of mTORC1 by leucine is potentiated by branched-chain amino acids and even more so by essential amino acids following resistance exercise. Am J Physiol Cell Physiol 310:C874–C884
Moore DR et al (2015) Protein ingestion to stimulate myofibrillar protein synthesis requires greater relative protein intakes in healthy older versus younger men. J Gerontol A Biol Sci Med Sci 70(1):57–62

Moro T et al (2018) Muscle protein anabolic resistance to essential amino acids does not occur in healthy older adults before or after resistance exercise training. J Nutr 148:900–909
Murphy S, West KP Jr, Greenough WB, Cherot E, Katz J, Clement L (1992) Impact of vitamin a supplementation on the incidence of infection in elderly nursing-home residents: a randomized controlled trial. Age Ageing 21:435–439
Murray RK (2009) Harper illustrated biochemistry. McGraw-Hill Medical, New York
Murton AJ (2015) Muscle protein turnover in the elderly and its potential contribution to the development of sarcopenia. Proc Nutr Soc 74(4):387–396
Nelson DL, Cox MM (2005) Lehninger – principles of biochemistry. W.H. Freeman and Company, New York
Paddon-Jones D et al (2004) Amino acid ingestion improves muscle protein synthesis in the young and elderly. Am J Physiol Endocrinol Metab 286:E321–E328
Park S et al (2020) Anabolic response to essential amino acid plus whey protein composition is greater than whey protein alone in young healthy adults. J Int Soc Sports Nutr 17(1):9
Pascual-Fernandez J et al (2020) Sarcopenia: molecular pathways and potential targets for intervention. Int J Mol Sci 21(22):8844
Pellegrino MA et al (2008) Amino acid supplementation counteracts metabolic and functional damage in the diabetic rat heart. Am J Cardiol 101(11):S48–S56
Pietrocola F et al (2015) Acetyl coenzyme a: a central metabolite and second messenger. Cell Metab 21(6):805–821
Rasmussen BB et al (2006) Insulin resistance of muscle protein metabolism in ageing. FASEB J 20(6):768–769
Rawson ES, Wehnert ML, Clarkson PM (1999) Effects of 30 days of creatine ingestion in older men. Eur J Appl Physiol Occup Physiol 80(2):139–144
Rodwell VWIRV, Bender DA, Botham KM, Kennelly PJ, Weil PA (2018) Metabolism of protein & amino acids. Harper's illustrated biochemistry. McGraw-Hill Education, New York, pp 643–725
Rondanelli M et al (2011) Effect of essential amino acid supplementation on quality of life, amino acid profile and strength in institutionalized elderly patients. Clin Nutr 30(5):571–577
Roschel H, Gualano B, Ostojic SM, Rawson ES (2001) Creatine supplementation and brain health. Nutrients 13:586
Russell RM (2000) The ageing process as a modifier of metabolism. Am J Clin Nutr 72-(2 Suppl):529S–532S
Saez I, Vilchez D (2014) The mechanistic link between proteasome activity, aging and related disease. Curr Genomics 15:38–51
Sanvictores T, Farci F (2022) Biochemistry, primary protein structure. StatPearls Publishing, Treasure Island
Shin JW et al (2019) Molecular mechanisms of dermal ageing and antiageing approaches. Int J Mol Sci 20(9):2126
Skully R (2014) Essential nutrient requirements of the elderly. Nutr Diet Suppl 6:59–68
Solerte SB et al (2008) Nutritional supplements with oral amino acid mixtures increases whole-body lean mass and insulin sensitivity in elderly subjects with sarcopenia. Am J Cardiol 101: 69E–77E
Spencer ML et al (2004) NPDC-1, a novel regulator of neuronal proliferation, is degraded by the ubiquitin/proteasome system through a PEST degradation motif. J Biol Chem 279(35): 37069–37078
Stout JR et al (2007) Effects of creatine supplementation on the onset of neuromuscular fatigue threshold and muscle strength in elderly men and women (64–86 years). J Nutr Health Aging 11(6):459–464
Sullivan DH et al (2002) Protein-energy undernutrition and life-threatening complications among the hospitalized elderly. J Gen Intern Med 17:923–932
Suwannasom N et al (2020) Riboflavin: the health benefits of a forgotten natural vitamin. Int J Mol Sci 21(3):930

Tardy AL et al (2020) Vitamins and minerals for energy, fatigue and cognition: a narrative review of the biochemical and clinical evidence. Nutrients 12:228. https://doi.org/10.3390/nu12010228
Terry M, Gregorsz M, Roger CH, Jonathan PS, Alan H (2007) Creatine supplementation and cognitive performance in elderly individuals. Aging Neuropsychol Cogn 14(5):517–528
Timmerman KL, Volpi E (2008) Amino acid metabolism and regulatory effects in ageing. Curr Opin Clin Nutr Metab Care 11(1):45–49
Timmerman KL et al (2010) Pharmacological vasodilation improves insulin-stimulated muscle protein anabolism but not glucose utilization in older adults. Diabetes 59(11):2764–2771
Traylor DA et al (2018) Perspective: protein requirements and optimal intakes in ageing: are we ready to recommend more than the recommended daily allowance? Adv Nutr 9(3):171–182
Van der Loo B, Labugger R, Aebischer CP et al (2004) Age-related changes of vitamin A status. J Cardiovasc Pharmacol 43:26–30
Vaquero MP, Sánchez-Muniz FJ, Carbajal A, García-Linares MC, García-Fernández MC, García-Arias MT (2004) Mineral and vitamin status in elderly persons from Northwest Spain consuming an Atlantic variant of the Mediterranean diet. Ann Nutr Metab 48:125–133. https://doi.org/10.1159/000078374
Volpi E, Kobayashi H, Shefeld-Moore M, Mittendorfer B, Wolfe RR (2003) Essential amino acids are primarily responsible for the amino acid stimulation of muscle protein anabolism in healthy elderly adults. Am J Clin Nutr 78(2):250–258
Wilkes EA et al (2009) Blunting of insulin inhibition of proteolysis in legs of older subjects may contribute to age-related sarcopenia. Am J Clin Nutr 90(5):1343–1350
Wilkinson DJ et al (2018) Effects of leucine-enriched essential amino acid and whey protein bolus dosing upon skeletal muscle protein synthesis at rest and after exercise in older women. Clin Nutr 37(6 Pt A):2011–2021
Willoughby DS, Rosene JM (2003) Effects of oral creatine and resistance training on myogenic regulatory factor expression. Med Sci Sports Exerc 35(6):923–929
Wu G (2009) Amino acids: metabolism, functions, and nutrition. Amino Acids 37(1):1–17
Wurtman JJ et al (1988) Calorie and nutrient intakes of elderly and young subjects measured under identical conditions. J Gerontol 43(6):8174–8180
Yoshizawa F et al (1998) Effect of dietary protein on translation initiation in rat skeletal muscle and liver. Am J Phys 275:E814–E820
Zhou X et al (2018) The effects of electrical acupuncture and essential amino acid supplementation on sarcopenic obesity in male older adults: a randomized control study. Obes Facts 11(4): 327–334

Chapter 15
Ageing, Metabolic Dysfunction, and the Therapeutic Role of Antioxidants

Ana L. Santos and Sanchari Sinha

Abstract The gradual ageing of the world population has been accompanied by a dramatic increase in the prevalence of obesity and metabolic diseases, especially type 2 diabetes. The adipose tissue dysfunction associated with ageing and obesity shares many common physiological features, including increased oxidative stress and inflammation. Understanding the mechanisms responsible for adipose tissue dysfunction in obesity may help elucidate the processes that contribute to the metabolic disturbances that occur with ageing. This, in turn, may help identify therapeutic targets for the treatment of obesity and age-related metabolic disorders. Because oxidative stress plays a critical role in these pathological processes, antioxidant dietary interventions could be of therapeutic value for the prevention and/or treatment of age-related diseases and obesity and their complications. In this chapter, we review the molecular and cellular mechanisms by which obesity predisposes individuals to accelerated ageing. Additionally, we critically review the potential of antioxidant dietary interventions to counteract obesity and ageing.

Keywords Ageing · Obesity · Metabolic syndrome · Oxidative stress · Inflammation · Nutraceuticals · Dietary interventions

Introduction

From a clinical standpoint, obesity is defined as a body mass index (BMI) between 30 and 39.9 kg/m^2 and is characterised by the accumulation of excess body fat (Kopelman 2000; Ogden et al. 2006). The condition reflects chronic excess energy due to decreased energy expenditure and increased energy intake (Hill et al. 2012),

A. L. Santos (✉)
IdISBA – Fundación de Investigación Sanitaria de las Islas Baleares, Palma, Spain
e-mail: alsantos@ua.pt

S. Sinha
Independent Science Writer, Kolkata, India

J. R. Harris, V. I. Korolchuk (eds.), *Biochemistry and Cell Biology of Ageing: Part IV, Clinical Science*, Subcellular Biochemistry 103,
https://doi.org/10.1007/978-3-031-26576-1_15

resulting from a complex and not yet well-defined interaction between genetic, metabolic, and lifestyle factors. In recent decades, the incidence of obesity has increased dramatically, a trend that is expected to continue in the coming years. In the USA, 86.3% of adults will likely be overweight or obese by 2030 (Wang et al. 2008), which could significantly burden the health care system.

Globally, obesity is estimated to be the fifth leading cause of death (Secord and Gehrig 2012). Over 2.8 million people are estimated to die from obesity and associated complications yearly (WHO 2021a). Type 2 diabetes (T2D), cardiovascular disease, breast and colon cancer, gallbladder disease, fatty liver disease, sleep apnoea and other respiratory problems, arthritis, and infertility are the most common health complications associated with obesity (Pi-Sunyer 2009). The term "metabolic syndrome" describes the co-occurrence of obesity, dyslipidaemia, hypertension, and diabetes (Eckel et al. 2005). It is estimated that cardiovascular disease and all-cause mortality are 2-fold and 1.5-fold higher, respectively, in individuals with metabolic syndrome (Engin 2017).

Interestingly, the adipose tissue dysfunction associated with obesity shares many similarities with the adipose tissue dysfunction associated with normal ageing. These similarities include redox imbalance, altered mitochondrial function, accumulation of damaged macromolecules, impaired immunity, and systemic inflammation (Ahima 2009; Minamino et al. 2009; Tchkonia et al. 2010; Santos and Sinha 2021). Based on these similarities, obesity has been proposed to represent an accelerated form of adipose tissue ageing (Tchkonia et al. 2010).

Therefore, a better understanding of the mechanistic links between ageing and obesity, particularly at the adipose tissue level, could contribute to the development of therapeutic and preventive strategies to improve longevity and quality of life in ageing populations.

Adipose Tissue and the Pathophysiology of Obesity

Adipose tissue in mammals occurs in two distinct forms, brown adipose tissue (BAT) and white adipose tissue (WAT), which have different morphological and functional characteristics (Fantuzzi 2005; Saely et al. 2012). BAT is present in significant amounts in infants and gradually disappears or becomes inactive with age. In adults, BAT is found in small amounts in the neck, subclavicular area, and near the heart (Nedergaard et al. 2007). Due to extensive vascularisation and numerous mitochondria expressing uncoupling protein 1 (UCP1), BAT specialises in heat production by nonshivering thermogenesis (Saely et al. 2012; Gordon et al. 2019). Nonshivering thermogenesis maintains core body temperature at cold ambient temperatures. During this process, UCP1 uncouples oxidative phosphorylation from ATP synthesis in mitochondria, releasing the proton motive force in the form of heat (Argyropoulos and Harper 2002).

WAT is the primary long-term energy reserve in mammals, synthesising triglycerides by lipogenesis when energy intake exceeds energy output. The human

WAT can mainly be divided into visceral and subcutaneous, depending on the anatomical location. It is estimated that approximately 80% of WAT is present in the subcutaneous compartments of the body, while 10–20% is present in the visceral compartments around the mesentery and omentum. Small amounts of perivascular adipose tissue are also present around the blood vessels. The liver, muscles, and joints also contain adipose tissue (Logue et al. 2019). WAT undergoes lipolysis, which releases free fatty acids that are oxidised and provide energy to the body during periods of increased energy demand. Following energy intake, lipolysis is attenuated, primarily due to the potent antilipolytic effect of insulin (Duncan et al. 2007).

Under certain circumstances, white fat cells, or adipocytes, acquire some of the properties of brown adipocytes, leading to the production of beige adipocytes with intermediate properties (Barbatelli et al. 2010). Beige adipocytes can also arise from unique precursor cells (Wu et al. 2012a). In mice, beige adipocytes are found in the white inguinal fat depot (Vitali et al. 2012), whereas in adult humans, they are located in the cervical and supraclavicular fat depots (Virtanen et al. 2009; Cypess et al. 2013). Under normal conditions, beige and white adipocytes are morphologically indistinguishable. However, when cold or adrenergic signals stimulate beige adipocytes, they adopt brown adipocyte-like characteristics, including the accumulation of many small lipid droplets and high mitochondrial content (Sharp et al. 2012; Wu et al. 2012a; Nedergaard and Cannon 2014). Beige adipocytes, like brown adipocytes, exhibit high expression of UCP1, which increases thermogenesis and energy expenditure (Ikeda et al. 2018). Activated brown and beige adipocytes can take up large amounts of lipids and glucose, serving as metabolic sinks, eliminating excessive amounts of nutrients in the blood and contributing to whole-body fat and glucose metabolism and insulin sensitivity (Kajimura et al. 2015). White, brown, and beige adipocytes work together to regulate the body's energy balance. In addition to its role in fat storage, adipose tissue has autocrine, paracrine, and endocrine functions in the brain, muscle, liver, vasculature, kidney, and bone that are critical to the regulation of energy homeostasis (Mohamed-Ali et al. 1998); its involvement in immunity is also increasingly recognised (Schäffler and Schölmerich 2010).

During periods of abundant energy intake, adipocytes, the primary cell type of adipose tissue, synthesise fatty acids through lipogenesis and store them as triglycerides in lipid droplets (Luo and Liu 2016). This prevents lipotoxicity from circulating fatty acids or the accumulation of triglycerides in other organs. Besides adipocytes and pre-adipocytes, adipose tissue comprises a matrix of connective tissue, nervous tissue, stromal vascular cells, and immune cells. Together, these components coordinate various biological functions, including energy metabolism, neuroendocrine function, and immune response (Frayn et al. 2003; Kershaw and Flier 2004; Grant and Dixit 2015).

Adipose tissue stromal cells promote pre-adipocyte proliferation and differentiation and secrete adipokines, which are adipose tissue-derived cytokines and hormonal factors (Frayn et al. 2003; Guerre-Millo 2004). Adipokines include pro-inflammatory cytokines, cytokine-related proteins, and several other

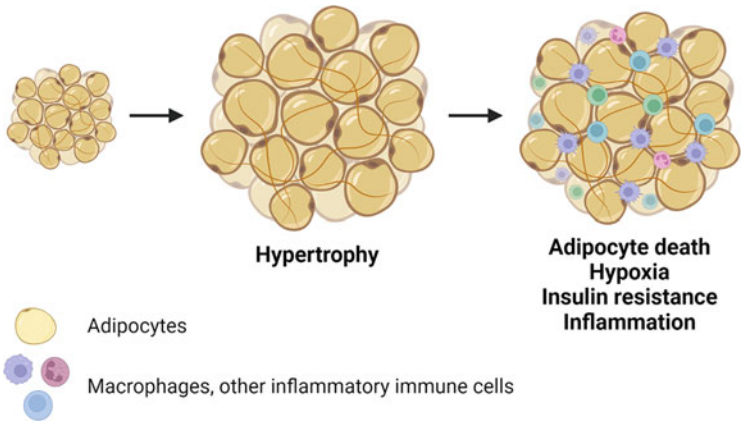


Fig. 15.1 Changes in adipose tissue with obesity. The development of obesity is associated with an increase in the number and size of adipocytes. Growing adipocytes produce increasing amounts of pro-inflammatory cytokines that can lead to low-grade chronic inflammation. As adipose tissue expands, it compresses blood vessels, allowing less oxygen to reach the tissue and causing hypoxia. Increased levels of inflammatory cytokines and oxygen deprivation contribute to adipocyte death. Simultaneously, the matrix of adipose tissue becomes increasingly dense and stiff. These and other pathological changes that occur in adipose tissue during obesity contribute to obesity-related complications such as T2D and insulin resistance, as well as an increased risk of cancer, particularly breast cancer, in obese individuals. Image used with permission from Elsevier. Image originally in Rosen and Spiegelman (2014)

biologically active proteins that exhibit hormone-like effects and regulate appetite, energy expenditure, fat storage, and insulin secretion and sensitivity, among others (Blüher and Mantzoros 2015; Luo and Liu 2016). Some adipokines, such as adiponectin, adipsin, and leptin, are almost exclusively produced by adipocytes, and their concentration correlates with the amount of adipose tissue and BMI (Wajchenberg 2000; Pan et al. 2014). Other adipokines are produced almost exclusively by the stromal vascular and matrix fractions of the hypertrophic adipose tissue, primarily by resident macrophages. These include tumour necrosis factor-alpha (TNF-α), interleukins (ILs), and monocyte chemoattractant protein-1 (MCP-1) (Weisberg et al. 2003; Fain 2006). The secretion of adipokines depends on the size and location of the fat depot. While subcutaneous adipose tissue secretes greater amounts of metabolically beneficial adipokines such as leptin and adiponectin, visceral adipose tissue produces greater amounts of pro-inflammatory adipokines (Fain 2006).

Fat accumulation depends on the balance between lipogenesis and lipolysis (triglyceride degradation) (Kersten 2001). The expansion of adipose tissue associated with obesity is caused by an increase in the number (hyperplasia) and mass/size (hypertrophy) of adipocytes as a result of enhanced lipogenesis and adipogenesis (Fig. 15.1) (Rosen and Spiegelman 2014; Haczeyni et al. 2018). Additionally, increased local vascularisation and proliferation of pre-adipocytes in the stromal

vascular fraction of adipose depots promote adipose tissue growth (Gesta et al. 2007).

Mature obese adipocytes exhibit increased production of pro-inflammatory and insulin-resistant adipokines such as IL-6 and MCP-1 and a decrease in the production of anti-inflammatory and insulin-sensitising adipokines (Weisberg et al. 2003; Unamuno et al. 2018). As a result of sustained chemotactic stimulation, monocytes leave the circulation and migrate to the accumulating fat, where they develop into macrophages (Kanda et al. 2006). Macrophages that settle in adipose tissue secrete cytokines (e.g. IL-6 and TNF-α) that cause further infiltration of macrophages and inflammation. These actions result in increased production of pro-inflammatory adipokines and a reduced response of adipocytes to insulin (Suganami et al. 2005). Over time, persistent low-grade inflammation caused by obesity can lead to T2D, hypertension, dyslipidaemia, atherosclerosis, thrombosis, cardiovascular disease, and even cancer (Wellen and Hotamisligil 2005; Ellulu et al. 2017). In the brain, obesity can lead to neuroinflammation, impaired blood–brain barrier (BBB) integrity, and alterations in neuronal structure, synaptic plasticity, and cognitive function (Nguyen et al. 2014).

Ageing and Age-Related Diseases

The average life expectancy of the world population has increased significantly in recent decades (Christensen et al. 2009). In the USA, the average life expectancy is estimated to have increased by more than 30 years since the 1900s (Bunker et al. 1994). By 2050, the proportion of people over 60 years of age worldwide will be 22%, and 400 million people will be 80 years of age or older (Harper 2014). Unfortunately, this increase in life expectancy has not been accompanied by a corresponding increase in healthspan, defined as the number of years lived without chronic age-related diseases. Therefore, to extend the human healthspan, it is crucial to understand the basic mechanisms of ageing and related diseases.

Biological theories of ageing fall into three main categories. Evolutionary ageing theories postulate that ageing results from decreasing natural selection forces with increasing chronological age (Medawar 1952; Williams 1966). As organisms age, especially after the onset of reproduction, there is a decline in reproductive output that selection can act on to discriminate between more suitable and less suitable genotypes. Accordingly, selection cannot "see" deleterious mutations whose effects are confined to late-life stages after they have been passed on to offspring. Developmental (or programmed) theories of ageing posit that ageing follows a genetically determined schedule similar to that underlying growth, development, and maturity. Despite considerable effort, no gene mutation has yet been found to abolish the ageing process, questioning the notion that ageing is based on a genetic programme (Kirkwood and Melov 2011). Conversely, according to error theories, ageing results from the lifetime accumulation of damage to molecules, eventually leading to physiological decline (Muller et al. 2007). Although convenient, the division of

theories of ageing into different categories is artificial, and ageing is probably the result of overlapping genetic and non-genetic mechanistic and evolutionary forces (Wensink and Cohen 2021).

From a biological perspective, ageing is a lifelong, complex phenomenon characterised by a continuous decrease in the efficiency of cellular processes (Harman 2001). As a result, cells become less able to recover from internal and external damage, leading to tissue dysfunction, increased organism vulnerability to disease, and, ultimately, death (Niccoli and Partridge 2012). Ageing is the leading risk factor for many diseases, including cancer, cardiovascular disease, and neurodegenerative diseases such as Alzheimer's and Parkinson's disease (Kennedy et al. 2014). Accordingly, as the world population ages, the prevalence of age-related diseases is expected to increase substantially (Mattson and Magnus 2006; Niccoli and Partridge 2012). For example, in 2021, an estimated 50 million people worldwide were affected by dementia. This number is estimated to increase to 78 million by 2030 and 139 million by 2050 (WHO 2021b).

In elderly individuals, Alzheimer's disease (AD) is the most prevalent neurodegenerative disease and is responsible for more than 75% of cases of dementia. AD is characterised by severe disturbances in cognitive function and behaviour (Nussbaum and Ellis 2003). Estimates suggest that approximately 10% of people over 65 have AD. The prevalence of AD doubles every 5 years after age 65, and by age 85, the incidence reaches approximately 50% (Alzheimer's Association 2019).

AD is a complex disease that is neuropathologically characterised by the presence of senile plaques and intracellular neurofibrillary tangles (NFTs). Senile or amyloid plaques consist mainly of Aβ-peptides formed by proteolytic cleavage of the β-amyloid precursor protein (APP) by α-, β-, and γ-secretases (Seubert et al. 1993). Intracellular NFTs consist of hyperphosphorylated tau amyloid fibrils (Serrano-Pozo et al. 2011). Approximately 5–7% of AD cases are familial (Campion et al. 1999) and are associated with alterations in genes encoding presenilin 1 (PSEN 1), presenilin 2 (PSEN 2), APP, and apolipoprotein E (APOE) (Nikolac Perkovic and Pivac 2019).

However, most AD cases (>90–95%) occur sporadically and manifest in people 65 years and older. In addition to the accumulation of Aβ plaques and NFTs, sporadic AD is associated with various other pathological, metabolic, and neurochemical changes, including altered brain metabolism (Mosconi et al. 2008; Liu and Zhang 2014), impaired BBB function (Zenaro et al. 2017), activation of microglia and astrocytes (McGeer and McGeer 1998; Heneka et al. 2010), development of T2D and metabolic syndrome (Razay et al. 2007), and oxidative stress (Chen and Zhong 2014).

Aβ accumulation and aggregation trigger several cytotoxic effects, including the impairment of mitochondrial activity resulting in increased generation of reactive oxygen species (ROS) and loss of intracellular calcium homeostasis (De Felice et al. 2007; Quintana et al. 2020). Additionally, Aβ accumulation promotes the release of cytokines (Rubio-Perez and Morillas-Ruiz 2012) and changes in actin cytoskeleton dynamics (Bamburg and Bloom 2009). Tau hyperphosphorylation and NFT formation further promote protein misfolding and inhibit the function of protein repair

systems. Together with impaired cell bioenergetics (Blass et al. 2000) and antioxidant defence mechanisms (Tönnies and Trushina 2017), these processes cause neuronal dysfunction and, eventually, death (Guo et al. 2020).

Parkinson's disease (PD) is the second most common neurodegenerative disease after AD. Clinical manifestations of PD include motor symptoms such as akinesia, tremors, rigidity, and postural instability (Kalia and Lang 2015) and nonmotor symptoms such as autonomic and cognitive dysfunction (Schapira et al. 2017). The median age of diagnosis for PD is 65. At this age, the prevalence of PD is approximately 1% and increases to nearly 5% by the age of 85 (Tysnes and Storstein 2017).

From a histopathological perspective, PD is characterised by the loss of dopaminergic neurons in the *substantia nigra*. Neuronal degeneration is accompanied by the build-up of inclusion bodies in the cytoplasm composed of the protein α-synuclein, called Lewy bodies (Wong and Krainc 2017). Under physiological conditions, monomeric α-synuclein is widely distributed in the brain and participates in the regulation of neurotransmitter release, neuronal plasticity, and normal synaptic function. However, α-synuclein has a propensity to aggregate into higher molecular weight structures, such as oligomers, protofibrils, and eventually fibrils, which are the principal components of Lewy bodies (Spillantini et al. 1997). Oxidised and nitrated α-synuclein are also important components of Lewy bodies associated with PD and some forms of AD (Giasson et al. 2000). Abnormal accumulation of α-synuclein in neuritic processes appears to be the driving force in the pathogenesis of PD, and these deposits occur early in the course of PD (Wong and Krainc 2017).

Mechanistic Similarities Between Ageing and Obesity

There are many similarities between the adipose tissue dysfunction that is characteristic of obesity and that of normal ageing. These include increased oxidative stress and chronic low-level inflammation accompanied by insulin resistance, elevated pro-inflammatory, chemotactic, and procoagulant protein levels, and lipotoxicity (Tchkonia et al. 2010; Pérez et al. 2016; Trim et al. 2018). Interestingly, obesity is also accompanied by dysfunction at the neuronal level, which has similarities to that associated with ageing, including neuroinflammation, decreased grey and white matter volume, decreased hippocampal volume, and decreased neurogenesis, as well as structural changes in the frontal and temporal lobes and altered neuronal connectivity and dopamine release in reward circuits (Jagust et al. 2005; Cai 2013). Obesity and overweight in middle age have been linked to a higher incidence of dementia in old age (Elias et al. 2003; Whitmer et al. 2007), and several cognitive and executive functions are impaired in obese individuals (Dahl et al. 2010). The risk of age-related neurodegenerative diseases such as AD and PD can also be increased by obesity (Mazon et al. 2017). These commonalities and their underlying mechanisms are examined in more detail in the following sections.

Oxidative Stress and Inflammation in Ageing and Obesity

According to the "free radical theory of ageing" (Harman 1956), ageing and age-related dysfunction are caused by oxidative stress resulting from increased ROS production and decreased antioxidant activity with age. The finding that mitochondria are not only the main producers of ROS in the cell but also their primary targets was later summarised in the revised "mitochondrial free radical theory of ageing" (Harman 1972; de Grey 1997).

Both ROS and reactive nitrogen species (RNS) are produced under physiological conditions. It is estimated that as much as 2% of the overall electrons passing through the mitochondrial electron transport chain during aerobic respiration escape, especially in complexes I and III (Liu et al. 2002). The leaked electrons readily react with molecular oxygen, forming the superoxide anion (Fig. 15.2). In mitochondria, superoxide dismutase catalyses the conversion of superoxide into hydrogen peroxide and molecular oxygen. Unlike superoxide, hydrogen peroxide can readily diffuse to the cytoplasm and, in the presence of transition metals such as iron and copper, form highly reactive hydroxyl radicals through the Fenton and Haber-Weiss reactions (Halliwell and Gutteridge 1990). Additionally, superoxide can react with endogenous nitric oxide to form the RNS peroxynitrite, which then reacts with protein tyrosine residues to form nitrotyrosine or with other molecules to generate other RNS, such as nitrogen dioxide and nitrous trioxide (Beckman and Koppenol 1996).

With age, increased electron loss from the electron transport chain and decreased levels of cellular antioxidants lead to oxidative stress (Balaban et al. 2005). Because mitochondria lack protective histones and have limited DNA repair mechanisms, they are particularly susceptible to ROS-induced oxidative damage (Yakes and Van Houten 1997). The mutation rate in mitochondrial DNA (mtDNA) is up to 10 times higher than that of genomic DNA (Miquel 1991; Wallace 1992). Oxidative damage to mtDNA leads to further mitochondrial dysfunction and ROS production, creating a vicious cycle of electron loss and oxidative stress. Studies using mutator mice expressing an mtDNA polymerase deficient in proofreading ability have shown that impaired proofreading capacity accelerates the accumulation of somatic mutations in mtDNA, subsequently leading to respiratory defects and early ageing onset (Trifunovic et al. 2004).

In addition to DNA, ROS can attack proteins and lipids, especially polyunsaturated fatty acids. Since lipids are essential components of cell membranes and various lipoproteins, lipid oxidation can lead to membrane damage, altered lipid packing, and loss of cell integrity (Cosgrove et al. 1987; Runas and Malmstadt 2015). As a result, lipid peroxidation products and carbonylated proteins accumulate in aged animals (Ikeda et al. 1985; Brunk and Terman 2002a). This gradual accumulation of oxidative damage leads to the progressive cellular functional decline that characterises ageing (Ikeda et al. 1985; Stadtman 2001; Brunk and Terman 2002a). In some instances, decreased free radical production via antioxidant supplementation and increased resistance to oxidative stress have been associated with increased lifespan (Massie et al. 1984; Vanfleteren 1993; Larsen 1993; Brack

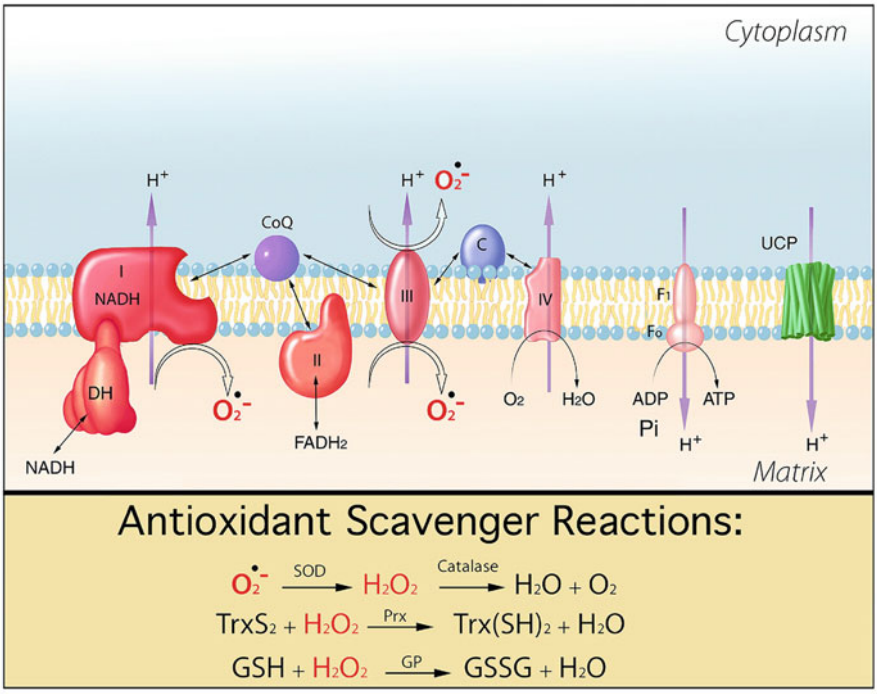


Fig. 15.2 Schematic representation of ROS production in the mitochondria. The mitochondrial electron transport chain is the main source of ROS in the cell, especially in complexes I and III. Superoxide ($O_2^{\bullet -}$) is produced as a by-product of respiration by the reaction of electrons leaked from the respiratory chain with oxygen. Superoxide can damage mitochondrial DNA, proteins, and lipids. To protect against oxidative damage, mitochondria contain several enzymatic and non-enzymatic antioxidants. The enzyme superoxide dismutase (SOD) catalyses the conversion of superoxide ($O_2^{\bullet -}$) to oxygen (O_2) and hydrogen peroxide (H_2O_2). The enzyme peroxiredoxin (Prx) catalyses the conversion of hydrogen peroxide (H_2O_2) into water (H_2O). Thioredoxins ($TrxS_2$), small redox proteins whose main function is the reduction of oxidised cysteine residues and the cleavage of disulphide bonds, keep peroxiredoxins in their reduced state. Glutathione peroxidase (GP) uses glutathione to reduce hydrogen peroxide (H_2O_2) to water (H_2O). The resulting oxidised glutathione (GSSG) can be returned to its active state by glutathione reductase, which uses NADPH as a cofactor. In addition to antioxidants, mitochondria also contain uncoupling proteins (UCP), which protect mitochondria from oxidative stress by dissipating the proton gradient at the inner mitochondrial membrane, resulting in a decrease in mitochondrial membrane potential and the production of ROS. Image used with permission from Elsevier. Image originally found in Balaban et al. (2005)

et al. 1997; Johnson et al. 2002; Flurkey et al. 2010). However, contradictory results have also been reported (reviewed by Shields et al. 2021) and, in some cases, antioxidant supplementation has even been associated with increased mortality (Bjelakovic et al. 2007). These conflicting results reflect the fact that ROS are not only harmful by-products of metabolism, but also play a critical role as signalling

molecules that contribute to the regulation of cell proliferation, differentiation, and death (Pizzino et al. 2017; Santos et al. 2018).

The brain consumes about 20% of the body's oxygen content, which, combined with low levels of endogenous antioxidants, high levels of iron and copper, and abundant polyunsaturated fatty acids, makes the central nervous system particularly vulnerable to oxidative damage (Cobley et al. 2018). Accordingly, oxidative stress is associated with the development of age-related neurodegenerative diseases, as evidenced by higher concentrations of lipid peroxidation products in the brain tissue of subjects with AD (Montine et al. 2002) and PD (Dexter et al. 1989). Significant carbonylation and nitration of proteins (Gonos et al. 2018) and increased levels of DNA oxidation products (Coppedè and Migliore 2015) have also been reported in different age-related neurodegenerative diseases.

Oxidative stress resulting from mitochondrial dysfunction is important in triggering age-related chronic inflammation, a process termed "inflammageing" (Franceschi and Campisi 2014). This process is characterised by increased serum levels of inflammatory markers, including C-reactive protein (CRP) and serum amyloid A, and pro-inflammatory cytokines, including the interleukins IL-1 and IL-6 and TNF-α (Ferrucci and Fabbri 2018). These inflammatory signals, in turn, stimulate the immune system and further promote ROS formation (Zuo et al. 2019). In addition to oxidative stress, genetic susceptibility, cell senescence, impaired autophagy, and changes in microbiota composition may contribute to inflammageing (Franceschi et al. 2018).

The involvement of oxidative stress and inflammation in the ageing process has been summarised in the oxidation-inflammation theory of ageing or "oxi-inflammageing" (De la Fuente and Miquel 2009). According to this theory, chronic oxidative stress and inflammation lead to protein, lipid, and DNA damage, which promotes the degeneration of various regulatory systems, such as the immune system. This, in turn, leads to organism dysfunction and increased morbidity and mortality in old age. Moreover, functional impairment of the immune system leads to excessive production of oxidative and pro-inflammatory compounds that further promote cellular damage and contribute to chronic oxidative stress and inflammation in ageing organisms. In support of the "oxi-inflammageing" theory, oxidative and inflammatory stress parameters in immune cells were found to be predictive markers of lifespan (Martínez de Toda et al. 2019).

Like ageing, obesity is associated with increased oxidative stress and inflammation. Increased oxidative stress in obesity has been attributed to increased caloric intake triggering mitochondrial dysfunction in fat cells. Consequently, higher levels of oxidative stress markers have been associated with a shorter lifespan in obese mice (Baur et al. 2006; Zhang et al. 2015), and increased oxidative stress biomarkers have also been found in the blood, skeletal muscle, and erythrocytes of obese animals and humans (Furukawa et al. 2004; Pihl et al. 2006). Similarly, rapid oxidation of low-density lipoprotein (LDL), which promotes atherosclerosis, was observed in obese individuals (Van Gaal et al. 1998), and higher urinary excretion of isoprostanes, another biomarker of lipid peroxidation, was positively correlated with BMI (Keaney et al. 2003). Obesity is also characterised by a state of chronic

inflammation, as evidenced by an increase in inflammatory biomarkers (Visser et al. 1999; Schmatz et al. 2017), increased expression of inflammation-related genes (Lee et al. 2005; Nair et al. 2005; van Dijk et al. 2009), and infiltration and accumulation of immune cells, including macrophages and T cells, in the visceral adipose tissue of obese individuals (Weisberg et al. 2003; Kratz et al. 2014). The pro-inflammatory environment associated with obesity can also affect insulin receptors and cause the development of peripheral insulin resistance (Evans et al. 2005).

The redox-sensitive transcription factor nuclear factor kappa B (NF-κB) is an essential regulator of immune and inflammatory responses activated during normal ageing, obesity, and other pathological processes (Zhang et al. 2017; Yu et al. 2020). NF-κB usually exists in an inactive form in the cytoplasm. However, in response to oxidative stress, NF-κB is activated and migrates to the nucleus, binding to DNA and activating the expression of various pro-oxidant and pro-inflammatory genes. Most NF-κB-activated genes, including interleukins, chemokines, TNF-α, and the enzymes cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS), can, in turn, further activate NF-κB, which stimulates additional ROS and cytokine production, promoting inflammation (Liu et al. 2017a). Conversely, antioxidants inhibit NF-κB activation (Pinkus et al. 1996). NF-κB can also be activated by obesity-associated hypoxia resulting from fat cell hypertrophy, which further contributes to chronic inflammation in the adipose tissue of obese individuals (Griffin 2022). Additionally, NF-κB activation has been implicated in impaired insulin signalling, T2D, and atherosclerosis (Baker et al. 2011b). The importance of NF-κB in ageing is supported by the observation that inhibition of NF-κB signalling increases lifespan in flies, an effect associated, at least to some extent, with increased adipokinetic hormone signalling (Shaposhnikov et al. 2011; Moskalev and Shaposhnikov 2011; Kounatidis et al. 2017). Similarly, inactivation of IKKβ/NF-κB signalling in the hypothalamus protected against obesity, hypothalamic insulin resistance, and glucose intolerance in mice fed a high-fat diet (HFD) (Zhang et al. 2008; Benzler et al. 2015), while increased NF-κB signalling led to stress in the endoplasmic reticulum (ER), which in turn accelerated the onset of obesity (Zhang et al. 2008).

Increased oxidative stress and chronic inflammation have been linked to obesity-related comorbidities, such as diabetes, cardiovascular disease, kidney damage, and cancer, which contribute to the dramatically increased mortality rate of obese individuals (Fernández-Sánchez et al. 2011). Changes in the composition of the gut microbiota may also contribute to obesity and insulin resistance (Jiao et al. 2018). For example, an imbalance in the composition and diversity of the gut microbiota was found to cause the release of lipopolysaccharides from Gram-negative bacteria through the gut mucosa into the bloodstream (Lee et al. 2020). Lipopolysaccharides in the blood, in turn, triggered chronic, low-grade inflammation that activated Toll-like receptor 4 (TLR4) and promoted the development of insulin resistance associated with obesity (Saad et al. 2016). Obesity-related chronic oxidative stress and inflammation may also alter BBB function and ultimately contribute to the early onset of neurodegenerative diseases in obese individuals (Roh et al. 2017).

Hallmarks of Ageing in Obesity

The processes that drive ageing have been summarised in nine hallmarks, divided into three categories (Fig. 15.3) (López-Otín et al. 2013). DNA damage, telomere erosion, altered proteostasis, and epigenetic changes are among the events that trigger the ageing process and are therefore classified as "primary hallmarks" of ageing. Cell senescence, mitochondrial dysfunction, and altered nutrient sensing are considered "antagonistic hallmarks" of ageing because they initially protect the organism from damage, but their role becomes increasingly negative as the effects of the primary hallmarks progress. "Integrative hallmarks", such as altered intercellular communication and stem cell depletion, are responsible for the ageing phenotype by directly affecting homeostasis once damage accumulation becomes irreversible (López-Otín et al. 2013). These hallmarks are directly or indirectly associated with increased oxidative stress and inflammation, the two major causes

Fig. 15.3 The hallmarks of ageing. The hallmarks of ageing refer to the nine major changes that occur in the body as we age. These changes include increased cell senescence, impaired mitochondrial function resulting in enhanced ROS production, altered nutrient sensing, impaired proteostasis, epigenetic changes, telomere shortening, DNA damage, stem cell exhaustion, and impaired intracellular communication resulting in, for instance, increased inflammation. Image used with permission from Elsevier. Image originally in López-Otín et al. (2013)

of ageing and obesity. Accordingly, some, if not all, of these hallmarks have also been associated with obesity, further supporting the role of obesity in accelerating ageing, as discussed below.

Genomic Instability

The term genomic instability describes the accumulation of genetic damage caused by endogenous (e.g. DNA replication errors) and exogenous (e.g. UV radiation) factors throughout the lifespan. This genetic damage includes single and double DNA strand breaks, DNA adducts, and DNA crosslinks. Although somatic cells can accumulate a large number of DNA lesions daily, most of them are corrected by an extensive DNA repair system (Niedernhofer et al. 2018). Age-related reduction in DNA repair capacity leads to the accumulation of somatic mutations that can trigger carcinogenesis and dysfunction at the cellular, tissue, and organismal levels (Chen et al. 2007; Hoeijmakers 2009; Niedernhofer et al. 2018).

The importance of genomic instability in ageing is evidenced by accelerated ageing phenotypes in mice with genetically altered defective DNA repair proteins (Hasty et al. 2003; Hasty 2005). Similarly, human progeroid syndromes, which are characterised by a premature ageing phenotype and the early onset of age-related diseases such as cancer or T2D (Navarro et al. 2006), are caused by defects in genes involved in maintaining genomic stability (Martin and Oshima 2000).

Consistent with the critical role of genome instability in ageing, overexpression of DNA repair genes in *Drosophila melanogaster* prolonged lifespan (Shaposhnikov et al. 2015; Garschall et al. 2017). Similarly, the expression of DNA repair genes is increased in long-lived mammals compared with their short-lived counterparts (MacRae et al. 2015). Likewise, whole-genome sequencing analysis of 16 mammalian species with different lifespans and body masses revealed a correlation between longer lifespans and lower mutation rates (Cagan et al. 2022). Together, these observations highlight the importance of genome stability in ageing.

Several studies have also indicated a link between genomic instability and obesity. *In vitro*, acute treatment of human primary myoblasts with an environment similar to obesity (high glucose, insulin, and palmitate) elicited DNA damage (Dungan et al. 2020). *In vivo*, obese Zucker rats showed substantially increased levels of DNA damage in various organs compared with their lean counterparts (Azzarà et al. 2017). The number of DNA double-strand breaks in peripheral cells was also higher in obese children than in their normal-weight counterparts (Scarpato et al. 2011). Likewise, obese and overweight males were found to have altered sperm DNA integrity, which may explain the reduction in fertility associated with obesity (Chavarro et al. 2010; Fariello et al. 2012). Similarly, DNA damage was significantly greater in obese women than in non-obese women (Zaki et al. 2018a, b; Włodarczyk et al. 2018). Oxidative stress and chronic inflammation seem to be the underlying mechanisms responsible for increased DNA damage in obesity (Włodarczyk and Nowicka 2019), as evidenced by a significant relationship between DNA damage and serum levels of CRP in obese women (Włodarczyk et al. 2018).

These observations suggest that obesity may trigger cancer by inducing genomic instability and promoting tumourigenesis (Sieber et al. 2003).

Telomere Attrition

Somatic cells can undergo only a few replications and therefore have a limited lifespan. This replicative lifespan, commonly referred to as the Hayflick limit (Hayflick 1965), is related to the shortening of telomeres at the extremities of chromosomes with each cell division (Levy et al. 1992). In embryonic and germline cells, some stem cells, and most cancer cells, the enzyme telomerase is responsible for maintaining telomere length, whereas in most somatic cells telomerase is not present (Shay and Wright 2006).

Although telomere shortening is associated with the normal ageing process (Blasco 2007), pathological dysfunction of telomerase accelerates ageing, as shown by the premature ageing phenotype of telomerase-deficient mice (Rudolph et al. 1999). Stress factors such as inflammation and oxidative stress can accelerate telomere loss (von Zglinicki 2002; Jurk et al. 2014), and older adults with shorter telomeres have twice the mortality rate of people with longer telomeres (Cawthon et al. 2003). Additionally, people with shorter telomeres have a significantly higher risk of developing various age-related diseases, including insulin resistance, osteoarthritis, atherosclerosis, coronary heart disease, and atrial fibrillation, than people with longer telomeres (Demissie et al. 2006; Aviv 2012; Kuszel et al. 2015; Scheller Madrid et al. 2016; Gavia-García et al. 2021). Telomere length has also been inversely correlated with the risk of cardiovascular disease and T2D (Willeit et al. 2014; Haycock et al. 2014; D'Mello et al. 2015).

An association between abdominal obesity in humans and decreased telomere length has also been reported (Nordfjall et al. 2008; Lee et al. 2011). Indeed, compared with their lean counterparts, the telomeres of obese women were 240 bp shorter, corresponding to an age difference of approximately 8.8 years (Valdes et al. 2005). Obesity-associated oxidative stress, increased production of pro-inflammatory cytokines, and insulin resistance are thought to be responsible for the accelerated telomere attrition in obesity (Gardner et al. 2005; Al-Attas et al. 2010; Tzanetakou et al. 2012). These results suggest that obesity contributes to the acceleration of ageing by promoting telomere erosion.

Epigenetic Alterations

The term "epigenetics" describes the control of gene expression by mechanisms that do not involve modifications of the genetic code (Skvortsova et al. 2018). Epigenetic changes associated with ageing include chromatin remodelling, DNA methylation, and histone modifications. These and other epigenetic changes contribute to the loss of heterochromatin in aged cells (Zhang et al. 2020). The predictable nature of the changes in DNA methylation with age can be used as an "epigenetic clock" to

estimate a person's age (Horvath 2013; Chen et al. 2016; Levine et al. 2018). ROS and chronic inflammation can influence age-related epigenetic changes and the epigenetic clock (Pal and Tyler 2016), and some epigenetic changes, such as histone modifications and non-coding RNAs, have been shown to affect lifespan (Merkwirth et al. 2016). Similarly, several age-related diseases, including AD and PD (Berson et al. 2018), have been associated with the acceleration of "epigenetic ageing", that is, the difference between a person's epigenetic age and chronological age (Horvath and Raj 2018).

Obesity has also been associated with accelerated epigenetic ageing. Indeed, each 10-point increase in BMI can reportedly lead to an acceleration of ageing by up to 2.7 years (Horvath et al. 2014). Several epigenetic changes associated with obesity have also been reported. For example, obesity was found to increase histone H3 acetylation of the inflammatory mediators TNF-α and Ccl2/MCP-1 (Mikula et al. 2014). The expression of histone deacetylase class III sirtuin 1 (SIRT1) was lower in the adipose tissue of obese individuals than in lean individuals (Mariani et al. 2018). SIRT1 is involved in the cellular response to oxidative stress, glucose and lipid homeostasis, and the regulation of insulin sensitivity. Accordingly, decreased SIRT1 expression has been associated with insulin resistance and increased inflammation (Alcendor et al. 2007; Yoshizaki et al. 2009; Grabowska et al. 2017; Meng et al. 2020; Yang et al. 2022). Interestingly, in women, there was a positive age-related correlation between SIRT1 activity and metabolic rate, suggesting that SIRT1 may be a biomarker of ageing, although this correlation was not seen in men (Lee and Yang 2017). Maternal HFD has been reported to decrease the level and activity of histone deacetylase 1 (HDAC1) in the fetal liver, suggesting that HFD-induced maternal obesity may alter the structure of fetal chromatin by modifying histones (Aagaard-Tillery et al. 2008).

Obesity-associated inflammation also induces overexpression of the microRNA miR-155 in adipocytes, which promotes further inflammation, possibly by targeting peroxisome proliferator-activated receptor gamma (PPARγ) (Karkeni et al. 2016). In HFD-induced obese mice, knockdown of the microRNA miR-146b was found to improve insulin resistance and reduce body weight and adiposity by suppressing the SIRT1/FOXO1 signalling cascade (Ahn et al. 2013). In contrast, the microRNA miR-148a was found to promote adipogenesis by suppressing Wnt1 signalling (Shi et al. 2015). Elevated levels of miR-148a are considered to be biomarkers of obesity (Shi et al. 2015), and single-nucleotide polymorphisms in this microRNA have been associated with obesity (Aryal et al. 2017). Interestingly, gastric bypass shifted the methylation pattern of adipose tissue from an obese to a lean methylation pattern (Benton et al. 2015). This suggests that weight loss interventions can reverse the epigenetic changes associated with obesity.

Loss of Proteostasis

Proteostasis refers to the maintenance of protein homeostasis in terms of conformation, concentration, localisation, and turnover. Proteostasis is essential for cell

function, and loss of proteostasis causes the build-up of damaged proteins. The maintenance of proteostasis is achieved through the coordination of multiple interconnected networks that tightly regulate the rate of protein synthesis, chaperone-mediated protein folding, and the degradation of unfolded polypeptides through proteasome-mediated and autophagy-mediated pathways (Labbadia and Morimoto 2015).

One of the best-characterised networks responsible for proteostasis is the unfolded protein response (UPR) (Schröder and Kaufman 2005). The cytosol, ER, and mitochondria contain a network of proteins that are differentially regulated during stress to maintain proteostasis (Walter and Ron 2011). The ER plays a critical role in the coordinated folding, processing, and transport of at least one-third of the proteome (Ellgaard and Helenius 2003). Environmental perturbations of ER homeostasis, such as those caused by hypoxia, glucose deficiency or excess, and oxidative stress, can alter protein folding, leading to an accumulation of misfolded proteins in ER and ER stress (Xu et al. 2005; Malhotra and Kaufman 2007). Severe or prolonged ER stress can eventually lead to apoptotic cell death (Szegezdi et al. 2006). To alleviate ER stress, the UPR is activated to degrade misfolded proteins, promote proper protein folding, and restore ER homeostasis (Walter and Ron 2011). Conversely, the mitochondrial UPR (UPRmt) is activated when mitochondrial ROS levels are high, respiratory chain function is impaired, or there is an imbalance between mitochondrial and nuclear-encoded respiratory chain subunits. The UPRmt triggers the transcription and translation of chaperones and proteases to refold or destroy damaged mitochondrial proteins (Haynes and Ron 2010; Jovaisaite et al. 2014).

With ageing, changes in protein synthesis, turnover, and repair increase the propensity of proteins to become dysfunctional, unfold, and aggregate (Morimoto 2008; Taylor and Dillin 2011). The oxidation of proteins by ROS, the formation of which also increases during ageing, is one of the major causes of protein misfolding and aggregation (Morimoto 2008; Tyedmers et al. 2010). Many UPR chaperones themselves can also be oxidatively damaged during ageing, further impairing their function (Nuss et al. 2008). The accumulation of oxidised and damaged proteins with ageing poses a challenge to maintaining proteostasis (Labbadia and Morimoto 2015), and persistent impairment of proteostasis ultimately activates pro-inflammatory signalling pathways and apoptosis (Szegezdi et al. 2006; Zhang and Kaufman 2008). Pathological accumulation of protein aggregates due to loss of proteostasis has been observed in several age-related diseases, such as PD, AD, and Huntington's disease (Powers et al. 2009). Conversely, long-lived organisms appear to have more stable proteomes and active proteostasis networks than short-lived ones (Pérez et al. 2009; Salmon et al. 2009; Rodriguez et al. 2012; Treaster et al. 2014), and activation of components of the proteostasis network prolongs both lifespan and healthspan in certain organisms (Labbadia and Morimoto 2014; Vilchez et al. 2014; Morimoto and Cuervo 2014), underscoring the importance of proteostasis in ageing and age-related diseases.

Obesity is also associated with altered proteostasis. Compared to lean, insulin-sensitive individuals, the adipose tissue of obese, insulin-resistant individuals

exhibits upregulation of proteins and genes associated with ER stress, as well as increased levels of oxidised and ubiquitinated proteins in their adipose tissue (Boden et al. 2008). The UPR also appears to be impaired in obese adipose tissue (Yilmaz 2017). Pathological nutrient excess may overwhelm the ER (Gregor and Hotamisligil 2007), and increased levels of free fatty acids, insulin, and oxidative stress may alter the proteasome in the liver and adipose tissue of obese individuals, which together may contribute to insulin resistance (Díaz-Ruiz et al. 2015).

Cellular Senescence

Cellular senescence is defined as a specific functional state of the cell characterised by the irreversible arrest of the cell cycle (Campisi and d'Adda di Fagagna 2007). Senescence can be triggered by shortened telomeres and various physicochemical signals, such as mitochondrial dysfunction, oxidative stress, DNA replication stress, and oncogene activation (Kuilman et al. 2010). These "stress signals" activate the tumour-suppressive signalling pathways p53/p21 and p16INK4a/pRB, eventually leading to cell growth arrest (Campisi and d'Adda di Fagagna 2007; Kuilman et al. 2010; Muñoz-Espín and Serrano 2014). Specific features of senescent cells include increased size and protein content, enlarged nuclei, increased activity of senescence-associated β-galactosidase (SA-β-gal), and increased expression of the cyclin-dependent kinase inhibitor p16INK4 (Kuilman et al. 2010).

The development of a senescence-associated secretory phenotype (SASP) is another feature of senescent cells (Coppe et al. 2010), characterised by the enhanced generation of inflammatory cytokines and chemokines, growth factors, extracellular matrix components, fibronectin, and ROS. The SASP is activated to eliminate senescent cells and promote tissue repair and regeneration. However, it also triggers further infiltration of immune cells, leading to even greater ROS production, inflammation, and cell death. The SASP can also cause senescence in functionally active neighbouring cells in a paracrine manner (Kuilman and Peeper 2009).

The observation that the number of senescent cells in tissues increases with age suggests that cell senescence plays an essential role in ageing (Hernandez-Segura et al. 2018). Accordingly, selective elimination of senescent cells was found to extend lifespan and healthspan (Baker et al. 2011a; Jeon et al. 2017). In some cases, the elimination of senescent cells also ameliorated age-related changes in metabolic function (Xu et al. 2015).

Senescence also plays an important role in obesity. Obese rats and humans have more senescent cells in their pre-adipocytes and endothelial cells than their lean counterparts (Minamino et al. 2009; Tchkonia et al. 2010; Xu et al. 2015). The adipose tissue of obese and diabetic individuals also shows increased levels of p53 and enhanced SA-β-gal activity (Yahagi et al. 2004; Minamino et al. 2009; Tchkonia et al. 2010). In obese individuals, cell senescence in adipose tissue stimulates immune cell infiltration, triggering pro-inflammatory effects on pre-adipocytes and increasing the production of inflammatory cytokines (Suganami et al. 2005; Tchkonia et al. 2010). Adipokines secreted by senescent cells in obese adipose

tissue may contribute to obesity-related metabolic disorders such as diabetes (Tchkonia et al. 2010). Accordingly, obese macrophages and adipocytes expressing high levels of p53 exhibited senescence, enhanced generation of inflammatory cytokines, and insulin resistance (Minamino et al. 2009).

Mitochondrial Dysfunction

Cells produce energy in the form of ATP by oxidative phosphorylation in mitochondria. During aerobic respiration, the leakage of electrons from the electron transport chain inevitably leads to the production of ROS. Mitochondrial ROS are effectively scavenged by mitochondrial and cytoplasmic antioxidant enzymes and small molecules under normal physiological conditions. However, with age, antioxidant capacity decreases while the production of ROS increases, leading to oxidative stress that further exacerbates mitochondrial dysfunction (Finkel and Holbrook 2000; Balaban et al. 2005).

Altered mitochondrial membrane fluidity and permeability and decreased membrane potential are associated with the reduced ATP synthesis and mitochondrial dysfunction that characterise ageing. The respiratory activity of mitochondrial enzyme complexes slowly decreases with age (Trounce et al. 1989; Paradies and Ruggiero 1991; Beal et al. 1993), while the production of ROS increases (Sawada and Carlson 1987; Sohal and Sohal 1991; Sohal and Dubey 1994; Capel et al. 2005; Cho et al. 2011; Tower 2015). Ageing impacts mitochondrial gene expression, as evidenced by a decrease in mitochondrial mRNA transcription levels, resulting from decreased mRNA transcription and/or increased mRNA instability with age (Barazzoni et al. 2000; Welle et al. 2000; Short et al. 2005). Additionally, mtDNA copy number is also reduced with age (Barazzoni et al. 2000; Short et al. 2005; Lanza and Nair 2010), contributing to the decline in mitochondrial gene transcripts and proteins. Similarly, mitochondrial mass decreases with age, possibly due to the dysfunction of key regulators of mitochondrial biogenesis, including adenosine monophosphate-activated protein kinase (AMPK) and peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α) (Chistiakov et al. 2014; Regmi et al. 2014; Ji and Kang 2015). Moreover, aged tissues exhibit a higher proportion of enlarged mitochondria and fewer ovoid mitochondria (El'darov et al. 2015; Leduc-Gaudet et al. 2015), as well as increased mitochondrial disorganisation resulting from impaired mitochondrial fission (Brunk and Terman 2002b). Together, the age-related decline in mitochondrial function contributes to the dysregulation of cellular energy homeostasis, which promotes ageing and age-related diseases (Sun et al. 2016a).

Brain mitochondrial dysfunction is considered a critical factor in the development of age-related neurodegenerative diseases, including AD and PD (Lin and Beal 2006). Impaired mitochondrial function is an early characteristic of AD neurons, as evidenced by reduced mitochondrial respiration, decreased pyruvate dehydrogenase concentration and activity, and abnormal mitochondrial dynamics (Blass 2000). The observation of mitochondrial complex I dysfunction in post-mortem brain tissue

from individuals with PD and in animal models also suggests the involvement of impaired mitochondrial function in the pathogenesis of PD (Schapira et al. 1990; Schapira 2008; Exner et al. 2012; Michel et al. 2016).

Mitochondrial dysfunction is also seen in obesity. Obese fat cells show an altered mitochondrial profile with changes in the number, structure, and function of mitochondria (Kusminski and Scherer 2012; de Mello et al. 2018). Microarray studies have found that several mitochondrial genes critical for mitochondrial function and oxidative phosphorylation, including peroxisome proliferator-activated receptor alpha (PPARα), oestrogen-related receptor alpha (ERRα), and PGC-1α, are downregulated in insulin-resistant mice fed an HFD and in the T2D *db*/*db* mouse model (Keller and Attie 2010; Devarakonda et al. 2011), reflecting the impairment of mitochondrial biogenesis in obese insulin-resistant mice. Excess ROS generation in obesity has been associated with increased muscle activity and mechanical stress necessary to carry additional weight, leading to rapid respiration and oxygen consumption and increased mitochondrial electron leakage (Manna and Jain 2015). Excessive nutrient processing by mitochondria may also lead to uncoupling of oxidative phosphorylation, increased ROS generation, and impaired mitochondrial function (Fan et al. 2010). Furthermore, abdominal obesity in rodents and humans has been associated with impaired mitochondrial biogenesis, altered mitochondrial function, reduced mitochondrial gene expression, and decreased ATP generation (Wlodek and Gonzales 2003; Wisløff et al. 2005; Choo et al. 2006; Nisoli et al. 2007; Bódis and Roden 2018). Interestingly, Lindinger et al. (2010) found that mtDNA content in human omental adipose tissue was significantly higher in obese subjects than in non-obese subjects and that there was a positive correlation between mtDNA content and several anthropometric parameters, such as BMI, waist circumference, and the amount of body fat.

The connection between mitochondrial dysfunction and obesity has been demonstrated in mice and humans. In mice, age-related dysfunction of mitochondrial complex IV resulted in decreased fatty-acid oxidation, increased lipid accumulation, and enlargement of WAT (Soro-Arnaiz et al. 2016). Mutations in mitofusin 2 (MFN2), a mitochondrial protein that plays an important role in mitochondrial fusion, have been implicated in mitochondrial dysfunction and increased adipocyte hyperplasia in obese individuals (Rocha et al. 2017). In Zucker rats and obese humans, MFN2 protein expression in skeletal muscle mitochondrial fractions was significantly lower than that in lean counterparts (Bach et al. 2003). Mutations in mitochondrial tRNA genes have also been linked to metabolic disorders and diabetes (Mezghani et al. 2010; Liu et al. 2015).

Adiponectin is critical for lipid and glucose metabolism and exhibits antiatherogenic and anti-inflammatory properties as well as insulin-sensitising effects (Kershaw and Flier 2004). In skeletal muscle, adiponectin stimulates fatty-acid oxidation by increasing mitochondrial biogenesis and the expression of PPAR-α-responsive genes (Yamauchi et al. 2003; Civitarese et al. 2006). Proper mitochondrial function is crucial for adiponectin production, and mitochondrial dysfunction in obese adipose tissue has been associated with decreased adiponectin synthesis (Koh et al. 2007). Mitochondrial dysfunction has also been connected to the inhibition of

the tumour suppressor protein p53, which is already decreased in obesity (Chen et al. 2006), further contributing to the increased risk of certain cancers in obese individuals (Compton et al. 2011). Furthermore, obesity-associated adipokines may contribute to tumourigenesis by promoting mitochondrial dysfunction and the transition to a glycolytic phenotype (Bournat and Brown 2010).

Mitochondrial dysfunction has also been implicated in the development of insulin resistance in peripheral tissues (Szendroedi et al. 2011), possibly due to decreased mitochondrial biogenesis and functional capacity (Petersen et al. 2004; Højlund et al. 2008; Pinti et al. 2019). Additionally, diabetes and obesity-related insulin resistance have been associated with impaired mitochondrial function in the brain, which may contribute to obesity-related cognitive decline (Pipatpiboon et al. 2012; Pintana et al. 2014; Pratchayasakul et al. 2015; Wang et al. 2015; Sun et al. 2016b; Sa-Nguanmoo et al. 2016).

Deregulated Nutrient Sensing

Nutrient sensing pathways are responsible for the ability of cells to recognise and adapt to the surrounding nutrient environment. The insulin/insulin-like growth factor (IGF1) pathway informs cells of the presence of glucose. This pathway begins with one or more tyrosine kinase-type membrane receptors that vary among species (Carlberg et al. 2016). Stimulation of these receptors by glucose activates phosphoinositide 3-kinase (PI3K) and protein kinase B (PKB, also known as Akt)-mediated pathways that lead to the phosphorylation of one or more members of the forkhead box (FOX) family of transcription factors, including forkhead box O (FOXO). FOXO regulates the expression of genes involved in cell death, cell cycle arrest, and stress resistance. Upon phosphorylation by Akt, FOXO translocates from the nucleus to the cytosol, disrupting the transcription of FOXO-regulated genes (Taguchi and White 2008). In lower organisms, including *D. melanogaster*, disrupting the IGF1/PI3K/Akt signal transduction pathway leads to an extended lifespan (Kenyon 2005; Kirkwood 2005). An evolutionarily conserved amino acid sensing pathway has evolved in parallel with glucose sensing pathways. Amino acids bind to Rag GTPases on the cell surface, which phosphorylate and activate Rag proteins and then bind to and activate the mammalian or mechanistic target of rapamycin (mTOR) (Shaw 2008). mTOR then phosphorylates the protein's eukaryotic initiation factor 4E-binding protein 1 (4E-BP1) and ribosomal protein S6 kinase (S6K), increasing protein synthesis (Wullschleger et al. 2006; Saxton and Sabatini 2017; Liu and Sabatini 2020). The insulin/IGF1 pathway can interact with the mTOR/S6K pathway via Akt (Manning and Toker 2017). Inhibition of the mTOR/S6K pathway has been found to prolong the life expectancy of organisms ranging from yeast to mice (Vellai et al. 2003; Kapahi et al. 2004; Powers et al. 2006; Harrison et al. 2009; Miller et al. 2011; Wilkinson et al. 2012).

Perhaps the most substantial evidence for the involvement of nutrient sensing in ageing is the fact that caloric restriction (CR), that is, reducing daily caloric intake by 10–40% without malnutrition and without altering the intake of essential nutrients,

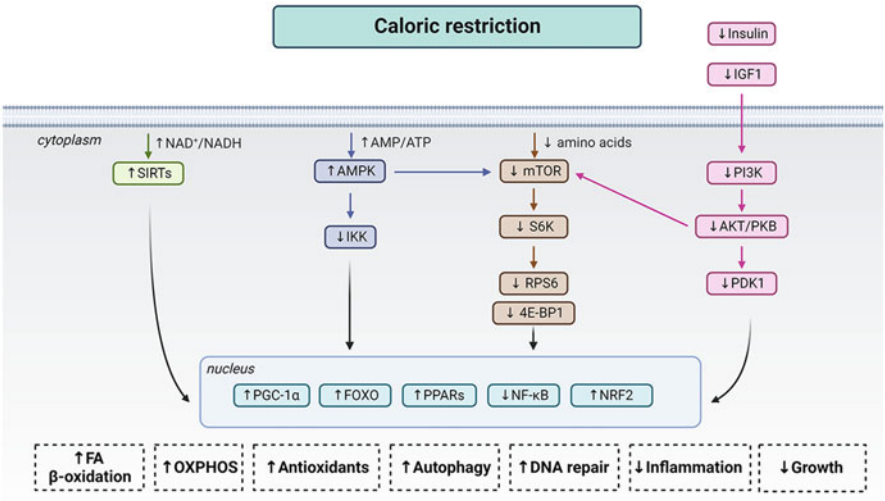


Fig. 15.4 Overview of the main mechanisms by which caloric restriction elicits health benefits. During caloric restriction, increased NAD^+ levels activate sirtuins (SIRTs), whereas increased AMP levels activate AMPK. Conversely, mTOR and IGF1 activity is decreased in response to reduced nutrient levels. As a result, the transcriptional regulators PGC-1α, FOXO, PPARs, and Nrf2 are activated, while NF-κB activity is decreased. The result is increased expression of genes involved in mitochondrial biogenesis, fatty-acid (FA) β-oxidation, gluconeogenesis, stress resistance, and damage repair, thereby reducing growth and inflammation and promoting cell survival. Created with Biorender.com

including vitamins and minerals, remains one of the most robust non-genetic interventions for extending lifespan and healthspan in several model organisms (Heilbronn and Ravussin 2003). CR influences several age-associated signalling pathways involved in the modulation of growth, metabolism, damage response and repair, inflammation, autophagy, and proteostasis (Fig. 15.4) (López-Lluch and Navas 2016; Most et al. 2017). Under CR, increased AMP levels activate the energy sensor AMP-activated protein kinase (AMPK), eliciting an increase in the NAD^+/NADH ratio that activates sirtuins (SIRTs). In turn, SIRTs activate AMPK via a positive feedback loop. Through phosphorylation and deacetylation, AMPK and SIRTs activate FOXO and PGC-1α, promoting the expression of antioxidant enzymes and mitophagy-related signalling pathways. PGC-1α also induces the expression of genes involved in oxidative phosphorylation and mitochondrial biogenesis. Additionally, PGC-1α activates peroxisomal proliferator activator receptors (PPARs), which regulate fatty-acid oxidation. AMPK, SIRTs, FOXO, and PGC-1α work together to extend lifespan under CR through a positive feedback loop (López-Lluch and Navas 2016). The upregulation of SIRTs in response to CR also leads to the deacetylation of several transcription factors, resulting in the suppression of NF-κB and thus eliciting antioxidant and anti-inflammatory effects (Imai et al. 2000; Brunet et al. 2004; Cantó and Auwerx 2012). Moreover, CR inhibits mTOR,

activating autophagy and thereby contributing to its lifespan- and healthspan-extending effects (Chung and Chung 2019).

Obesity is associated with an increase in the amount of circulating insulin and IGF1. Circulating insulin binds cell surface insulin receptors, leading to increased activity of the PI3K/Akt/mTOR pathway (Laplante and Sabatini 2012). Obesity is also associated with an abnormal increase in the activity of stress-activated c-Jun amino-terminal kinase (JNK) (Solinas and Becattini 2017), which promotes cell growth and proliferation and suppresses autophagy (Raman et al. 2007). Inflammatory cytokines, which are elevated in obese individuals, further activate the mTOR pathway, possibly by increasing phosphatidic acid levels in the cell, triggering the production of transcription factors that regulate the synthesis of even more pro-inflammatory cytokines (Conn and Qian 2011). Chronic hyperactivation of mTOR by hyperinsulinaemia can contribute to insulin resistance in obesity by activating S6K, leading to desensitisation of the PI3K/Akt pathway (Um et al. 2004; Khamzina et al. 2005; Tremblay et al. 2007). The mTOR pathway also promotes adipocyte differentiation and hypertrophy, as well as lipogenesis, contributing to fat accumulation (Lamming and Sabatini 2013).

Blocking the mTOR pathway with rapamycin or its homologues ("rapalogues") prolongs lifespan and promotes health in various model organisms (Ramos et al. 2012; Wilkinson et al. 2012; Flynn et al. 2013; Johnson et al. 2013b; Bitto et al. 2016). Consistent with the mechanistic similarities between ageing and obesity, rapamycin also counteracts obesity by affecting adipogenesis, lipogenesis, lipolysis, and thermogenesis (Zoncu et al. 2011; Cai et al. 2016). *In vitro*, rapamycin inhibits primary human adipocyte differentiation and protects against nutrient-mediated insulin resistance in both fat and skeletal muscle cells (Bell et al. 2000; Chang et al. 2009). In mice on an HFD, blocking mTOR with rapamycin was found to reduce obesity and prevent weight gain (Chang et al. 2009). The anti-obesity effect of rapamycin appears to result from decreased food intake, decreased uptake of lipoproteins into tissues, decreased insulin secretion, and increased energy expenditure through lipolysis (Yeh et al. 1995; Bell et al. 2000; Zhang et al. 2009a; Chakrabarti et al. 2010). Rapamycin has also been shown to prevent diabetic complications such as retinopathy, nephropathy, and coronary heart disease (Keogh et al. 2004; Lloberas et al. 2006; Kolosova et al. 2012).

Stem Cell Exhaustion

Stem cells represent a group of self-renewing cells with multidirectional differentiation potential. Stem cells arise during embryogenesis and are maintained throughout the lifespan of an organism. To ensure that stem cells survive throughout an organism's lifespan, a small percentage of these cells must be kept in a dormant state, known as quiescence, which ensures their survival by limiting the number of divisions the cells can undergo (van Velthoven and Rando 2019). This quiescence is lifted in response to tissue damage so that new cells can be rapidly generated to replace damaged tissue without overloading the replication limits of individual stem

cells. Loss of quiescence in hematopoietic, muscular, or neural stem cells is associated with reduced stem cell self-renewal capacity, stem cell pool depletion, and impaired tissue regeneration (Morrison and Spradling 2008). During ageing, the combined effect of telomere shortening and genomic instability reduces the self-renewal capacity of stem cells and their ability to produce differentiated daughter cells (Ermolaeva et al. 2018). The shrinkage of the stem cell pool with age leads to a decline in tissue regenerative and/or repair potential and contributes to increased susceptibility to disease (Ren et al. 2017).

The stem cell pool is also affected by obesity. For example, stem cell-based hematopoietic and osteogenic regeneration is impaired in obesity (Ambrosi et al. 2017) due to the proliferation of adipose tissue in the bone marrow leading to skeletal deterioration, alterations in the bone marrow microenvironment, and increased ER stress in mesenchymal stem cells (MSCs) (da Silva et al. 2016; Tencerova et al. 2018; Ulum et al. 2018). In addition, MSCs derived from the adipose tissue of obese individuals show reduced proliferation and migratory capacity, possibly due to the chronic oxidative and inflammatory environment associated with obesity (Pérez et al. 2013). Conversely, excessive differentiation of MSCs into the adipocyte lineage leads to increased numbers of adipocytes (hyperplasia) in adipose tissue (Hausman et al. 2001). These stem cells may upregulate inflammatory genes that affect angiogenic and adipogenic differentiation in obese individuals (Oñate et al. 2012, 2013). In mutant obese rats (WNIN/Ob), adipose tissue- and bone marrow-derived MSCs also exhibited increased inflammation (Madhira et al. 2011, 2012).

Altered Intercellular Communication

Effective intercellular communication is critical for organ development, stress response, cell survival, tissue development, differentiation, and cell proliferation. Well-coordinated mechanisms are required to generate, distribute, and receive molecular signals. Cells communicate with each other via various chemical signals, including hormones, neurotransmitters, cytokines, and growth factors. Different communication mechanisms are used for different processes, allowing for a variety of communication patterns, including endocrine (mediated by blood-based messengers), paracrine (between individual cells or groups of cells nearby), and neurocrine (mediated by neurotransmitters released from nerve terminals). Additionally, neuronal communication between neurons occurs via synapses and direct communication via gap junctions (Cooper 2000).

Ageing alters intercellular communication, including neuroendocrine and immunological communication (Russell and Kahn 2007), microbiota–host interactions (Li et al. 2016b; Marchesi et al. 2016; Aleman and Valenzano 2019), and neurotransmitter regulation and release (Aprikyan and Gekchyan 1988; Fordyce and Wehner 1993). A recent analysis of the intercellular communication processes disrupted with age in mice revealed that the most widespread changes that occurred with age included the upregulation of immune responses and inflammation and the

downregulation of angiogenesis and extracellular matrix organisation, growth, and development (Lagger et al. 2021).

Inflammation is one of the most important and well-studied intercellular communication processes that changes during ageing. As mentioned earlier, during ageing, the chronic release of SASP factors, the incapacity of the immune system to remove senescent cells, and the hyperactivation of the pro-inflammatory NF-κB pathway result in low-grade chronic sterile inflammation termed "inflammageing" (Franceschi 2007). Obesity is also accompanied by chronic metabolic inflammation, or meta-inflammation, resulting from the increased release of pro-inflammatory adipokines such as leptin and adiponectin, as well as TNF-α, IL-6, and IL-1 (Hotamisligil et al. 1995; Trayhurn and Wood 2004). In addition to increased adipose tissue inflammation, obesity is associated with persistent macrophage infiltration and altered lipid metabolism resulting from changes in the methylation profile of lymphocytes (Jacobsen et al. 2016). Accordingly, time-course microarray analyses of the BAT of mice fed an HFD for up to 24 weeks compared with mice on a normal diet revealed alterations in gene networks associated with lipid metabolism, development, and immunity (McGregor et al. 2013).

Chronic inflammation associated with obesity also leads to increased deposition and remodelling of the extracellular matrix of the adipose tissue (Williams et al. 2015; Lin et al. 2016). While extracellular matrix remodelling is necessary for adipose tissue to expand (Hausman and Richardson 2004), the extracellular matrix of obese stromal cells is denser and stiffer than that of their lean counterparts, which can contribute to an increased risk of breast cancer in obese individuals (Seo et al. 2015; Druso and Fischbach 2018; Ling et al. 2020).

Overexpression of angiogenic factors has also been associated with obesity. During weight gain, angiogenesis is essential for adipose tissue expansion. The direct link between obesity and angiogenesis was demonstrated in experiments in which the inhibition of angiogenesis prevented obesity and mitigated weight gain in obese mice (Rupnick et al. 2002). Likewise, increased levels of angiogenesis markers were found in obese patients compared with control subjects (Piecuch et al. 2019; Wiewiora et al. 2019). Interestingly, weight loss surgery was found to alter the proangiogenic profile and reduce angiogenesis biomarkers in obese patients 12 months after surgery (Wiewiora et al. 2020). Chronic inflammation has also been associated with increased tumour angiogenesis (Gu et al. 2011; Arendt et al. 2013; Kolb et al. 2016), suggesting that increased angiogenesis may play a role in the higher incidence of breast cancer in obese individuals (Fukumura et al. 2016; Kolb et al. 2019).

Dietary Interventions Against Ageing, Age-Related Diseases, and Metabolic Syndrome

Given the critical role that ROS and free radicals play in ageing, age-related diseases, and metabolic disorders, reducing oxidative stress could lead to slowing, preventing, attenuating, and/or reversing these pathological processes. The prevention of disease through dietary interventions, either by adopting a specific diet or by taking nutritional supplements, has been proposed as a therapeutic approach to improve human health. In the following sections, we critically review the epidemiological, preclinical, and clinical evidence for the therapeutic benefits of various dietary interventions against age-related diseases and metabolic disorders, particularly obesity.

Nutraceuticals

In recent years, nutraceuticals, that is, foods or food components with health benefits, including antioxidant and anti-inflammatory properties, have received considerable attention because of their purported therapeutic benefits against various diseases, including age-related diseases. The mechanisms underlying the benefits of nutraceuticals are diverse and sometimes not entirely clear. They include reducing inflammation, lowering cholesterol, strengthening the immune system, improving brain function, and modulating the gut microbiota. Many nutraceuticals reported to have beneficial effects on age-related diseases may also counteract obesity by regulating food intake, reducing lipogenesis and promoting lipolysis, attenuating inflammatory responses, and suppressing oxidative stress.

Polyphenols are well-known bioactive phytochemicals with antioxidant and anti-inflammatory effects that may be useful for treating age-related diseases, obesity, metabolic syndrome, and cancer, among others. The following sections provide an overview of the existing evidence for the therapeutic benefits of some of the most commonly studied polyphenols in ageing, age-related diseases, and obesity.

Curcumin

Curcumin (turmeric) is a well-studied polyphenol found in the rhizome of the plant *Curcuma longa*. Reported health benefits of curcumin include reducing inflammation and the risk of heart disease, improving brain function, and lowering cholesterol and blood sugar levels. Therefore, curcumin is considered to have therapeutic potential against various diseases, including age-related neurodegenerative diseases and diabetes (Strimpakos and Sharma 2008; Hatcher et al. 2008; Fuloria et al. 2022).

Curcumin can not only directly scavenge ROS but also upregulate cytoprotective and antioxidant proteins and is therefore considered a bifunctional antioxidant (Dinkova-Kostova and Talalay 2008; Calabrese et al. 2008; Cory et al. 2018). The

antioxidant properties of curcumin are attributed to its conjugated structure composed of two methoxylated phenols attached to a β-diketone moiety (Masuda et al. 2001). Curcumin can scavenge various types of ROS and RNS, including hydroxyl radicals, superoxide anions, singlet oxygen, peroxyl radicals, nitric oxide, and peroxynitrite (Sreejayan and Rao 1997; Das and Das 2002; Kim et al. 2003; Barzegar and Moosavi-Movahedi 2011).

Several *in vitro* and *in vivo* studies have demonstrated the antioxidant properties of curcumin. Curcumin has been shown to prevent lipid peroxidation, protect against radiation-induced DNA damage, and reduce the formation of protein carbonyls and nitrotyrosine (Reddy and Lokesh 1994; Sreejayan and Rao 1994; Srinivasan et al. 2006; Dkhar and Sharma 2010, 2013; Abrahams et al. 2019). However, at least *in vitro*, some studies have also reported that high concentrations of curcumin (10–100 μM) induce ROS production and DNA damage (Sreejayan and Rao 1997; Das and Das 2002; Kim et al. 2003; Cao et al. 2006; Mendonça et al. 2009; Barzegar and Moosavi-Movahedi 2011).

Curcumin has various molecular targets that may be responsible for its numerous pharmaceutical properties. An important target of curcumin in the context of ageing and obesity is the transcription factor NF-κB, a key regulator of oxidative stress and inflammation (Gupta et al. 2013). *In vitro*, curcumin was reported to reduce inflammation by inhibiting COX-2 activity and suppressing NF-κB activation, presumably by preventing phosphorylation of inhibitory factor IκB kinase (IKK) (Surh et al. 2001; Ukil et al. 2003; Goel et al. 2008). *In vivo*, curcumin has also been shown to inhibit the metabolism of arachidonic acid in the mouse epidermis, reducing inflammation by downregulating signalling pathways involving COX-2 and lipoxygenase (Huang et al. 1991).

Curcumin also acts on the Nrf2-Keap1-ARE pathway, which is critical for preserving redox balance and metabolic homeostasis and regulating inflammation (Serafini et al. 2019; Shin et al. 2020). Under normal homeostatic conditions, Keap1 binds to Nrf2, targeting it for degradation by the proteasome. This maintains Nrf2 at a low level in the cytoplasm (Kobayashi et al. 2004; Cullinan et al. 2004; Zhang et al. 2004). Under redox stress, Keap1 is oxidised and releases Nrf2, which then translocates to the nucleus, where it binds small Maf (musculoaponeurotic fibrosarcoma) proteins (sMAF). Nrf2-sMAF heterodimers bind to antioxidant response elements (ARE) in the promoters of genes encoding a network of cooperating proteins that protect against numerous diseases, including neurodegenerative and metabolic disorders (Cuadrado et al. 2018; Yamamoto et al. 2018). Curcumin disrupts the binding between Keap1 and Nrf2, allowing Nrf2 to enter the nucleus and activate the transcription of ARE-carrying genes. This leads to the production of antioxidant enzymes and anti-inflammatory mediators and activates the proteasome and various transcription factors involved in mitochondrial biogenesis (Calabrese et al. 2008; Tufekci et al. 2011; Esatbeyoglu et al. 2012).

Brain-derived neurotrophic factor (BDNF), which is under the control of the transcriptional regulator cAMP-response element binding protein (CREB), plays a crucial role in neuronal cell survival, neuronal integrity, and synaptic plasticity (Finkbeiner et al. 1997; Finkbeiner 2000; Huang and Reichardt 2003). Increased

oxidative stress associated with ageing is accompanied by decreased BDNF expression, leading to cognitive decline (Castelli et al. 2019). Conversely, an increase in BDNF ameliorates learning and memory deficits (Wu et al. 2004; Erickson et al. 2010). D-galactose-induced learning and memory impairments in aged mice were attenuated by curcumin due to increased CREB and BDNF levels (Nam et al. 2014). Likewise, the treatment of rats with chronic unpredictable stress-induced cognitive deficits with curcumin also restored the levels of BDNF and altered the expression of proteins of the extracellular signal-regulated kinase (ERK) signalling pathway (Liu et al. 2014b), which modulates the activities of CREB and NF-κB (Enserink et al. 2002; Emery and Eiden 2012).

The therapeutic benefits of curcumin have also been associated with its effect on SIRTs (Xiao et al. 2016). In a surgically induced osteoarthritis animal model, curcumin reduced oxidative stress and ER stress and inhibited apoptosis by increasing the levels of the deacetylase SIRT1 (Feng et al. 2019). In turn, SIRT1 reduced the levels of ER stress-responsive proteins, including the phosphorylated protein kinase R-like ER kinase (PERK), the phosphorylated alpha subunit of eukaryotic translation initiation factor 2 (eIF2α), and C/EBP homologous protein (CHOP), thereby attenuating apoptosis and preventing further progression of osteoarthritis (Feng et al. 2019). In an experimental stroke model, curcumin was reported to exert neuroprotective effects by activating SIRT1, decreasing brain levels of the pro-inflammatory markers TNF-α and IL-6, and increasing the activity of mitochondrial complex I and the levels of mitochondrial cytochrome c (Miao et al. 2016).

The therapeutic benefits of curcumin may also be mediated by its effects on mTOR signalling (Beevers et al. 2009). For instance, curcumin was shown to alleviate inflammation, synovial hyperplasia, and other symptoms in a rat model of rheumatoid arthritis by suppressing Akt/mTOR signalling and decreasing the levels of pro-inflammatory markers, including IL-1β, TNF-α, and matrix metalloproteinases 1 and 3 (Dai et al. 2018).

The effects of curcumin on longevity vary by model organism, sex, and genotype. In *Caenorhabditis elegans*, curcumin was found to extend lifespan by decreasing intracellular ROS levels and lipofuscin accumulation during ageing (Liao et al. 2011). In the fruit fly *D. melanogaster*, curcumin was shown to extend lifespan, an effect attributed, at least to some extent, to its antioxidant activities and its capacity to modulate the expression of several genes related to ageing, including genes of the mTOR and JNK pathways (Lee et al. 2010; Soh et al. 2013). In mammals, the curcumin metabolite tetrahydrocurcumin extended the average lifespan of male C57BL/6 mice (Kitani et al. 2007). In contrast, feeding curcumin to 4- or 12-month-old F1 hybrid mice or mice of genetically heterogeneous backgrounds did not prolong lifespan (Strong et al. 2013; Spindler et al. 2013).

Beyond lifespan, beneficial effects of curcumin on health parameters have also been reported. In aged rats, curcumin treatment improved muscle mass and function (Receno et al. 2019). In rodent models of accelerated ageing (Sun et al. 2013; Nam et al. 2014) and healthy older rodents (Dong et al. 2012; Belviranlı et al. 2013; Yu et al. 2013), curcumin improved cognitive performance, while in middle-aged rhesus

monkeys, long-term administration of curcumin improved fine motor function (Moore et al. 2018).

In vitro and *in vivo* studies have demonstrated the neuroprotective effects of curcumin in AD. The underlying neuroprotective mechanisms are multifactorial and include stimulation of neurogenesis and neuronal differentiation, reduction of Aβ and tau accumulation and aggregation, modulation of the levels and activity of β-secretase and acetylcholinesterase, promotion of amyloid clearance, attenuation of mitochondrial dysfunction, activation of the UPR, and attenuation of neuroinflammation and oxidative stress (Zhu et al. 2004; Giri et al. 2004; Begum et al. 2008; Ahmed and Gilani 2009; Ishrat et al. 2009; Shytle et al. 2009; Hamaguchi et al. 2010; Wang et al. 2013; Eckert et al. 2013; Tiwari et al. 2014; Hagl et al. 2015; Reddy et al. 2016; Akinyemi et al. 2017; Chen et al. 2018; Sala de Oyanguren et al. 2020). *In vivo*, curcumin has been reported to attenuate or reverse cognitive deficits associated with neurotoxicity of β-amyloid peptides by suppressing glial activity (Wang et al. 2013) and reducing brain concentrations of amyloid plaques, oxidised proteins, and isoprostanes (Frautschy et al. 2001; Lim et al. 2001).

In preclinical studies, curcumin was shown to reduce and restore PD-related motor impairment (Khajavi and Lupski 2008; Spinelli et al. 2015; Khatri and Juvekar 2016). Mechanisms underlying the therapeutic effects of curcumin in PD include promotion of neurogenesis and neuron differentiation, prevention of α-synuclein aggregation, attenuation of neuroinflammation, enhancement of antioxidant defences, reduction of oxidative stress through ROS/RNS scavenging and/or metal chelation, regulation of autophagy, promotion of amyloidogenic protein degradation, and modulation of dopamine levels (Sharma and Nehru 2018; Heebkaew et al. 2019; Tabatabaei Mirakabad et al. 2020; Doytchinova et al. 2020; Song et al. 2020).

Clinical studies in humans have shown that curcumin is well tolerated and non-toxic even at high doses, suggesting that it could be used over an extended period without significant side effects (Zhang et al. 2009a). However, clinical trials have shown conflicting results regarding the benefits of curcumin on cognitive function. In healthy middle-aged people, a 4-week intake of a lipidated form of curcumin (80 mg/day) had several beneficial effects, including a reduction in plasma β-amyloid protein (DiSilvestro et al. 2012). Likewise, Cox et al. (2015) reported that a 4-week supplementation (400 mg/day) with a solid lipid formulation of curcumin (Longvida®) improved working memory and attention. In contrast, other studies have found that the intake of curcumin (up to 4 g/day) for 3–12 months did not affect cognitive function compared with the placebo group (Baum et al. 2008; Rainey-Smith et al. 2016; Santos-Parker et al. 2018). Similarly, a 24-week placebo-controlled study with AD patients found no biochemical evidence for the efficacy of curcumin at up to 4 g/day (Ringman et al. 2012).

Systematic reviews and meta-analyses of randomised controlled trials have revealed that curcumin supplementation increased BDNF levels by an average of 21.8% in adults (Sarraf et al. 2019) and that curcumin may improve cognitive

function in some patient populations but not in others (Zhu et al. 2019; Tsai et al. 2021).

Overall, the currently available evidence in humans does not allow for a generalisation of the results, as only a few studies have been conducted, the sample sizes are relatively small, and the results vary greatly from study to study. The insolubility of curcumin in water, its low bioavailability, and the difficulty in crossing the BBB are some possible reasons for the inability of curcumin to exert therapeutic effects in humans.

Theracurmin® is a highly bioavailable nanoformulation of curcumin. In the 5XFAD mouse model of AD, it was reported that treatment with Theracurmin® improved recognition ability and spatial memory compared with vehicle-treated controls. The authors attributed this effect to the ability of Theracurmin® to increase synaptic component expression and prevent neuronal cell damage due to oxidative stress or microglial activation (Kim et al. 2019). In the first double-blind, placebo-controlled, long-term (18-month) study of Theracurmin® (90 mg curcumin twice daily) in adults without dementia, Theracurmin® intake was found to significantly improve memory and attention, which was associated with reductions in amyloid and tau levels in brain regions that modulate mood and memory, as assessed using positron emission tomography (Small et al. 2018). The development of improved delivery systems and the synthesis of curcumin analogues that can mimic its neuroprotective effects but reach the brain more efficiently are ongoing (Maiti and Dunbar 2018).

There are also reports of curcumin's ability to promote weight loss and improve insulin signalling, suggesting that it may be useful in treating obesity and metabolic syndrome. Mechanisms responsible for the benefits of curcumin in weight management include attenuation of mitochondrial dysfunction, oxidative stress, and inflammation, promotion of mitochondrial biogenesis, reduction of adiposity and lipid storage, and induction of fatty-acid oxidation. Additionally, curcumin was reported to reduce atherogenic risk in patients with diabetes by lowering insulin resistance, triglycerides, and visceral and total body fat (Weisberg et al. 2008; Chuengsamarn et al. 2014; Prasad et al. 2014).

Several clinical trials have investigated the potential of curcumin to treat obesity. In one study, obese subjects were treated with curcumin (1 g/day) for 30 days, along with a bioavailability enhancer (piperine) (Mohammadi et al. 2013). Compared with controls, weight, BMI, and body fat were not affected by curcumin treatment, but serum triglyceride levels were significantly reduced, suggesting improved insulin activity. Conversely, Di Pierro et al. (2015) reported that curcumin (800 mg twice daily for 1 month) complexed with piperine promoted weight loss and reduced omental adipose tissue in overweight people with metabolic syndrome. Saraf-Bank et al. (2019) also found that curcumin (500 mg/day for 10 weeks) was effective in lowering body weight, BMI, waist circumference, triglycerides, LDL, and total cholesterol levels in obese and overweight adolescent girls. Positive effects of curcumin (1 g/day for 4 weeks) on the levels of inflammatory cytokines and oxidative stress biomarkers in the serum of obese subjects have also been reported (Ganjali et al. 2014).

Epigallocatechin Gallate

The most abundant polyphenolic catechin in green tea is epigallocatechin-3-gallate (EGCG). Health benefits attributed to EGCG include reducing inflammation, boosting the immune system, lowering cholesterol and blood pressure, and protecting against heart disease and cancer (Singh et al. 2011).

The beneficial effects of EGCG have been attributed to its antioxidant activity. EGCG is a potent free radical scavenger because it contains eight free hydroxyl groups. The phenolic hydroxyl groups on the aromatic rings of EGCG are also associated with its iron-chelating activity (Rice-Evans et al. 1995; Fujisawa and Kadoma 2006; Intra and Kuo 2007; Perron and Brumaghim 2009; Hatcher et al. 2009; Azman et al. 2014; Amadi et al. 2019). However, *in vitro*, EGCG was found to exhibit pro-oxidant and cytotoxic effects at high concentrations (>50 μM) and in the presence of Fe(III) (reviewed by Kim et al. 2014b). The health benefits of EGCG have also been attributed to its ability to induce mitohormesis, an adaptive response that promotes mitochondrial biogenesis through a steady increase in mitochondrial ROS production, leading to the upregulation of stress resistance proteins (Zhang et al. 2009b; Xiong et al. 2018).

Several ageing-related signalling pathways have been implicated in the beneficial effects of EGCG on lifespan and healthspan. For instance, activation of AMPK and subsequent effects on glucose metabolism have been linked to the lifespan-extending properties of EGCG in *D. melanogaster* (Wagner et al. 2015). However, Lopez et al. (2014) reported that EGCG-induced lifespan extension negatively impacted male fertility, as evidenced by a lower number of offspring and increased mating latency in EGCG-treated flies. In *C. elegans*, EGCG was found to prolong life expectancy and improve health by enhancing the nuclear accumulation of the FOXO orthologue Dauer-independent factor 16 (DAF-16) and increasing the expression of the DAF-16 target gene *sod-3* encoding the antioxidant enzyme superoxide dismutase (Bartholome et al. 2010). More recently, Tian et al. (2021) identified a role for complex I inhibition in the life-prolonging effects of EGCG. However, in other studies, the extension of the *C. elegans* lifespan by EGCG occurred only under stress conditions but not under standard culture conditions, suggesting that the observed lifespan extension was due to the upregulation of proteins related to stress resistance (Zhang et al. 2009b).

Green tea catechins were found to prolong the life expectancy of male C57BL/6 mice (Kitani et al. 2007) and Wistar rats (Niu et al. 2013) by attenuating inflammation and oxidative stress, possibly through inhibition of NF-κB signalling via FOXO3a and SIRT1 (Niu et al. 2013). EGCG was also found to prolong the lifespan of rats fed an HFD by ameliorating lipid metabolism and attenuating inflammation and oxidative stress (Yuan et al. 2020).

The therapeutic benefits of EGCG in cognitive dysfunction have also been reported. EGCG has been shown to attenuate lipid- and fructose-related cognitive deficits (Mi et al. 2017) and mitigate long-term memory loss in ischaemic rats by reducing oxidative stress and inflammation (Wu et al. 2012b). In mouse models of

AD, EGCG slowed cognitive degeneration (Rezai-Zadeh et al. 2008; Chang et al. 2015) and improved spatial memory (Haque et al. 2006). In the amyloid-β-protein precursor/presenilin 1 (APP/PS1) mouse model of AD, EGCG reduced spatial memory impairment by improving hippocampal insulin signalling (Jia et al. 2013). EGCG intake and exercise, both individually and in combination, also reduced cognitive dysfunction in a transgenic mouse model of AD (Walker et al. 2015). The mechanisms underlying the therapeutic benefits of EGCG in AD include inhibition of Aβ- and tau-protein aggregation and reduction in the amount of aggregated proteins, alleviation of oxidative stress, metal chelation, induction of α-secretase activity, and reduction of acetylcholinesterase activity and neuroinflammation (Abbas and Wink 2010).

Beneficial effects of EGCG in PD have also been reported. These benefits are attributed to EGCG's ability to mitigate damage to dopaminergic neurons, promote neurogenesis, inhibit iNOS production, chelate metals, reduce α-synuclein aggregation, attenuate mitochondrial dysfunction and neuroinflammation, and exert anti-apoptotic effects (Wang et al. 2012; Zhou et al. 2018). Tea polyphenols restored locomotor activity in paraquat-treated *D. melanogaster* models of PD (Jimenez-Del-Rio et al. 2010). EGCG also attenuated functional and neurochemical deficits in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-treated mice by modulating the levels of the iron export protein ferroportin in the midbrain and alleviating oxidative stress (Xu et al. 2017). In the 6-hydroxydopamine (6-OHDA) mouse model of PD, green tea or EGCG extracts reversed pathological and behavioural changes and improved cognitive dysfunction through antioxidant and anti-inflammatory properties (Bitu Pinto et al. 2015). In another study using the same PD model, EGCG-mediated neuroprotection and attenuation of motor abnormalities were attributed to decreased α-synuclein expression and reduced mTOR, Akt, and glycogen synthase kinase-3 beta (GSK3-β) levels (Zhou et al. 2019). In the rotenone-induced mouse model of PD, EGCG treatment attenuated motor impairments by decreasing levels of RNS, lipid peroxides, and neuroinflammation, as well as markers of apoptosis, and increasing the activity of several electron transport chain enzymes and catecholamine levels in the striatum (Tseng et al. 2020). In monkeys with PD, oral administration of tea polyphenols reduced motor impairments and attenuated dopamine depletion and damage to dopaminergic neurons (Chen et al. 2015a).

Epidemiological studies have associated regular consumption of green tea (at least 2 cups/day) with a reduced risk of cognitive decline and AD (Mandel et al. 2012; Polito et al. 2018). Green tea consumption has also been linked to a decrease in all-cause mortality and cardiovascular mortality, but not mortality associated with cancer (Mandel et al. 2012; Polito et al. 2018). Other epidemiological studies have shown a reduced prevalence of T2D in green tea drinkers (Iso et al. 2006). However, despite promising preclinical evidence, human clinical trials have been unable to demonstrate significant benefits of green tea catechins, particularly EGCG, on neurodegenerative diseases (Levin et al. 2019). For example, in an elderly Japanese cohort, a daily intake of 220 mg of catechins for 12 months significantly

lowered the levels of oxidative stress markers but did not improve cognitive function compared with the placebo group (Ide et al. 2016).

The effects of EGCG on obesity and metabolic syndrome have also been reported. For example, in Sprague–Dawley rats, EGCG induced acute weight loss within days of treatment (Choo 2003). Similarly, EGCG was reported to significantly reduce or prevent weight gain in lean and obese Zucker rats (Kao et al. 2000). In C57BL/6J mice, tea catechin administration was shown to substantially reduce weight gain and visceral and liver fat, mitigate the development of hyperinsulinaemia and hyperleptinaemia, and increase liver β-oxidation (Murase et al. 2002). In the same mouse model, diet-induced increases in body weight and plasma levels of glucose, triglycerides, and leptin were also prevented by EGCG supplementation (Wolfram et al. 2005).

In an HFD-induced obesity model, EGCG supplementation significantly reduced weight gain, visceral fat weight, and fasting blood glucose levels and attenuated hyperlipidaemia-induced atherosclerosis and systemic organ damage (Bose et al. 2008). In an obese fructose-fed hamster model treated with EGCG, plasma adiponectin levels were increased, and triacylglycerol levels were decreased (Li et al. 2006). Additionally, in spontaneously hypertensive rats with insulin resistance and obesity, EGCG treatment improved metabolism and cardiovascular function (Potenza et al. 2007). In the *db*/*db* diabetes mouse model, EGCG also attenuated glucose intolerance (Ortsäter et al. 2012; Wein et al. 2013).

The mechanisms that may explain the beneficial effects of EGCG in weight control include alteration of the gut microbiome, decreased adipocyte differentiation and proliferation, prevention of fat uptake from the gut, and increased energy expenditure (Hursel and Westerterp-Plantenga 2010; Meydani and Hasan 2010; Chen et al. 2019). In particular, EGCG appears to modulate energy expenditure by activating AMPK, stimulating fatty-acid oxidation, and reducing fat accumulation (Murase et al. 2009; Li et al. 2018).

Several epidemiological studies and randomised controlled trials have investigated the relationship between tea consumption and metabolic syndrome and diabetes with conflicting results. An 8-week randomised controlled clinical trial in overweight men found that 400 mg of EGCG twice a day did not affect insulin sensitivity or secretion, or glucose tolerance (Brown et al. 2009). However, in another study, 12 weeks of daily intake of 625 mg of EGCG-containing catechins resulted in greater weight loss and lower fasting serum triglyceride levels than in subjects receiving a placebo (Maki et al. 2009).

Systematic reviews and meta-analyses of randomised controlled trials have found that green tea consumption can significantly reduce fasting blood sugar levels, body weight, and BMI (Xu et al. 2020) and improve insulin resistance in patients with T2D and lipid abnormalities (Liu et al. 2014a).

Resveratrol

Resveratrol (3,5,4′-trihydroxy-trans-stilbene) is a polyphenol commonly present in grapevine (*Vitis vinifera*), grape juice, and wine, especially red wine. Resveratrol has been linked to a lower risk of heart disease, cancer, inflammation, neurodegenerative disease, and improved blood sugar control. These therapeutic benefits are associated with its anti-inflammatory, antioxidant, and metal chelating properties (Candelario-Jalil et al. 2007; Sun et al. 2008; Robb et al. 2008; Gülçin 2010; Lee et al. 2012; Quincozes-Santos et al. 2014). However, the bioavailability of resveratrol in mammals is relatively low (Das et al. 2008), which limits its role as a direct ROS radical scavenger *in vivo* (Leonard et al. 2003). Moreover, some studies have identified cytotoxic effects of resveratrol which were attributed to resveratrol-induced ROS generation and subsequent activation of apoptosis (Juan et al. 2008).

Resveratrol also induces the activity of antioxidant enzymes such as superoxide dismutase, catalase, thioredoxin, and glutathione peroxidase (Floreani et al. 2003; Liu et al. 2017b; Salehi et al. 2018). Other cytoprotective mechanisms of resveratrol include stimulation of antioxidant enzyme activity and inhibition of ROS-forming enzymes, including NADPH oxidase and xanthine oxidase (Doré 2005; Deby-Dupont et al. 2005; Huang et al. 2008).

The beneficial effects of resveratrol in ageing and age-related diseases are mainly attributed to its ability to activate SIRTs (Baur 2010) and FOXO (Motta et al. 2004; van der Horst et al. 2004), leading to increased expression of the antioxidant enzymes superoxide dismutase and catalase, inhibition of ROS production, and attenuation of oxidative stress (Cheng et al. 2014; Cosín-Tomàs et al. 2019). Additionally, resveratrol promotes mitochondrial biogenesis and function by increasing the activity of PGC-1α (Lagouge et al. 2006; Parihar et al. 2015; Kumar and Lombard 2015). Activation of FOXO by resveratrol may also upregulate genes related to autophagy/mitophagy, thereby promoting the clearance of damaged proteins and mitochondria (Webb and Brunet 2014; Chaanine et al. 2016; Sebori et al. 2018). Moreover, resveratrol has been found to activate the transcription factor Nrf2, thereby promoting the synthesis of enzymatic and non-enzymatic antioxidants (Kode et al. 2008; Ungvari et al. 2010; Kim et al. 2010). Resveratrol also inhibits NF-κB and NF-κB-dependent genes, which may explain its anti-inflammatory effects (Tsai et al. 1999; Manna et al. 2000; Holmes-McNary and Baldwin 2000; Surh et al. 2001; Estrov et al. 2003).

Resveratrol has been reported to prolong the lifespan and/or healthspan of yeast (Howitz et al. 2003), *C. elegans* (Wood et al. 2004; Viswanathan et al. 2005; Collins et al. 2006), *D. melanogaster* (Bauer et al. 2004), and the turquoise killifish *Nothobranchius furzeri* (Valenzano et al. 2006), while no lifespan-extending effects were observed in resveratrol-fed *Daphnia* (Kim et al. 2014a). In mice, the effect of resveratrol on longevity is unclear. Some authors have reported that resveratrol does not affect life expectancy, while others have found a positive effect of resveratrol only under certain conditions, for instance, when mice were fed an HFD but not in

mice fed a normal diet (Pearson et al. 2008). Additionally, resveratrol has been reported to improve mice health by delaying vascular ageing (da Luz et al. 2012).

Resveratrol also exhibits neuroprotective properties due to its anticholinesterase activity and ability to eliminate free radicals and reduce oxidative stress, prevent the formation of pathogenic protein aggregates, modulate hippocampal plasticity, and attenuate neuroinflammation (Bhullar and Rupasinghe 2013; Kodali et al. 2015; Gomes et al. 2018). In senescence-accelerated mouse-prone 8 (SAMP8) mice, maternal resveratrol and vitamin D supplementation were found to attenuate cognitive impairment through effects on neuroinflammation, tau phosphorylation, and amyloidogenic signalling pathways, among others (Cheng et al. 2017; Izquierdo et al. 2019). Chronic administration of resveratrol also protected against oxidative stress and cognitive impairment associated with intrathecal injection of streptozotocin (Sharma and Gupta 2002).

In the APP/PS1 mouse model of AD, long-term treatment with resveratrol was found to reduce amyloid load and prevent memory loss by activating SIRT1- and AMPK-mediated signalling pathways (Porquet et al. 2014). Resveratrol has been reported to attenuate hippocampal neurodegeneration and cognitive deficits in an animal model with AD and tau pathology by reducing the acetylation of p53 and PGC-1α by SIRT1 (Kim et al. 2007). In the Tg2576 mouse model of AD, resveratrol-rich red wine was also reported to reduce the deterioration of spatial memory function (Wang et al. 2006).

The therapeutic benefits of resveratrol have also been reported in PD models. Resveratrol protected mice from MPTP-induced motor dysfunction, neuronal loss, and excess hydroxyl radicals (Lu et al. 2008) and decreased the expression of COX-2 and TNF-α in the *substantia nigra* of the 6-OHDA-induced PD rat model (Jin et al. 2008).

Clinical trials have examined the effects of resveratrol on cognitive function with varying results. In people with mild cognitive impairment, long-term (26 weeks) resveratrol supplementation (200 mg/day) mitigated cognitive decline and improved hippocampal functional connectivity (Witte et al. 2014; Köbe et al. 2017). Furthermore, resveratrol was shown to significantly increase the cerebrovascular response to hypercapnic and cognitive stimuli in postmenopausal women and improve general cognitive performance (Wightman et al. 2014). Conversely, in individuals with mild-to-moderate AD, cerebrospinal fluid and plasma Aβ-40 levels decreased more in the placebo group than in the resveratrol-treated group (Turner et al. 2015).

The therapeutic benefits of resveratrol have also been reported in obesity and metabolic syndrome and attributed to the ability of resveratrol to activate AMPK, leading to enhanced glucose uptake and fatty-acid oxidation (Zang et al. 2006). There are also reports that resveratrol promotes mitochondrial biogenesis and oxidative phosphorylation and reduces lipid accumulation by increasing the activity of SIRT1 and PGC-1α, reducing lipid synthesis, and increasing lipolysis in adipocytes (Li et al. 2016c; Majeed et al. 2021). Accordingly, mice on an HFD supplemented with resveratrol gained less body weight and accumulated less fat, triglycerides, and liver weight than mice on the same diet that did not receive resveratrol (Ahn et al. 2008).

Despite promising preclinical evidence, clinical trials have not yet yielded conclusive results on the therapeutic benefits of resveratrol in metabolic disorders. In some studies, chronic or acute resveratrol treatment improved endothelial function and cardiovascular health in obese adults (Wong et al. 2011, 2013). In non-insulin-dependent older adults (49–78 years old) with T2D, weekly treatment with synthetic trans-resveratrol (75, 150, and 300 mg) improved cerebrovascular function (Wong et al. 2016). In contrast, in another study of obese men, a 4-week treatment with resveratrol (500 mg trans-resveratrol thrice daily) did not affect endogenous glucose production, turnover or oxidation rates, insulin sensitivity, or body composition (Poulsen et al. 2013). In non-obese postmenopausal women, 12-week resveratrol intake (75 mg/day) also had no effects on body composition, metabolic parameters, inflammatory markers, or insulin sensitivity. Additionally, no effects were observed on its putative targets (e.g. AMPK, SIRT1, or PGC-1α) in the skeletal muscle or adipose tissue of the subjects (Yoshino et al. 2012).

A review of the literature by Shaito et al. (2020) concluded that resveratrol is generally safe, but that adverse effects may occur in some people at high doses, most commonly gastrointestinal symptoms such as nausea, vomiting, and diarrhoea, as well as headaches, dizziness, and fatigue.

Quercetin

The polyphenol flavonoid quercetin (3,5,7,3′,4′-pentahydroxyflavone) can be found in fruits and vegetables, including capers, red onions, and kale. Potential health benefits attributed to quercetin include reducing inflammation, allergies, and cancer risk and improving heart and brain function (Salehi et al. 2020).

Quercetin has ROS scavenging activity due to the presence of several hydroxyl groups (Formica and Regelson 1995; Mira et al. 2002; Anjaneyulu and Chopra 2004; Okamoto 2005; Kampkötter et al. 2007, 2008; Terao 2009; Nabavi et al. 2012). In addition, quercetin protects against oxidative stress by chelating metals, mitigating ROS and RNS generation, and preventing lipid peroxidation (Bindoli et al. 1985; Mira et al. 2002; López-López et al. 2004). *In vitro*, some studies have reported that quercetin concentrations greater than 50 μM are cytotoxic, an effect that has been explored for cancer therapy (Chang et al. 2006; Chen et al. 2013a; Rauf et al. 2018). Moreover, quercetin, like other polyphenols, may have pro-oxidant effects under certain experimental conditions (Long et al. 2000). Quercetin was also reported to be genotoxic and mutagenic *in vitro* (Schimmer et al. 1988; Suzuki et al. 1991). However, subsequent studies have shown that the protective effects of quercetin *in vivo* outweigh its adverse effects and that quercetin is not genotoxic in mice at doses of up to 2000 mg/kg body weight (Harwood et al. 2007).

As with other polyphenols, the cytoprotective effects of quercetin are attributed to its ability to promote Nrf2 nuclear translocation and binding to ARE-containing genes, thereby activating the expression of detoxifying and antioxidant enzymes and the proteasome (Granado-Serrano et al. 2010; Si et al. 2011; Moreno-Ulloa et al. 2015; Li et al. 2016a). Quercetin may also modulate the activity of the

age-promoting IGF1/insulin and PI3K/Akt signalling pathways (Si et al. 2011; Pietsch et al. 2012). Additionally, the longevity-promoting effects of quercetin have been associated with increased stress resistance (Son et al. 2008; Pietsch et al. 2011), and in some model organisms, suppression of mTOR and IGF1/insulin signalling pathways by quercetin was reported to increase longevity through activation of heat shock factor 1 (Seo et al. 2013). Moreover, quercetin-induced inhibition of mTOR signalling promotes autophagy and suppresses protein synthesis and cell growth (Son et al. 2008; Pietsch et al. 2011; Johnson et al. 2013a). Quercetin also reduces inflammation by inhibiting TNF-α, NF-κB, mitogen-activated protein kinase (MAPK), COX-2, and MCP-1, among others (Comalada et al. 2005; Overman et al. 2011).

In the yeast *Saccharomyces cerevisiae*, quercetin increased lifespan and stress resistance (Belinha et al. 2007). Dietary supplementation with quercetin has also been shown to extend the life expectancy of *C. elegans* by up to 20%. This effect was at least partly attributed to the modulation of the insulin/IGF1 signalling pathway by quercetin, since recessive mutations in the *age-1* or *daf-2* genes abolished quercetin-induced lifespan extension (Saul et al. 2008; Pietsch et al. 2009). Increased translocation of DAF-16 to the nucleus induced by quercetin in *C. elegans* has also been implicated in its ability to extend lifespan (Kampkötter et al. 2008). In *D. melanogaster*, short-term quercetin ingestion at young and middle age increased lifespan in females; however, no such effect was observed in males (Proshkina et al. 2016).

Quercetin has also been reported to exert a rejuvenating effect on senescent fibroblasts and prolong their lifespan by acting as a proteasome activator (Chondrogianni et al. 2010). Screening of a library of natural products identified quercetin as a geroprotectant in a model of premature ageing (human Werner syndrome mesenchymal stem cells) (Geng et al. 2019). However, *in vivo* studies found that dietary supplementation with quercetin either significantly shortened the lifespan of mice (Jones and Hughes 1982) or had no significant effect (Spindler et al. 2013). Interestingly, the combination of quercetin with the senolytic drug dasatinib increased the average lifespan of C57BL/6 mice by 6.3% and improved physical function (Xu et al. 2018).

Several studies have also reported the neuroprotective effects of quercetin, attributed to its ability to reduce oxidative stress, attenuate neuroinflammation, activate autophagy, and protect against ischaemia, among others (Costa et al. 2016). In aged mice, quercetin ameliorated cognitive dysfunction caused by dietary intake of advanced glycation products (Yang et al. 2020). In a transgenic mouse model of AD, quercetin was reported to reduce β-amyloid and tau accumulation in the hippocampus and amygdala, improving cognitive function (Sabogal-Guáqueta et al. 2015). Quercetin-loaded exosomes also improved cognitive performance in mice with okadaic acid-induced AD (Qi et al. 2020). The combined administration of quercetin and fish oil was found to have a neuroprotective effect in rats with 3-nitropropionic acid-induced PD (Denny Joseph and Muralidhara 2013, 2015).

In a clinical trial of healthy elderly subjects, 24 weeks of continuous consumption of quercetin-rich onions (estimated daily intake of 50–100 mg of quercetin) was

found to reduce age-related cognitive decline, which was attributed to improved emotional state (Nishihira et al. 2021). Conversely, another study did not find significant effects of supplementation with quercetin (500 mg/day or 1000 mg/day) for 12 weeks on different cognitive parameters, including memory, reaction time, and attention (Broman-Fulks et al. 2012).

Quercetin has been reported to inhibit adipogenesis by activating AMPK and suppressing adipogenic factors, including PPAR-γ, and reduce hyperglycaemia by inhibiting the PI3k/Akt pathway (Vinayagam and Xu 2015). Quercetin also reduced body weight by alleviating inflammation through its effects on Nrf2, haem oxygenase-1, and NF-κB (Panchal et al. 2012). However, Wistar rats on an HFD that was also high in sucrose supplemented with quercetin showed a decrease in basal glucose and insulin levels but no differences in body weight, fat accumulation, or triacylglycerol levels (Arias et al. 2014). In another study, treatment of Wistar rats with quercetin or quercetin-iron complexes reduced oxidative stress markers and increased antioxidant protection due to quercetin's ability to scavenge ROS and free radicals (Imessaoudene et al. 2016). In addition, quercetin prevented and reduced HFD-induced liver damage in rats (Mamun et al. 2019; Kumar et al. 2019).

Quercetin, in doses of 100–500 mg over a period of 4–6 months, has been shown in several clinical trials to lower blood pressure and improve vascular function in both healthy and overweight to obese patients (Egert et al. 2009; Dower et al. 2015; Choi et al. 2015; Shi and Williamson 2016). In contrast, Brüll et al. (2017a) reported that acute ingestion of quercetin from onion skin extract had no effect on postprandial blood pressure and endothelial function in overweight to obese adults with hypertension.

The effects of quercetin on metabolic parameters have also been reported in the scientific literature. For example, Leyva-Soto et al. (2021) found that daily consumption of quercetin-enriched bread for 3 months resulted in improvements in biochemical parameters associated with metabolic syndrome, including total cholesterol, LDL cholesterol, total triglycerides, and fasting plasma glucose. Similarly, Khorshidi et al. (2018) found that quercetin intake (1000 mg/day for 12 weeks) led to improvements in metabolic and hormonal parameters, as well as reduced resistin plasma concentration and gene expression, in overweight or obese women with polycystic ovary syndrome. Cialdella-Kam et al. (2016) concluded that supplementation with a mixture of quercetin-rich flavonoids and fish oil for 10 weeks was associated with decreased levels of pro-inflammatory cytokines and increased levels of anti-inflammatory cytokines. Nishimura et al. (2019) reported that daily intake of quercetin-rich onion powder for 12 weeks resulted in significantly greater reductions in body weight and body fat mass than in the placebo group. However, Brüll et al. (2017b) found that quercetin (162 mg/day for 6 weeks) had no effect on serum leptin and adiponectin concentrations in overweight to obese hypertensive or prehypertensive patients compared with the placebo group.

A systematic review and meta-analysis of randomised controlled trials conducted by Tabrizi et al. (2020) concluded that quercetin supplementation might improve lipid profiles and reduce inflammatory markers in patients with metabolic syndrome

and related disorders. However, more research is needed to confirm quercetin's therapeutic benefits in managing obesity and metabolic syndrome in humans.

Anthocyanins

Another group of flavonoids with health-promoting properties are anthocyanins, which give fruits their red, purple, or blue colour (Milbury and Kalt 2010). Anthocyanins are found in a variety of fruits and vegetables, including plums, eggplant, red cabbage, and especially berries. Health benefits associated with the consumption of berry anthocyanins include improved cognitive function, regulation of glucose and lipid metabolism, and cardiovascular protection (Joseph et al. 1999; Youdim et al. 2000; Lau et al. 2007; Reis et al. 2016; Aboonabi and Aboonabi 2020). In addition, various anthocyanins have been shown to arrest the cell cycle or induce cell death in some cancer cell lines, and some *in vivo* studies have shown that anthocyanins have the potential to prevent and treat certain cancers; however, this has not yet been demonstrated in humans (reviewed by Lin et al. 2017).

Anthocyanins have been shown to directly and indirectly promote the activity of antioxidant enzymes, such as superoxide dismutase, by modulating Nrf2 activity (Tsuji et al. 2013; Curti et al. 2014). Anthocyanins have also been reported to attenuate pro-inflammatory signalling pathways by downregulating NF-κB and the expression of pro-inflammatory genes (Ye et al. 2010; Poulose et al. 2012).

Anthocyanins from various fruits have been reported to extend the lifespan of *C. elegans* by modulating the expression of the DAF-16/FOXO pathway (Chen et al. 2013b; Tambara et al. 2018; Wang et al. 2018). In *D. melanogaster* and zebrafish (*Danio rerio*), anthocyanins have also been reported to reduce oxidative stress and inflammatory markers (Mylnikov et al. 2005; Kim et al. 2012; Wang et al. 2016; Valenza et al. 2018). Life expectancy was significantly increased in cancer-prone $Trp53^{-/-}$ mice fed a diet high in anthocyanins (Butelli et al. 2008). In the APCMin cancer mouse model, an anthocyanin-enriched cherry extract combined with the non-steroidal anti-inflammatory drug sulindac was more effective than sulindac alone in preventing colon cancer (Bobe et al. 2006).

Dietary anthocyanins have been reported to exert neuroprotective effects, reducing age-related cognitive decline, improving cognitive performance, and even mitigating stress-related brain damage (Joseph et al. 1999; Casadesus et al. 2004; Petersen 2004; Rahman et al. 2008; Krikorian et al. 2010a; Albert et al. 2011). In the hippocampal cell line HT22, anthocyanin treatment attenuated cell death and normalised mitochondrial membrane potential and calcium levels associated with Aβ1-42 neurotoxicity (Badshah et al. 2015). *In vivo*, anthocyanins reversed the effect of Aβ on mitochondrial apoptotic pathway proteins and key AD markers, including Aβ peptide, APP protein, phosphorylated tau, and β-secretase 1 (Badshah et al. 2015). In another study, blueberries promoted microglial clearance of Aβ, inhibited Aβ1-42 aggregation, and suppressed microglial activation (Zhu et al. 2008). Extracts rich in anthocyanins and proanthocyanidins were also reported to

reverse mitochondrial dysfunction in dopaminergic cells and reduce microglial activity in cellular models of PD (Strathearn et al. 2014).

The neuroprotective effects of dietary anthocyanins have been attributed to their ability to reduce neuroinflammatory mediators such as IL-1 and TNF-α, protect neurons from oxidative stress, and modulate neuronal signalling, plasticity, and neurogenesis, at least to some extent through BDNF (Domitrovic 2011; Tsuda 2012; Mecocci et al. 2014; de Pascual-Teresa 2014). Interestingly, liquid chromatography-mass spectrometry (LC-MS) analyses of the brains of blueberry-fed rats showed that anthocyanins were localised in the cerebellum, cortex, hippocampus, and striatum, and their concentrations correlated with the performance of rats in the Morris water maze, suggesting a direct effect of anthocyanins on memory and learning (Andres-Lacueva et al. 2005).

Studies on the neuroprotective abilities of anthocyanins in humans are scarce. In a small cohort of older adults with early memory impairment, wild blueberry juice consumption daily for 12 weeks improved pairwise-associated learning and word-list recall (Krikorian et al. 2010b). In the Nurses' Health Study, high consumption of blueberries and strawberries was also associated with a delay in cognitive ageing of approximately 2.5 years (Devore et al. 2012). More recently, Bøhn et al. (2021) reported that 12 weeks of consumption of bilberry/red grape juice reduced plasma biomarkers of inflammation and tissue damage in older men with subjective memory impairment but had no significant effect on cognitive performance.

Anthocyanins have also been reported to protect against obesity and metabolic syndrome. For example, DeFuria et al. (2009) showed that mice fed an HFD and blueberries exhibited reduced adipocyte death and were protected from insulin resistance and hyperglycaemia. This protective effect was attributed to the impact of blueberry anthocyanins on stress signalling pathways such as MAPK and NF-κB. Moreover, blueberry supplementation attenuated the HFD-induced gene expression shift in the adipose tissue of mice (DeFuria et al. 2009). Conversely, Prior et al. (2008) reported that C57BL/6J obese mice fed an HFD plus anthocyanins in the form of whole blueberries had significantly more body weight, body fat, and epididymal fat mass than the control group. However, when the mice received purified anthocyanins, they gained less body weight and fat than the HFD control group (Prior et al. 2008). Moreover, supplementing an HFD with purified anthocyanins reversed the HFD-induced increase in serum cholesterol and triglyceride levels in C57BL/6 mice (Prior et al. 2009), indicating the potential of purified anthocyanins to prevent diet-induced dyslipidaemia and obesity. In the KKAy mouse model of leptin resistance, biotransformed blueberry juice protected mice from developing glucose intolerance and diabetes mellitus, reduced food intake and body weight, and significantly increased adiponectin levels (Vuong et al. 2009). Carey et al. (2019) reported that microglial activation and nitric oxide levels decreased and BDNF levels increased in mice fed an HFD supplemented with 4% freeze-dried blueberry extract.

Clinical studies in humans have shown some therapeutic benefits of anthocyanins in obesity and metabolic disorders. Based on three prospective cohort studies conducted among health professionals in the USA, a higher intake of flavonoids,

particularly anthocyanidins, was associated with better weight maintenance (Bertoia et al. 2016). Obese subjects with metabolic syndrome who consumed a blueberry drink containing 50 g of frozen blueberries daily (equivalent to 742 mg of anthocyanins) for 8 weeks showed significant reductions in blood pressure and plasma levels of oxidised LDL, malondialdehyde, and 4-hydroxynonenal compared with control subjects (Basu et al. 2010). Another study found that twice-daily consumption of blueberry bioactives (22.5 g) improved insulin sensitivity but had no effect on obesity or inflammatory biomarkers (Stull et al. 2010). In 30 overweight healthy subjects who received anthocyanin-rich Moro juice extract (400 mg/day) for 12 weeks, BMI decreased significantly compared with the control group (Cardile et al. 2015). In overweight and obese women, berry consumption for up to 35 days was also associated with reduced waist circumference and body weight (Lehtonen et al. 2011). In contrast, Wright et al. (2013) found that 4 weeks of consumption of anthocyanin- and phenolic acid-rich dried purple carrot had no significant effects on body mass, lipids, blood pressure, body composition, or inflammatory markers in overweight and obese adults.

A systematic review and meta-analysis of randomised controlled trials conducted by Park et al. (2021) concluded that supplementation with anthocyanins resulted in significant reductions in body weight, body mass index, and waist circumference, as well as triglycerides and LDL cholesterol.

Diets

An alternative to nutraceutical supplements is adopting a diet rich in foods with various bioactive components and pleiotropic benefits, such as the Mediterranean Dietary Approaches to Stop Hypertension (DASH) and Mediterranean-DASH Intervention for Neurodegenerative Delay (MIND) diets. These dietary patterns, typically characterised by a low glycaemic index, promote the consumption of foods rich in antioxidants and anti-inflammatory phytochemicals that can boost metabolism and prevent age-related cognitive decline by reducing inflammation and oxidative stress and improving glycaemic control. Reducing caloric intake through fasting diets also has well-documented benefits, including weight loss, increased mental clarity and focus, reduced inflammation, and improved cardiovascular health.

In the following sections, we critically review the health benefits of some of the best-studied diets in the context of age-related diseases and metabolic disorders.

Mediterranean Diet

The traditional Mediterranean diet consists of a high proportion of olive oil, vegetables, nuts, fruits, and grains, a moderate amount of fish, and little red or processed meat. The Mediterranean diet also recommends daily consumption of fruits and vegetables (3–8 servings) (Lin and Morrison 2002; Alonso et al. 2004; Crowe

et al. 2011). The Mediterranean diet has been reported to lower blood pressure and improve cardiovascular health (Kris-Etherton et al. 2001). Other health benefits include the prevention of diabetes, obesity, some cancers, and age-related cognitive decline (Guasch-Ferré and Willett 2021). Below, we discuss the health benefits of individual components of the Mediterranean diet and the Mediterranean diet as a whole.

The Mediterranean diet is characterised by a high fibre intake (41–62 g of fibre per person per day) (Saura-Calixto and Goñi 2009). The consumption of dietary fibre, especially cereal fibre, has been inversely correlated with cardiovascular disease risk (Lairon et al. 2005; Threapleton et al. 2013). A high fibre diet can contribute to a healthier cardiovascular system by promoting better glycaemic control and weight management and lowering inflammation and lipid levels (Good et al. 2008; Crowe et al. 2011; Widmer et al. 2015).

Nuts can help lower cholesterol levels and blood pressure and improve blood sugar control and vascular function. This, in turn, reduces the risk of T2D, cardiovascular disease, and other chronic diseases (Good et al. 2008; Crowe et al. 2011; Kingwell et al. 2014; Jamshed et al. 2015; Widmer et al. 2015). The health benefits of almonds, for instance, are well documented (reviewed by Kamil and Chen 2012). Almonds are rich in monounsaturated fatty acids, fibre, protein, and vitamins. A meta-analysis found that almonds in the diet lowered serum cholesterol levels, especially LDL cholesterol (Musa-Veloso et al. 2016). In randomised controlled clinical trials, almonds have also been reported to increase serum HDL cholesterol and lower triglycerides, LDL, and total cholesterol and the overall atherogenic index (Good et al. 2008; Crowe et al. 2011; Widmer et al. 2015). A cross-sectional analysis of data from the Nurses' Health Study and the Health Professionals Follow-Up Study found an association between frequent nut consumption and lower levels of the inflammatory biomarkers CRP, IL-6, and TNF-α (Yu et al. 2016).

The carotenoid lycopene, found in red fruits and vegetables such as tomatoes, is also an important component of the Mediterranean diet with antioxidant, anti-inflammatory, and antithrombotic properties (Burton-Freeman and Reimers 2010). The health benefits of lycopene include a reduced risk of certain cancers, such as prostate cancer, macular degeneration and cataracts, stroke, and improved immunity (Mohanty et al. 2002; Gupta et al. 2003; Luo and Wu 2011; Karppi et al. 2012; Chen et al. 2015b). Low-dose lycopene supplementation has also been suggested as a preventive measure to reduce the risk of cardiovascular disease (Sesso et al. 2012; Burton-Freeman and Sesso 2014).

Olive oil (*Olea europaea*) contains several antioxidants and anti-inflammatory compounds, including several polyphenols such as oleocanthal, and is a Mediterranean diet staple. Health benefits associated with olive oil include a lower risk of stroke, heart disease, cancer, AD, and obesity (Huang and Sumpio 2008; Foscolou et al. 2018). The consumption of nuts and extra virgin olive oil as part of the Mediterranean diet was found to improve cognitive function in the elderly (Martínez-Lapiscina et al. 2013; Valls-Pedret et al. 2015). Likewise, Mazza et al. (2018) observed an improvement in cognitive function in elderly individuals who consumed a Mediterranean diet with extra virgin olive oil instead of other oils for 1 year.

Magnesium is an essential cofactor for several enzymes involved in carbohydrate metabolism and the Krebs cycle (Saris et al. 2000). Several health conditions are caused by magnesium deficiency, including asthma, hypertension, heart disease, diabetes, migraines, osteoporosis, and anxiety (Ma et al. 1995; Guerrero-Romero and Rodriguez-Moran 2002). The Mediterranean diet contains several magnesium-rich components, such as vegetables, legumes, and nuts, and adherence to the Mediterranean diet is generally associated with high magnesium consumption. Several epidemiological studies have reported an association between increased magnesium intake and a lower risk of T2D (Ma et al. 1995; Kao et al. 1999).

Some studies have found that adherence to the Mediterranean diet may reduce the risk of developing AD and PD (Burton-Freeman and Reimers 2010; Yin et al. 2021; Ballarini et al. 2021; Wade et al. 2021; Strikwerda et al. 2021), while others have found no clear association between the Mediterranean diet and age-related neurodegeneration (Féart et al. 2009; Petersson and Philippou 2016). The Mediterranean diet has also been linked to improved weight control, a lower likelihood of obesity, and the prevention of T2D and insulin resistance (Ford and Mokdad 2001; Sargeant et al. 2001; Liese et al. 2003; Esposito et al. 2004; Schröder et al. 2004; Montonen et al. 2005; Panagiotakos et al. 2006; Schröder 2007).

Dietary Approaches to Stop Hypertension Diet

The DASH diet, developed by the National Heart, Lung, and Blood Institute, includes foods rich in potassium, calcium, and other minerals, lean protein, and fibre, which can lower blood pressure. The diet also restricts the consumption of red meat, dairy products, and sugary snacks and is low in sodium, saturated fat, and cholesterol (National Heart Lung and Blood Institute 2022). While the Mediterranean diet emphasises the consumption of olive oil and fish, the DASH diet favours low-fat dairy products, whole grains, and lean meats. As intended, adopting the DASH diet is associated with reduced blood pressure in hypertensive and non-hypertensive individuals (Appel et al. 1997).

Adherence to the DASH diet helps reduce oxidative stress and inflammation in the body and increases insulin sensitivity, reducing the risk of cardiovascular disease and dementia (Abbatecola et al. 2018). The DASH diet has also been reported to mitigate age-related cognitive decline, reduce the risk of AD, and improve global cognitive function in older adults (Tangney et al. 2014; Morris et al. 2015a; Berendsen et al. 2017). Similarly, long-term compliance with the DASH diet in older American women slowed cognitive decline (Berendsen et al. 2017). However, in the Women's Health Initiative Memory Study (WHIMS), which targeted the same population, no association was found between adherence to the DASH dietary pattern and mild cognitive impairment or dementia (Haring et al. 2016). Interestingly, in adults (20–65 years old) without a prior diagnosis of chronic disease, higher compliance to the DASH diet was linked to longer telomeres in women but not in men (Leung et al. 2018).

Although it was designed to reduce cardiometabolic risk, high adherence to the DASH diet has also been reported to impact body weight. A meta-analysis of 13 randomised clinical trials reported that adherence to the DASH diet reduced body weight, BMI, and waist circumference, especially in overweight/obese individuals (Soltani et al. 2016). Another study showed that compliance with the DASH diet reduced the risk of obesity in Chinese adults (Cheung et al. 2018). In overweight and obese women with polycystic ovary syndrome, adherence to the DASH diet significantly reduced body weight and BMI (Herriot et al. 2008). The adoption of the DASH diet was also reported to increase antioxidant capacity, especially in obese hypertensive patients (Lopes et al. 2003). Moreover, in individuals with T2D and obesity, the DASH diet was found to lower levels of the inflammatory marker CRP (Chiavaroli et al. 2019). There is also evidence that adherence to the DASH diet, combined with exercise and caloric restriction, may improve psychomotor performance in overweight or obese adults with hypertension (Smith et al. 2010).

Mediterranean-DASH Intervention for Neurodegenerative Delay Diet

The MIND diet was developed by researchers at Rush College Medical Center and the Harvard School of Public Health to prevent age-related cognitive decline. It combines the Mediterranean diet with the DASH diet. According to the MIND diet, weekly dietary staples should include fruits and vegetables, especially berries and green leafy vegetables, nuts, beans, and whole grains, with occasional fish and chicken consumption and minimal consumption of red meat, fat, high-fat dairy products, sweets, or fast food (Koch and Jensen 2016).

The MIND diet emphasises the consumption of several food components that have antioxidant and anti-inflammatory effects, which can help prevent dementia (Kang et al. 2005; Devore et al. 2012; Morris et al. 2018). Additionally, the MIND diet may reduce dementia risk by improving cardiovascular health, insulin resistance, and hypercholesterolaemia (Dinu et al. 2018; Chiavaroli et al. 2019). Adherence to the MIND diet has also been reported to reduce the incidence of symptoms of AD (Morris et al. 2015a) and PD (Agarwal et al. 2018; Metcalfe-Roach et al. 2021) in old age. Interestingly, Arjmand et al. (2022) reported that adherence to the MIND diet could improve cognitive performance in healthy obese women and reverse the adverse effects of obesity on the brain. Conversely, Berendsen et al. (2018) concluded that the MIND diet had only moderately positive effects on verbal memory in older women, but did not affect age-related cognitive decline.

The MIND diet seems to provide greater neuroprotection than the Mediterranean diet (Morris et al. 2015a, b; Hosking et al. 2019), while the Mediterranean diet, with its focus on healthy fats, appears to protect against AD better than the DASH diet (Morris et al. 2015a). A systematic review by Van den Brink et al. (2019) concluded that high compliance with the Mediterranean, DASH, or MIND diet was associated with attenuation of age-related cognitive decline and a lower risk of AD. Importantly, adherence to the MIND diet was associated with a significantly reduced risk of all-cause mortality in a longitudinal birth cohort study with 12 years

of follow-up (Corley 2022), denoting its potential as a public health measure to improve survival.

Because the MIND diet advocates the consumption of plant-based foods with a low glycaemic index and high fibre and water content (Buckland et al. 2008), it is plausible that it could also reduce the risk of obesity and metabolic dysfunction. However, the results of clinical trials investigating the relationship between adherence to the MIND diet and metabolic diseases are sparse and contradictory. For instance, Aminianfar et al. (2020) found no association between the adoption of the MIND diet and obesity in a group of Iranian adults (mean age of 36.8 ± 8.08 years). However, Mohammadpour et al. (2020) concluded that adherence to the MIND diet was associated with a lower risk of metabolic syndrome and general and abdominal obesity also in a group of Iranian adults (mean age of 47.7 ± 10.7 years).

Fasting Regimes

As mentioned earlier, caloric restriction (CR) describes a 10–40% reduction in daily caloric consumption without malnutrition while maintaining a daily intake of essential nutrients, including vitamins and minerals. CR is the only intervention known to slow ageing and extend lifespan in various animal models (Heilbronn and Ravussin 2003; Roth and Polotsky 2012). Several indices of cardiovascular health have been shown to improve with CR in laboratory animals and humans (Soare et al. 2014; Golbidi et al. 2017). CR has also been shown to delay or prevent several types of cancer, diabetes, and autoimmune diseases, delay age-related diseases, and improve immune function in animal models (Mercken et al. 2012; Flanagan et al. 2020).

The beneficial effects of CR are attributed to its capacity to mitigate oxidative stress and inflammation in the body by modulating the activity of various signalling pathways involved in the ageing process, as discussed above. Another important mechanism by which CR exerts its beneficial effects is by promoting ketosis, in which fatty acids released from adipose tissue are broken down in the liver to form ketone bodies, such as β-hydroxybutyrate. β-hydroxybutyrate has beneficial effects on age-related pathology, including improved glucose metabolism, reduced inflammation, and increased autophagy (Han et al. 2020). Additionally, CR might produce health benefits through the process of hormesis, a term used to describe the beneficial effects of low doses of stressors on an organism. This hypothesis is supported by the observation that low doses of various stressors increase longevity in several organisms, including yeast, worms, and flies, by activating stress response pathways and inducing autophagy and damage repair (Masoro 2006).

In several animal models, from yeast (Jiang et al. 2000) to *C. elegans* (Kaeberlein et al. 2006; Lee et al. 2006), *D. melanogaster* (Mair et al. 2003), and dogs (Kealy et al. 2002), CR has been found to increase lifespan and healthspan. In mice and rats, CR increased lifespan by up to 45% and 27%, respectively (Swindell 2012). However, there are also examples of animals in which CR does not increase lifespan, including the Mediterranean fruit fly *Ceratitis capitata* (Carey et al. 2006) or the housefly *Musca domestica* (Cooper et al. 2004), and in rodents, the life-prolonging

effect of CR is age- and genotype-dependent (Forster et al. 2003). Although it is not yet clear whether CR can prolong human life expectancy, Okinawans, who consume 20% fewer calories than mainland Japanese, are significantly less likely to develop stroke, cancer, and heart disease than mainlanders (Willcox et al. 2007).

In vitro, CR has been shown to delay the onset of cellular senescence in different cell types, including neurons, fibroblasts, and endothelial cells, through its antioxidant and anti-inflammatory effects. In middle-aged mice, dietary restriction resulted in reduced numbers of senescent cells in the liver and intestine, possibly mediated by CR-induced inhibition of mTOR/S6K1, resulting in improved mitochondrial function and reduced formation of ROS (Wang et al. 2010). Recently, Sbierski-Kind et al. (2022) attributed the delayed cell senescence associated with CR to its effects on the gut microbiome.

CR has also been shown to protect against age-related neurodegeneration. The neuroprotective effects of CR are attributed to its ability to improve mitochondrial function, reduce pathogenic protein accumulation, and attenuate neuroinflammation by inhibiting NF-κB and blocking the synthesis of inflammatory interleukins and TNF-α (Wang et al. 2005; Patel et al. 2005; Cerqueira et al. 2012; Lanza et al. 2012; Amigo et al. 2017; Fontana et al. 2021). In addition, CR has been found to increase levels of neurotrophic factors such as BDNF and GDNF (glial cell line-derived neurotrophic factor), promoting neurogenesis and synaptic plasticity and boosting antioxidant defences, leading to alleviation of neurochemical and behavioural deficits associated with neurodegenerative diseases (Duan et al. 2003; Maswood et al. 2004; Thrasivoulou et al. 2006).

Because higher caloric intake is associated with an increased risk of neurodegenerative disease (Luchsinger et al. 2002), CR may be a viable therapeutic option to prevent age-related neurodegeneration. CR was found to delay the onset of age-related neurodegenerative diseases in mice and nonhuman primates by improving lipid and glucose metabolism and decreasing systemic inflammation, in addition to its direct neuroprotective effects (Duan et al. 2003; Maswood et al. 2004; Wang et al. 2005; Qin et al. 2006; Colman et al. 2009, 2014; Anderson et al. 2009). In the 3xTgAD triple-transgenic mouse model of AD, CR reduced hippocampal Aβ and phosphorylated tau levels and ameliorated age-related behavioural deficits (Halagappa et al. 2007). CR was also found to reduce Aβ-plaque accumulation and astrocytic activation in the AD-transgenic mouse models APP (swe/ind) (J20) and APP (swe) + PS1(M146L) (APP + PS1) (Patel et al. 2005).

The beneficial effects of CR in PD have also been reported. CR attenuated MPTP-induced neurotoxicity in mice (Duan and Mattson 1999) and nonhuman primates (Maswood et al. 2004). Similarly, CR was found to protect against the loss of dopamine neurons in the lactacystin mouse model of PD (Coppens et al. 2017). In humans, short CR periods have been shown to enhance cognitive performance, particularly verbal memory, in older individuals (Witte et al. 2009).

CR can also promote weight loss, improve insulin sensitivity, and decrease the risk of developing T2D and cardiovascular disease (Wing et al. 1994; Janssen et al. 2002; Wing 2010). The mechanism by which CR leads to these benefits is thought to be a combination of decreased energy intake and increased energy expenditure

mediated by changes in the levels of the hormones leptin (Dubuc et al. 1998) and ghrelin (Cummings et al. 2002). Interestingly, Spadaro et al. (2022) recently reported that adipose tissue from healthy individuals exposed to 14% CR for 2 years underwent transcriptional reprogramming involving pathways that controlled mitochondrial bioenergetics, immune responses, and lifespan, revealing an important role for CR-induced immunometabolic effects in promoting healthspan.

Reporting on the results of "The Comprehensive Assessment of Long-term Effects of Reducing Intake of Energy (CALERIE)" trials, Dorling et al. (2021) found that caloric restriction had several positive health effects, including weight loss, reduced inflammation, improved insulin sensitivity, reduced blood pressure, and improved mood.

Intermittent fasting (IF) is a dietary strategy that involves alternating between periods of fasting and feeding without necessarily reducing the total amount of calories consumed. The most common IF regimen is to fast for 16 h and eat only in an 8-h window ("16/8"). Health benefits associated with IF include improved insulin sensitivity, reduced inflammation, weight loss, and prevention of age-related neurodegeneration (Patterson and Sears 2017; de Cabo and Mattson 2019; Varady et al. 2021).

The mechanisms underlying the health benefits of IF are similar to those of CR. They include the reduction of inflammation and oxidative damage, activation of autophagy, production of ketone bodies, and hormesis (Mattson et al. 2017). The neuroprotective effects of IF appear to be due to a reduction in inflammation and oxidative stress through activation of SIRT3 and Nrf2, a decrease in the levels of the stress hormone cortisol, an increase in the levels of neurotrophic factor BDNF, and enhanced neurogenesis and improved mitochondrial function (Dai et al. 2022).

IF may prevent vascular dementia by improving endothelial function and reducing inflammation and oxidative stress (Yoon and Song 2019). In rats with AD-induced oestrogen deficiency, IF prevented cognitive decline and improved energy metabolism and dyslipidaemia but exacerbated bone mineral density loss and insulin resistance (Shin et al. 2018). In the PDAPP-J20 mouse model of AD, regular food restriction increased autophagy in microglia and astrocytes, leading to reduced amyloid pathology and improved cognitive performance (Gregosa et al. 2019). IF has also been shown to have several benefits in experimental models of PD, including improved insulin sensitivity, increased levels of the ketone body β-hydroxybutyrate, and improved neuronal resistance to excitotoxicity, which protected against disease-related motor and cognitive decline (Kashiwaya et al. 2000; Włodarek 2019). In addition, IF was found to reduce α-synuclein load in the brainstem, leading to an improvement in motor function in mice with PD (Griffioen et al. 2013).

In a small cohort of healthy adults, Wegman et al. (2015) concluded that IF was well tolerated and was associated with a slight increase in gene expression of the sirtuin SIRT3 and a decrease in plasma insulin levels. In aged adults with mild cognitive impairment, IF improved cognitive function by increasing superoxide dismutase activity and reducing body weight, insulin, fasting blood glucose, malondialdehyde levels, and DNA damage (Ooi et al. 2020).

There is also growing evidence that IF may be an effective strategy to combat obesity. *In vitro* and *in vivo* studies have shown that IF can help reduce body fat mass, increase energy expenditure, improve insulin sensitivity and endothelial function, and lower blood triglyceride levels (Wilson et al. 2018; Liu et al. 2019a, b; Deng et al. 2020; Cui et al. 2022). However, at least in mice, incorrect feeding timing has been found to desynchronise peripheral clocks and induce obesity and other metabolic disturbances (Yasumoto et al. 2016), denoting the importance of the feeding window for the beneficial effects of IF.

Several clinical trials have demonstrated the effectiveness of IF in treating obesity and improving health outcomes, including reducing the risk of diabetes and cardiovascular disease. Heilbronn et al. (2005) found that in non-obese subjects, alternate-day fasting significantly decreased body weight and body fat and increased energy expenditure. In another study, Klempel et al. (2012) reported that IF combined with CR was effective in weight loss and cardiovascular protection in obese women, as evidenced by a reduction in the levels of key coronary heart disease risk indicators. In a small cohort of obese subjects, a modified alternate-day fasting regimen resulted in an 8% reduction in body weight and decreased waist circumference and triglyceride levels (Varady et al. 2009). In another study, alternate-day fasting was also found to reduce body weight, fat mass, triacylglycerols, leptin, and CRP and increase LDL particle size and plasma adiponectin in normal-weight and overweight individuals (Varady et al. 2013).

A systematic review of the literature concluded that IF is safe and well tolerated and may be an effective weight loss strategy (Welton et al. 2020). However, more long-term research is needed to understand the sustainability of IF in weight loss.

Overall, preclinical and clinical studies provide compelling evidence for the therapeutic benefits of fasting, either through CR or IF, against ageing and age-related diseases and syndromes of metabolic dysfunction. However, restricting calorie intake can lead to nutrient deficiencies, which can cause health problems. CR can also lead to muscle atrophy and weakness, which is particularly problematic in elderly individuals, for whom the loss of muscle mass is already a major health concern. This can be mitigated by combining CR with exercise (Chomentowski et al. 2009). IF is generally well tolerated (Gabel et al. 2019), but side effects such as gastrointestinal problems, headaches, dizziness, and dry mouth may occur; these side effects usually disappear over time (Cienfuegos et al. 2020).

The Longevity Diet

Based on a review of a variety of studies ranging from laboratory animals to epidemiological research in humans, Longo and Anderson (2022) proposed what they call a "longevity diet". The proposed diet takes into account not only the type of food but also the timing of food intake.

According to the authors, the ideal diet should provide approximately 30% of total energy needs from moderate to high amounts of unrefined carbohydrates, small but sufficient amounts of protein from plant sources, and adequate amounts of

vegetable fats. The diet should also include plenty of vegetables, legumes, whole grains, olive oil and nuts, dark chocolate, fish, small quantities of refined grains and sugars, minimal amounts of white meat, and no red meat. According to the authors, ideally, all meals should be eaten in an 11- to 12-h time window, allowing for a daily fasting period. The authors also note that a fasting-like diet or a 5-day fasting cycle every 3–4 months may help reduce the risk of diabetes, hypertension, and other diseases to improve overall health (Longo and Anderson 2022). The authors proposed the longevity diet as a valuable complement to standard health care that can be used as a preventative approach to reduce morbidity and maintain health into old age.

Conclusions

Obesity and ageing are characterised by increased oxidative stress and chronic inflammation. Interventions that target these processes have the potential not only to mitigate the adipose tissue dysfunction associated with obesity and prevent associated comorbidities, but also to alleviate the metabolic disturbances that accompany normal ageing.

Despite the extensive preclinical evidence for the therapeutic role of antioxidants in the treatment of ageing, age-related diseases, and metabolic disorders such as obesity, convincing evidence for such benefits in humans is still lacking.

A substantial number of epidemiological studies have inversely correlated disease incidence with the consumption of antioxidant-rich fruits and vegetables. However, these results cannot be used as evidence for a causal relationship between antioxidant consumption and improved health, and antioxidant supplements cannot offset the harmful effects of a Western diet and a sedentary lifestyle. Instead, a diet rich in anti-inflammatory and antioxidant-rich foods and an overall healthy lifestyle should be the most important strategies for maintaining a normal weight, preventing obesity-related health complications, and extending lifespan and healthspan.

References

Aagaard-Tillery KM, Grove K, Bishop J et al (2008) Developmental origins of disease and determinants of chromatin structure: maternal diet modifies the primate fetal epigenome. J Mol Endocrinol 41:91–102. https://doi.org/10.1677/JME-08-0025

Abbas S, Wink M (2010) Epigallocatechin gallate inhibits beta amyloid oligomerization in *Caenorhabditis elegans* and affects the *daf*-2/insulin-like signaling pathway. Phytomedicine 17:902–909. https://doi.org/10.1016/j.phymed.2010.03.008

Abbatecola AM, Russo M, Barbieri M (2018) Dietary patterns and cognition in older persons. Curr Opin Clin Nutr Metab Care 21:10–13. https://doi.org/10.1097/MCO.0000000000000434

Aboonabi A, Aboonabi A (2020) Anthocyanins reduce inflammation and improve glucose and lipid metabolism associated with inhibiting nuclear factor-kappaB activation and increasing PPAR-γ

gene expression in metabolic syndrome subjects. Free Radic Biol Med 150:30–39. https://doi.org/10.1016/j.freeradbiomed.2020.02.004

Abrahams S, Haylett WL, Johnson G et al (2019) Antioxidant effects of curcumin in models of neurodegeneration, aging, oxidative and nitrosative stress: a review. Neuroscience 406:1–21. https://doi.org/10.1016/j.neuroscience.2019.02.020

Agarwal P, Wang Y, Buchman AS et al (2018) MIND diet associated with reduced incidence and delayed progression of Parkinsonism in old age. J Nutr Health Aging 22:1211–1215. https://doi.org/10.1007/s12603-018-1094-5

Ahima RS (2009) Connecting obesity, aging and diabetes. Nat Med 15:996–997. https://doi.org/10.1038/nm0909-996

Ahmed T, Gilani A-H (2009) Inhibitory effect of curcuminoids on acetylcholinesterase activity and attenuation of scopolamine-induced amnesia may explain medicinal use of turmeric in Alzheimer's disease. Pharmacol Biochem Behav 91:554–559. https://doi.org/10.1016/j.pbb.2008.09.010

Ahn J, Cho I, Kim S et al (2008) Dietary resveratrol alters lipid metabolism-related gene expression of mice on an atherogenic diet. J Hepatol 49:1019–1028. https://doi.org/10.1016/j.jhep.2008.08.012

Ahn J, Lee H, Jung CH et al (2013) MicroRNA-146b promotes adipogenesis by suppressing the SIRT1-FOXO1 cascade. EMBO Mol Med 5:1602–1612. https://doi.org/10.1002/emmm.201302647

Akinyemi AJ, Okonkwo PK, Faboya OA et al (2017) Curcumin improves episodic memory in cadmium induced memory impairment through inhibition of acetylcholinesterase and adenosine deaminase activities in a rat model. Metab Brain Dis 32:87–95. https://doi.org/10.1007/s11011-016-9887-x

Al-Attas OS, Al-Daghri NM, Alokail MS et al (2010) Adiposity and insulin resistance correlate with telomere length in middle-aged Arabs: the influence of circulating adiponectin. Eur J Endocrinol 163:601–607. https://doi.org/10.1530/EJE-10-0241

Albert MS, DeKosky ST, Dickson D et al (2011) The diagnosis of mild cognitive impairment due to Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. Alzheimers Dement 7:270–279. https://doi.org/10.1016/j.jalz.2011.03.008

Alcendor RR, Gao S, Zhai P et al (2007) Sirt1 regulates aging and resistance to oxidative stress in the heart. Circ Res 100:1512–1521. https://doi.org/10.1161/01.RES.0000267723.65696.4a

Aleman FDD, Valenzano DR (2019) Microbiome evolution during host aging. PLoS Pathog 15: e1007727. https://doi.org/10.1371/journal.ppat.1007727

Alonso A, de la Fuente C, Martín-Arnau AM et al (2004) Fruit and vegetable consumption is inversely associated with blood pressure in a Mediterranean population with a high vegetable-fat intake: the Seguimiento Universidad de Navarra (SUN) Study. Br J Nutr 92:311–319. https://doi.org/10.1079/BJN20041196

Alzheimer's Association (2019) 2019 Alzheimer's disease facts and figures. Alzheimer's Dement 15:321–387. https://doi.org/10.1016/j.jalz.2019.01.010

Amadi CN, Offor SJ, Frazzoli C, Orisakwe OE (2019) Natural antidotes and management of metal toxicity. Environ Sci Pollut Res Int 26:18032–18052. https://doi.org/10.1007/s11356-019-05104-2

Ambrosi TH, Scialdone A, Graja A et al (2017) Adipocyte accumulation in the bone marrow during obesity and aging impairs stem cell-based hematopoietic and bone regeneration. Cell Stem Cell 20:771–784.e6. https://doi.org/10.1016/j.stem.2017.02.009

Amigo I, Menezes-Filho SL, Luévano-Martínez LA et al (2017) Caloric restriction increases brain mitochondrial calcium retention capacity and protects against excitotoxicity. Aging Cell 16:73–81. https://doi.org/10.1111/acel.12527

Aminianfar A, Hassanzadeh Keshteli A, Esmaillzadeh A, Adibi P (2020) Association between adherence to MIND diet and general and abdominal obesity: a cross-sectional study. Nutr J 19: 15. https://doi.org/10.1186/s12937-020-00531-1

Anderson RM, Shanmuganayagam D, Weindruch R (2009) Caloric restriction and aging: studies in mice and monkeys. Toxicol Pathol 37:47–51. https://doi.org/10.1177/0192623308329476

Andres-Lacueva C, Shukitt-Hale B, Galli RL et al (2005) Anthocyanins in aged blueberry-fed rats are found centrally and may enhance memory. Nutr Neurosci 8:111–120. https://doi.org/10.1080/10284150500078117

Anjaneyulu M, Chopra K (2004) Quercetin, an anti-oxidant bioflavonoid, attenuates diabetic nephropathy in rats. Clin Exp Pharmacol Physiol 31:244–248. https://doi.org/10.1111/j.1440-1681.2004.03982.x

Appel LJ, Moore TJ, Obarzanek E et al (1997) A clinical trial of the effects of dietary patterns on blood pressure. N Engl J Med 336:1117–1124. https://doi.org/10.1056/NEJM199704173361601

Aprikyan GV, Gekchyan KG (1988) Release of neurotransmitter amino acids from rat brain synaptosomes and its regulation in aging. Gerontology 34:35–40. https://doi.org/10.1159/000212928

Arendt LM, McCready J, Keller PJ et al (2013) Obesity promotes breast cancer by CCL2-mediated macrophage recruitment and angiogenesis. Cancer Res 73:6080–6093. https://doi.org/10.1158/0008-5472.CAN-13-0926

Argyropoulos G, Harper M-E (2002) Uncoupling proteins and thermoregulation. J Appl Physiol 92:2187–2198. https://doi.org/10.1152/japplphysiol.00994.2001

Arias N, Macarulla MT, Aguirre L et al (2014) Quercetin can reduce insulin resistance without decreasing adipose tissue and skeletal muscle fat accumulation. Genes Nutr 9:361. https://doi.org/10.1007/s12263-013-0361-7

Arjmand G, Abbas-Zadeh M, Eftekhari MH (2022) Effect of MIND diet intervention on cognitive performance and brain structure in healthy obese women: a randomized controlled trial. Sci Rep 12:2871. https://doi.org/10.1038/s41598-021-04258-9

Aryal B, Singh AK, Rotllan N et al (2017) MicroRNAs and lipid metabolism. Curr Opin Lipidol 28:273–280. https://doi.org/10.1097/MOL.0000000000000420

Aviv A (2012) Genetics of leukocyte telomere length and its role in atherosclerosis. Mutat Res 730:68–74. https://doi.org/10.1016/j.mrfmmm.2011.05.001

Azman NAM, Peiró S, Fajarí L et al (2014) Radical scavenging of white tea and its flavonoid constituents by electron paramagnetic resonance (EPR) spectroscopy. J Agric Food Chem 62:5743–5748. https://doi.org/10.1021/jf501707p

Azzarà A, Chiaramonte A, Filomeni E et al (2017) Increased level of DNA damage in some organs of obese Zucker rats by γ-H2AX analysis. Environ Mol Mutagen 58:477–484. https://doi.org/10.1002/em.22115

Bach D, Pich S, Soriano FX et al (2003) Mitofusin-2 determines mitochondrial network architecture and mitochondrial metabolism. A novel regulatory mechanism altered in obesity. J Biol Chem 278:17190–17197. https://doi.org/10.1074/jbc.M212754200

Badshah H, Kim TH, Kim MO (2015) Protective effects of anthocyanins against amyloid beta-induced neurotoxicity *in vivo* and *in vitro*. Neurochem Int 80:51–59. https://doi.org/10.1016/j.neuint.2014.10.009

Baker DJ, Wijshake T, Tchkonia T et al (2011a) Clearance of p16Ink4a-positive senescent cells delays ageing-associated disorders. Nature 479:232–236. https://doi.org/10.1038/nature10600

Baker RG, Hayden MS, Ghosh S (2011b) NF-κB, inflammation, and metabolic disease. Cell Metab 13:11–22. https://doi.org/10.1016/j.cmet.2010.12.008

Balaban RS, Nemoto S, Finkel T (2005) Mitochondria, oxidants, and aging. Cell 120:483–495. https://doi.org/10.1016/j.cell.2005.02.001

Ballarini T, Melo van Lent D, Brunner J et al (2021) Mediterranean diet, Alzheimer disease biomarkers and brain atrophy in old age. Neurology 96:e2920–e2932. https://doi.org/10.1212/WNL.0000000000012067

Bamburg JR, Bloom GS (2009) Cytoskeletal pathologies of Alzheimer disease. Cell Motil Cytoskeleton 66:635–649. https://doi.org/10.1002/cm.20388

Barazzoni R, Short KR, Nair KS (2000) Effects of aging on mitochondrial DNA copy number and cytochrome c oxidase gene expression in rat skeletal muscle, liver, and heart. J Biol Chem 275: 3343–3347. https://doi.org/10.1074/jbc.275.5.3343

Barbatelli G, Murano I, Madsen L et al (2010) The emergence of cold-induced brown adipocytes in mouse white fat depots is determined predominantly by white to brown adipocyte transdifferentiation. Am J Physiol Endocrinol Metab 298:E1244–E1253. https://doi.org/10.1152/ajpendo.00600.2009

Bartholome A, Kampkötter A, Tanner S et al (2010) Epigallocatechin gallate-induced modulation of FoxO signaling in mammalian cells and *C. elegans*: FoxO stimulation is masked via PI3K/Akt activation by hydrogen peroxide formed in cell culture. Arch Biochem Biophys 501:58–64. https://doi.org/10.1016/j.abb.2010.05.024

Barzegar A, Moosavi-Movahedi AA (2011) Intracellular ROS protection efficiency and free radical-scavenging activity of curcumin. PLoS One 6:e26012. https://doi.org/10.1371/journal.pone.0026012

Basu A, Du M, Leyva MJ et al (2010) Blueberries decrease cardiovascular risk factors in obese men and women with metabolic syndrome. J Nutr 140:1582–1587. https://doi.org/10.3945/jn.110.124701

Bauer JH, Goupil S, Garber GB, Helfand SL (2004) An accelerated assay for the identification of lifespan-extending interventions in *Drosophila melanogaster*. Proc Natl Acad Sci U S A 101: 12980–12985. https://doi.org/10.1073/pnas.0403493101

Baum L, Lam CWK, Cheung SK-K et al (2008) Six-month randomized, placebo-controlled, double-blind, pilot clinical trial of curcumin in patients with Alzheimer disease. J Clin Psychopharmacol 28:110–113. https://doi.org/10.1097/jcp.0b013e318160862c

Baur JA (2010) Resveratrol, sirtuins, and the promise of a DR mimetic. Mech Ageing Dev 131: 261–269. https://doi.org/10.1016/j.mad.2010.02.007

Baur JA, Pearson KJ, Price NL et al (2006) Resveratrol improves health and survival of mice on a high-calorie diet. Nature 444:337–342. https://doi.org/10.1038/nature05354

Beal MF, Hyman BT, Koroshetz W (1993) Do defects in mitochondrial energy metabolism underlie the pathology of neurodegenerative diseases? Trends Neurosci 16:125–131. https://doi.org/10.1016/0166-2236(93)90117-5

Beckman JS, Koppenol WH (1996) Nitric oxide, superoxide, and peroxynitrite: the good, the bad, and ugly. Am J Physiol 271:C1424–C1437. https://doi.org/10.1152/ajpcell.1996.271.5.C1424

Beevers CS, Chen L, Liu L et al (2009) Curcumin disrupts the Mammalian target of rapamycin-raptor complex. Cancer Res 69:1000–1008. https://doi.org/10.1158/0008-5472.CAN-08-2367

Begum AN, Jones MR, Lim GP et al (2008) Curcumin structure-function, bioavailability, and efficacy in models of neuroinflammation and Alzheimer's disease. J Pharmacol Exp Ther 326: 196–208. https://doi.org/10.1124/jpet.108.137455

Belinha I, Amorim MA, Rodrigues P et al (2007) Quercetin increases oxidative stress resistance and longevity in *Saccharomyces cerevisiae*. J Agric Food Chem 55:2446–2451. https://doi.org/10.1021/jf063302e

Bell A, Grunder L, Sorisky A (2000) Rapamycin inhibits human adipocyte differentiation in primary culture. Obes Res 8:249–254. https://doi.org/10.1038/oby.2000.29

Belviranlı M, Okudan N, Atalık KEN, Öz M (2013) Curcumin improves spatial memory and decreases oxidative damage in aged female rats. Biogerontology 14:187–196. https://doi.org/10.1007/s10522-013-9422-y

Benton MC, Johnstone A, Eccles D et al (2015) An analysis of DNA methylation in human adipose tissue reveals differential modification of obesity genes before and after gastric bypass and weight loss. Genome Biol 16:8. https://doi.org/10.1186/s13059-014-0569-x

Benzler J, Ganjam GK, Pretz D et al (2015) Central inhibition of IKKβ/NF-κB signaling attenuates high-fat diet-induced obesity and glucose intolerance. Diabetes 64:2015–2027. https://doi.org/10.2337/db14-0093

Berendsen AAM, Kang JH, van de Rest O et al (2017) The dietary approaches to stop hypertension diet, cognitive function, and cognitive decline in american older women. J Am Med Dir Assoc 18:427–432. https://doi.org/10.1016/j.jamda.2016.11.026

Berendsen AM, Kang JH, Feskens EJM et al (2018) Association of long-term adherence to the mind diet with cognitive function and cognitive decline in American women. J Nutr Health Aging 22: 222–229. https://doi.org/10.1007/s12603-017-0909-0

Berson A, Nativio R, Berger SL, Bonini NM (2018) Epigenetic regulation in neurodegenerative diseases. Trends Neurosci 41:587–598. https://doi.org/10.1016/j.tins.2018.05.005

Bertoia ML, Rimm EB, Mukamal KJ et al (2016) Dietary flavonoid intake and weight maintenance: three prospective cohorts of 124,086 US men and women followed for up to 24 years. BMJ 352: i17. https://doi.org/10.1136/bmj.i17

Bhullar KS, Rupasinghe HP (2013) Polyphenols: multipotent therapeutic agents in neurodegenerative diseases. Oxid Med Cell Longev 2013. https://doi.org/10.1155/2013/891748

Bindoli A, Valente M, Cavallini L (1985) Inhibitory action of quercetin on xanthine oxidase and xanthine dehydrogenase activity. Pharmacol Res Commun 17:831–839. https://doi.org/10.1016/0031-6989(85)90041-4

Bitto A, Ito TK, Pineda VV et al (2016) Transient rapamycin treatment can increase lifespan and healthspan in middle-aged mice. Elife 5. https://doi.org/10.7554/eLife.16351

Bitu Pinto N, da Silva AB, Neves KRT et al (2015) Neuroprotective properties of the standardized extract from *Camellia sinensis* (green tea) and its main bioactive components, epicatechin and epigallocatechin gallate, in the 6-OHDA model of Parkinson's disease. Evid Based Complement Alternat Med 2015:161092. https://doi.org/10.1155/2015/161092

Bjelakovic G, Nikolova D, Gluud LL et al (2007) Mortality in randomized trials of antioxidant supplements for primary and secondary prevention: systematic review and meta-analysis. JAMA 297:842–857. https://doi.org/10.1001/jama.297.8.842

Blasco MA (2007) Telomere length, stem cells and aging. Nat Chem Biol 3:640–649. https://doi.org/10.1038/nchembio.2007.38

Blass JP (2000) The mitochondrial spiral. An adequate cause of dementia in the Alzheimer's syndrome. Ann N Y Acad Sci 924:170–183. https://doi.org/10.1111/j.1749-6632.2000.tb05576.x

Blass JP, Sheu RK, Gibson GE (2000) Inherent abnormalities in energy metabolism in Alzheimer disease. Interaction with cerebrovascular compromise. Ann N Y Acad Sci 903:204–221. https://doi.org/10.1111/j.1749-6632.2000.tb06370.x

Blüher M, Mantzoros CS (2015) From leptin to other adipokines in health and disease: facts and expectations at the beginning of the 21st century. Metabolism 64:131–145. https://doi.org/10.1016/j.metabol.2014.10.016

Bobe G, Wang B, Seeram NP et al (2006) Dietary anthocyanin-rich tart cherry extract inhibits intestinal tumorigenesis in APCMin mice fed suboptimal levels of sulindac. J Agric Food Chem 54:9322–9328. https://doi.org/10.1021/jf0612169

Boden G, Duan X, Homko C et al (2008) Increase in endoplasmic reticulum stress-related proteins and genes in adipose tissue of obese, insulin-resistant individuals. Diabetes 57:2438–2444. https://doi.org/10.2337/db08-0604

Bódis K, Roden M (2018) Energy metabolism of white adipose tissue and insulin resistance in humans. Eur J Clin Invest 48:e13017. https://doi.org/10.1111/eci.13017

Bøhn SK, Myhrstad MCW, Thoresen M et al (2021) Bilberry/red grape juice decreases plasma biomarkers of inflammation and tissue damage in aged men with subjective memory impairment – a randomized clinical trial. BMC Nutr 7:75. https://doi.org/10.1186/s40795-021-00482-8

Bose M, Lambert JD, Ju J et al (2008) The major green tea polyphenol, (-)-epigallocatechin-3-gallate, inhibits obesity, metabolic syndrome, and fatty liver disease in high-fat-fed mice. J Nutr 138:1677–1683. https://doi.org/10.1093/jn/138.9.1677

Bournat JC, Brown CW (2010) Mitochondrial dysfunction in obesity. Curr Opin Endocrinol Diabetes Obes 17:446–452. https://doi.org/10.1097/MED.0b013e32833c3026

Brack C, Bechter-Thüring E, Labuhn M (1997) *N*-acetylcysteine slows down ageing and increases the life span of *Drosophila melanogaster*. Cell Mol Life Sci 53:960–966. https://doi.org/10.1007/pl00013199

Broman-Fulks JJ, Canu WH, Trout KL, Nieman DC (2012) The effects of quercetin supplementation on cognitive functioning in a community sample: a randomized, placebo-controlled trial. Ther Adv Psychopharmacol 2:131–138. https://doi.org/10.1177/2045125312445894

Brown AL, Lane J, Coverly J et al (2009) Effects of dietary supplementation with the green tea polyphenol epigallocatechin-3-gallate on insulin resistance and associated metabolic risk factors: randomized controlled trial. Br J Nutr 101:886–894. https://doi.org/10.1017/S0007114508047727

Brüll V, Burak C, Stoffel-Wagner B et al (2017a) Acute intake of quercetin from onion skin extract does not influence postprandial blood pressure and endothelial function in overweight-to-obese adults with hypertension: a randomized, double-blind, placebo-controlled, crossover trial. Eur J Nutr 56:1347–1357. https://doi.org/10.1007/s00394-016-1185-1

Brüll V, Burak C, Stoffel-Wagner B et al (2017b) No effects of quercetin from onion skin extract on serum leptin and adiponectin concentrations in overweight-to-obese patients with (pre-) hypertension: a randomized double-blinded, placebo-controlled crossover trial. Eur J Nutr 56:2265–2275. https://doi.org/10.1007/s00394-016-1267-0

Brunet A, Sweeney LB, Sturgill JF et al (2004) Stress-dependent regulation of FOXO transcription factors by the SIRT1 deacetylase. Science 303:2011–2015. https://doi.org/10.1126/science.1094637

Brunk UT, Terman A (2002a) Lipofuscin: mechanisms of age-related accumulation and influence on cell function. Free Radic Biol Med 33:611–619. https://doi.org/10.1016/s0891-5849(02)00959-0

Brunk UT, Terman A (2002b) The mitochondrial-lysosomal axis theory of aging: accumulation of damaged mitochondria as a result of imperfect autophagocytosis. Eur J Biochem 269:1996–2002. https://doi.org/10.1046/j.1432-1033.2002.02869.x

Buckland G, Bach A, Serra-Majem L (2008) Obesity and the Mediterranean diet: a systematic review of observational and intervention studies. Obes Rev 9:582–593. https://doi.org/10.1111/j.1467-789X.2008.00503.x

Bunker JP, Frazier HS, Mosteller F (1994) Improving health: measuring effects of medical care. Milbank Q 72:225–258. https://doi.org/10.2307/3350295

Burton-Freeman B, Reimers K (2010) Tomato consumption and health: emerging benefits. Am J Lifestyle Med 5:182–191. https://doi.org/10.1177/1559827610387488

Burton-Freeman BM, Sesso HD (2014) Whole food versus supplement: comparing the clinical evidence of tomato intake and lycopene supplementation on cardiovascular risk factors. Adv Nutr 5:457–485. https://doi.org/10.3945/an.114.005231

Butelli E, Titta L, Giorgio M et al (2008) Enrichment of tomato fruit with health-promoting anthocyanins by expression of select transcription factors. Nat Biotechnol 26:1301–1308. https://doi.org/10.1038/nbt.1506

Cagan A, Baez-Ortega A, Brzozowska N et al (2022) Somatic mutation rates scale with lifespan across mammals. Nature 604:517–524. https://doi.org/10.1038/s41586-022-04618-z

Cai D (2013) Neuroinflammation and neurodegeneration in overnutrition-induced diseases. Trends Endocrinol Metab 24:40–47. https://doi.org/10.1016/j.tem.2012.11.003

Cai H, Dong LQ, Liu F (2016) Recent advances in adipose mTOR signaling and function: therapeutic prospects. Trends Pharmacol Sci 37:303–317. https://doi.org/10.1016/j.tips.2015.11.011

Calabrese V, Bates TE, Mancuso C et al (2008) Curcumin and the cellular stress response in free radical-related diseases. Mol Nutr Food Res 52:1062–1073. https://doi.org/10.1002/mnfr.200700316

Campion D, Dumanchin C, Hannequin D et al (1999) Early-onset autosomal dominant Alzheimer disease: prevalence, genetic heterogeneity, and mutation spectrum. Am J Hum Genet 65:664–670. https://doi.org/10.1086/302553

Campisi J, d'Adda di Fagagna F (2007) Cellular senescence: when bad things happen to good cells. Nat Rev Mol Cell Biol 8:729–740. https://doi.org/10.1038/nrm2233

Candelario-Jalil E, de Oliveira ACP, Gräf S et al (2007) Resveratrol potently reduces prostaglandin E2 production and free radical formation in lipopolysaccharide-activated primary rat microglia. J Neuroinflammation 4:25. https://doi.org/10.1186/1742-2094-4-25

Cantó C, Auwerx J (2012) Targeting sirtuin 1 to improve metabolism: all you need is NAD(+)? Pharmacol Rev 64:166–187. https://doi.org/10.1124/pr.110.003905

Cao J, Jia L, Zhou H-M et al (2006) Mitochondrial and nuclear DNA damage induced by curcumin in human hepatoma G2 cells. Toxicol Sci 91:476–483. https://doi.org/10.1093/toxsci/kfj153

Capel F, Rimbert V, Lioger D et al (2005) Due to reverse electron transfer, mitochondrial H2O2 release increases with age in human vastus lateralis muscle although oxidative capacity is preserved. Mech Ageing Dev 126:505–511. https://doi.org/10.1016/j.mad.2004.11.001

Cardile V, Graziano ACE, Venditti A (2015) Clinical evaluation of Moro (*Citrus sinensis* (L.) Osbeck) orange juice supplementation for the weight management. Nat Prod Res 29:2256–2260. https://doi.org/10.1080/14786419.2014.1000897

Carey AN, Gildawie KR, Rovnak A et al (2019) Blueberry supplementation attenuates microglia activation and increases neuroplasticity in mice consuming a high-fat diet. Nutr Neurosci 22:253–263. https://doi.org/10.1080/1028415X.2017.1376472

Carey JR, Papadopoulos N, Kouloussis N et al (2006) Age-specific and lifetime behavior patterns in *Drosophila melanogaster* and the Mediterranean fruit fly, *Ceratitis capitata*. Exp Gerontol 41:93–97. https://doi.org/10.1016/j.exger.2005.09.014

Carlberg C, Ulven SM, Molnár F (2016) Nutritional signaling and aging. In: Carlberg C, Ulven SM, Molnár F (eds) Nutrigenomics: how science works. Springer International Publishing, Cham, pp 105–120

Casadesus G, Shukitt-Hale B, Stellwagen HM et al (2004) Modulation of hippocampal plasticity and cognitive behavior by short-term blueberry supplementation in aged rats. Nutr Neurosci 7:309–316. https://doi.org/10.1080/10284150400020482

Castelli V, Benedetti E, Antonosante A et al (2019) Neuronal cells rearrangement during aging and neurodegenerative disease: metabolism, oxidative stress and organelles dynamic. Front Mol Neurosci 12:132. https://doi.org/10.3389/fnmol.2019.00132

Cawthon RM, Smith KR, O'Brien E et al (2003) Association between telomere length in blood and mortality in people aged 60 years or older. Lancet (London) 361:393–395. https://doi.org/10.1016/S0140-6736(03)12384-7

Cerqueira FM, Cunha FM, Laurindo FRM, Kowaltowski AJ (2012) Calorie restriction increases cerebral mitochondrial respiratory capacity in a NO•-mediated mechanism: impact on neuronal survival. Free Radic Biol Med 52:1236–1241. https://doi.org/10.1016/j.freeradbiomed.2012.01.011

Chaanine AH, Kohlbrenner E, Gamb SI et al (2016) FOXO3a regulates BNIP3 and modulates mitochondrial calcium, dynamics, and function in cardiac stress. Am J Physiol Heart Circ Physiol 311:H1540–H1559. https://doi.org/10.1152/ajpheart.00549.2016

Chakrabarti P, English T, Shi J et al (2010) Mammalian target of rapamycin complex 1 suppresses lipolysis, stimulates lipogenesis, and promotes fat storage. Diabetes 59:775–781. https://doi.org/10.2337/db09-1602

Chang G-R, Chiu Y-S, Wu Y-Y et al (2009) Rapamycin protects against high fat diet-induced obesity in C57BL/6J mice. J Pharmacol Sci 109:496–503. https://doi.org/10.1254/jphs.08215fp

Chang X, Rong C, Chen Y et al (2015) (-)-Epigallocatechin-3-gallate attenuates cognitive deterioration in Alzheimer's disease model mice by upregulating neprilysin expression. Exp Cell Res 334:136–145. https://doi.org/10.1016/j.yexcr.2015.04.004

Chang Y-F, Chi C-W, Wang J-J (2006) Reactive oxygen species production is involved in quercetin-induced apoptosis in human hepatoma cells. Nutr Cancer 55:201–209. https://doi.org/10.1207/s15327914nc5502_12

Chavarro JE, Toth TL, Wright DL et al (2010) Body mass index in relation to semen quality, sperm DNA integrity, and serum reproductive hormone levels among men attending an infertility clinic. Fertil Steril 93:2222–2231. https://doi.org/10.1016/j.fertnstert.2009.01.100

Chen BH, Marioni RE, Colicino E et al (2016) DNA methylation-based measures of biological age: meta-analysis predicting time to death. Aging (Albany NY) 8:1844–1865. https://doi.org/10.18632/aging.101020

Chen C, Chang Y-C, Liu C-L et al (2006) Leptin-induced growth of human ZR-75-1 breast cancer cells is associated with up-regulation of cyclin D1 and c-Myc and down-regulation of tumor suppressor p53 and p21WAF1/CIP1. Breast Cancer Res Treat 98:121–132. https://doi.org/10.1007/s10549-005-9139-y

Chen J-H, Hales CN, Ozanne SE (2007) DNA damage, cellular senescence and organismal ageing: causal or correlative? Nucleic Acids Res 35:7417–7428. https://doi.org/10.1093/nar/gkm681

Chen M, Du Z-Y, Zheng X et al (2018) Use of curcumin in diagnosis, prevention, and treatment of Alzheimer's disease. Neural Regen Res 13:742–752. https://doi.org/10.4103/1673-5374.230303

Chen M, Wang T, Yue F et al (2015a) Tea polyphenols alleviate motor impairments, dopaminergic neuronal injury, and cerebral α-synuclein aggregation in MPTP-intoxicated parkinsonian monkeys. Neuroscience 286:383–392. https://doi.org/10.1016/j.neuroscience.2014.12.003

Chen P, Zhang W, Wang X et al (2015b) Lycopene and risk of prostate cancer: a systematic review and meta-analysis. Medicine (Baltimore) 94:e1260. https://doi.org/10.1097/MD.0000000000001260

Chen S-F, Nien S, Wu C-H et al (2013a) Reappraisal of the anticancer efficacy of quercetin in oral cancer cells. J Chin Med Assoc 76:146–152. https://doi.org/10.1016/j.jcma.2012.11.008

Chen T, Liu AB, Sun S et al (2019) Green tea polyphenols modify the gut microbiome in db/db Mice as Co-abundance groups correlating with the blood glucose lowering effect. Mol Nutr Food Res 63:e1801064. https://doi.org/10.1002/mnfr.201801064

Chen W, Müller D, Richling E, Wink M (2013b) Anthocyanin-rich purple wheat prolongs the life span of *Caenorhabditis elegans* probably by activating the DAF-16/FOXO transcription factor. J Agric Food Chem 61:3047–3053. https://doi.org/10.1021/jf3054643

Chen Z, Zhong C (2014) Oxidative stress in Alzheimer's disease. Neurosci Bull 30:271–281. https://doi.org/10.1007/s12264-013-1423-y

Cheng J, Rui Y, Qin L et al (2017) Vitamin D combined with resveratrol prevents cognitive decline in SAMP8 mice. Curr Alzheimer Res 14:820–833. https://doi.org/10.2174/1567205014666170207093455

Cheng Y, Takeuchi H, Sonobe Y et al (2014) Sirtuin 1 attenuates oxidative stress via upregulation of superoxide dismutase 2 and catalase in astrocytes. J Neuroimmunol 269:38–43. https://doi.org/10.1016/j.jneuroim.2014.02.001

Cheung LTF, Chan RSM, Ko GTC et al (2018) Diet quality is inversely associated with obesity in Chinese adults with type 2 diabetes. Nutr J 17:63. https://doi.org/10.1186/s12937-018-0374-6

Chiavaroli L, Viguiliouk E, Nishi SK et al (2019) DASH dietary pattern and cardiometabolic outcomes: an umbrella review of systematic reviews and meta-analyses. Nutrients 11:338. https://doi.org/10.3390/nu11020338

Chistiakov DA, Sobenin IA, Revin VV et al (2014) Mitochondrial aging and age-related dysfunction of mitochondria. Biomed Res Int 2014:238463. https://doi.org/10.1155/2014/238463

Cho J, Hur JH, Walker DW (2011) The role of mitochondria in *Drosophila* aging. Exp Gerontol 46:331–334. https://doi.org/10.1016/j.exger.2010.08.010

Choi E-Y, Lee H, Woo JS et al (2015) Effect of onion peel extract on endothelial function and endothelial progenitor cells in overweight and obese individuals. Nutrition 31:1131–1135. https://doi.org/10.1016/j.nut.2015.04.020

Chomentowski P, Dubé JJ, Amati F et al (2009) Moderate exercise attenuates the loss of skeletal muscle mass that occurs with intentional caloric restriction-induced weight loss in older, overweight to obese adults. J Gerontol A Biol Sci Med Sci 64:575–580. https://doi.org/10.1093/gerona/glp007

Chondrogianni N, Kapeta S, Chinou I et al (2010) Anti-ageing and rejuvenating effects of quercetin. Exp Gerontol 45:763–771. https://doi.org/10.1016/j.exger.2010.07.001
Choo H-J, Kim J-H, Kwon O-B et al (2006) Mitochondria are impaired in the adipocytes of type 2 diabetic mice. Diabetologia 49:784–791. https://doi.org/10.1007/s00125-006-0170-2
Choo JJ (2003) Green tea reduces body fat accretion caused by high-fat diet in rats through beta-adrenoceptor activation of thermogenesis in brown adipose tissue. J Nutr Biochem 14:671–676. https://doi.org/10.1016/j.jnutbio.2003.08.005
Christensen K, Doblhammer G, Rau R, Vaupel JW (2009) Ageing populations: the challenges ahead. Lancet (London) 374:1196–1208. https://doi.org/10.1016/S0140-6736(09)61460-4
Chuengsamarn S, Rattanamongkolgul S, Phonrat B et al (2014) Reduction of atherogenic risk in patients with type 2 diabetes by curcuminoid extract: a randomized controlled trial. J Nutr Biochem 25:144–150. https://doi.org/10.1016/j.jnutbio.2013.09.013
Chung KW, Chung HY (2019) The effects of calorie restriction on autophagy: role on aging intervention. Nutrients 11. https://doi.org/10.3390/nu11122923
Cialdella-Kam L, Nieman DC, Knab AM et al (2016) A mixed flavonoid-fish oil supplement induces immune-enhancing and anti-inflammatory transcriptomic changes in adult obese and overweight women-a randomized controlled trial. Nutrients 8. https://doi.org/10.3390/nu8050277
Cienfuegos S, Gabel K, Kalam F et al (2020) Effects of 4- and 6-h time-restricted feeding on weight and cardiometabolic health: a randomized controlled trial in adults with obesity. Cell Metab 32:366–378.e3. https://doi.org/10.1016/j.cmet.2020.06.018
Civitarese AE, Ukropcova B, Carling S et al (2006) Role of adiponectin in human skeletal muscle bioenergetics. Cell Metab 4:75–87. https://doi.org/10.1016/j.cmet.2006.05.002
Cobley JN, Fiorello ML, Bailey DM (2018) 13 reasons why the brain is susceptible to oxidative stress. Redox Biol 15:490–503. https://doi.org/10.1016/j.redox.2018.01.008
Collins JJ, Evason K, Kornfeld K (2006) Pharmacology of delayed aging and extended lifespan of *Caenorhabditis elegans*. Exp Gerontol 41:1032–1039. https://doi.org/10.1016/j.exger.2006.06.038
Colman RJ, Anderson RM, Johnson SC et al (2009) Caloric restriction delays disease onset and mortality in rhesus monkeys. Science 325:201–204. https://doi.org/10.1126/science.1173635
Colman RJ, Beasley TM, Kemnitz JW et al (2014) Caloric restriction reduces age-related and all-cause mortality in rhesus monkeys. Nat Commun 5:3557. https://doi.org/10.1038/ncomms4557
Comalada M, Camuesco D, Sierra S et al (2005) *In vivo* quercitrin anti-inflammatory effect involves release of quercetin, which inhibits inflammation through down-regulation of the NF-kappaB pathway. Eur J Immunol 35:584–592. https://doi.org/10.1002/eji.200425778
Compton S, Kim C, Griner NB et al (2011) Mitochondrial dysfunction impairs tumor suppressor p53 expression/function. J Biol Chem 286:20297–20312. https://doi.org/10.1074/jbc.M110.163063
Conn CS, Qian S-B (2011) mTOR signaling in protein homeostasis: less is more? Cell Cycle 10:1940–1947. https://doi.org/10.4161/cc.10.12.15858
Cooper G (2000) Signaling molecules and their receptors. In: The cell: a molecular approach. Sinauer Associates
Cooper TM, Mockett RJ, Sohal BH et al (2004) Effect of caloric restriction on life span of the housefly, *Musca domestica*. FASEB J 18:1591–1593. https://doi.org/10.1096/fj.03-1464fje
Coppe J-P, Desprez P-Y, Krtolica A, Campisi J (2010) The senescence-associated secretory phenotype: the dark side of tumor suppression. Annu Rev Pathol 5:99–118. https://doi.org/10.1146/annurev-pathol-121808-102144
Coppedè F, Migliore L (2015) DNA damage in neurodegenerative diseases. Mutat Res 776:84–97. https://doi.org/10.1016/j.mrfmmm.2014.11.010
Coppens J, Bentea E, Bayliss JA et al (2017) Caloric restriction protects against lactacystin-induced degeneration of dopamine neurons independent of the ghrelin receptor. Int J Mol Sci 18. https://doi.org/10.3390/ijms18030558

Corley J (2022) Adherence to the MIND diet is associated with 12-year all-cause mortality in older adults. Public Health Nutr 25:358–367. https://doi.org/10.1017/S1368980020002979

Cory H, Passarelli S, Szeto J et al (2018) The role of polyphenols in human health and food systems: a mini-review. Front Nutr 5:87. https://doi.org/10.3389/fnut.2018.00087

Cosgrove JP, Church DF, Pryor WA (1987) The kinetics of the autoxidation of polyunsaturated fatty acids. Lipids 22:299–304. https://doi.org/10.1007/BF02533996

Cosín-Tomàs M, Senserrich J, Arumí-Planas M et al (2019) Role of resveratrol and selenium on oxidative stress and expression of antioxidant and anti-aging genes in immortalized lymphocytes from Alzheimer's disease patients. Nutrients 11. https://doi.org/10.3390/nu11081764

Costa LG, Garrick JM, Roquè PJ, Pellacani C (2016) Mechanisms of neuroprotection by quercetin: counteracting oxidative stress and more. Oxid Med Cell Longev 2016:2986796. https://doi.org/10.1155/2016/2986796

Cox KHM, Pipingas A, Scholey AB (2015) Investigation of the effects of solid lipid curcumin on cognition and mood in a healthy older population. J Psychopharmacol 29:642–651. https://doi.org/10.1177/0269881114552744

Crowe FL, Roddam AW, Key TJ et al (2011) Fruit and vegetable intake and mortality from ischaemic heart disease: results from the European Prospective Investigation into Cancer and Nutrition (EPIC)-Heart study. Eur Heart J 32:1235–1243. https://doi.org/10.1093/eurheartj/ehq465

Cuadrado A, Manda G, Hassan A et al (2018) Transcription factor NRF2 as a therapeutic target for chronic diseases: a systems medicine approach. Pharmacol Rev 70:348–383. https://doi.org/10.1124/pr.117.014753

Cui J, Lee S, Sun Y et al (2022) Alternate day fasting improves endothelial function in type 2 diabetic mice: role of adipose-derived hormones. Front Cardiovasc Med 9:925080. https://doi.org/10.3389/fcvm.2022.925080

Cullinan SB, Gordan JD, Jin J et al (2004) The Keap1-BTB protein is an adaptor that bridges Nrf2 to a Cul3-based E3 ligase: oxidative stress sensing by a Cul3-Keap1 ligase. Mol Cell Biol 24:8477–8486. https://doi.org/10.1128/MCB.24.19.8477-8486.2004

Cummings DE, Weigle DS, Frayo RS et al (2002) Plasma ghrelin levels after diet-induced weight loss or gastric bypass surgery. N Engl J Med 346:1623–1630. https://doi.org/10.1056/NEJMoa012908

Curti V, Capelli E, Boschi F et al (2014) Modulation of human miR-17-3p expression by methyl 3-O-methyl gallate as explanation of its *in vivo* protective activities. Mol Nutr Food Res 58:1776–1784. https://doi.org/10.1002/mnfr.201400007

Cypess AM, White AP, Vernochet C et al (2013) Anatomical localization, gene expression profiling and functional characterization of adult human neck brown fat. Nat Med 19:635–639. https://doi.org/10.1038/nm.3112

D'Mello MJJ, Ross SA, Briel M et al (2015) Association between shortened leukocyte telomere length and cardiometabolic outcomes: systematic review and meta-analysis. Circ Cardiovasc Genet 8:82–90. https://doi.org/10.1161/CIRCGENETICS.113.000485

da Luz PL, Tanaka L, Brum PC et al (2012) Red wine and equivalent oral pharmacological doses of resveratrol delay vascular aging but do not extend life span in rats. Atherosclerosis 224:136–142. https://doi.org/10.1016/j.atherosclerosis.2012.06.007

da Silva SV, Renovato-Martins M, Ribeiro-Pereira C et al (2016) Obesity modifies bone marrow microenvironment and directs bone marrow mesenchymal cells to adipogenesis. Obesity (Silver Spring) 24:2522–2532. https://doi.org/10.1002/oby.21660

Dahl A, Hassing LB, Fransson E et al (2010) Being overweight in midlife is associated with lower cognitive ability and steeper cognitive decline in late life. J Gerontol A Biol Sci Med Sci 65:57–62. https://doi.org/10.1093/gerona/glp035

Dai Q, Zhou D, Xu L, Song X (2018) Curcumin alleviates rheumatoid arthritis-induced inflammation and synovial hyperplasia by targeting mTOR pathway in rats. Drug Des Devel Ther 12:4095–4105. https://doi.org/10.2147/DDDT.S175763

Dai S, Wei J, Zhang H et al (2022) Intermittent fasting reduces neuroinflammation in intracerebral hemorrhage through the Sirt3/Nrf2/HO-1 pathway. J Neuroinflammation 19:122. https://doi.org/10.1186/s12974-022-02474-2

Das KC, Das CK (2002) Curcumin (diferuloylmethane), a singlet oxygen $^1O_2$ quencher. Biochem Biophys Res Commun 295:62–66. https://doi.org/10.1016/s0006-291x(02)00633-2

Das S, Lin H-S, Ho PC, Ng K-Y (2008) The impact of aqueous solubility and dose on the pharmacokinetic profiles of resveratrol. Pharm Res 25:2593–2600. https://doi.org/10.1007/s11095-008-9677-1

de Cabo R, Mattson MP (2019) Effects of intermittent fasting on health, aging, and disease. N Engl J Med 381:2541–2551. https://doi.org/10.1056/NEJMra1905136

De Felice FG, Velasco PT, Lambert MP et al (2007) Abeta oligomers induce neuronal oxidative stress through an N-methyl-D-aspartate receptor-dependent mechanism that is blocked by the Alzheimer drug memantine. J Biol Chem 282:11590–11601. https://doi.org/10.1074/jbc.M607483200

de Grey AD (1997) A proposed refinement of the mitochondrial free radical theory of aging. BioEssays 19:161–166. https://doi.org/10.1002/bies.950190211

De la Fuente M, Miquel J (2009) An update of the oxidation-inflammation theory of aging: the involvement of the immune system in oxi-inflamm-aging. Curr Pharm Des 15:3003–3026. https://doi.org/10.2174/138161209789058110

de Mello AH, Costa AB, Engel JDG, Rezin GT (2018) Mitochondrial dysfunction in obesity. Life Sci 192:26–32. https://doi.org/10.1016/j.lfs.2017.11.019

de Pascual-Teresa S (2014) Molecular mechanisms involved in the cardiovascular and neuroprotective effects of anthocyanins. Arch Biochem Biophys 559:68–74. https://doi.org/10.1016/j.abb.2014.04.012

Deby-Dupont G, Mouithys-Mickalad A, Serteyn D et al (2005) Resveratrol and curcumin reduce the respiratory burst of *Chlamydia*-primed THP-1 cells. Biochem Biophys Res Commun 333:21–27. https://doi.org/10.1016/j.bbrc.2005.05.073

DeFuria J, Bennett G, Strissel KJ et al (2009) Dietary blueberry attenuates whole-body insulin resistance in high fat-fed mice by reducing adipocyte death and its inflammatory sequelae. J Nutr 139:1510–1516. https://doi.org/10.3945/jn.109.105155

Demissie S, Levy D, Benjamin EJ et al (2006) Insulin resistance, oxidative stress, hypertension, and leukocyte telomere length in men from the Framingham Heart Study. Aging Cell 5:325–330. https://doi.org/10.1111/j.1474-9726.2006.00224.x

Deng Y, Liu W, Wang J et al (2020) Intermittent fasting improves lipid metabolism through changes in gut microbiota in diet-induced obese mice. Med Sci Monit Int Med J Exp Clin Res 26:e926789. https://doi.org/10.12659/MSM.926789

Denny Joseph KM, Muralidhara (2013) Enhanced neuroprotective effect of fish oil in combination with quercetin against 3-nitropropionic acid induced oxidative stress in rat brain. Prog Neuropsychopharmacol Biol Psychiatry 40:83–92. https://doi.org/10.1016/j.pnpbp.2012.08.018

Denny Joseph KM, Muralidhara (2015) Combined oral supplementation of fish oil and quercetin enhances neuroprotection in a chronic rotenone rat model: relevance to Parkinson's disease. Neurochem Res 40:894–905. https://doi.org/10.1007/s11064-015-1542-0

Devarakonda S, Gupta K, Chalmers MJ et al (2011) Disorder-to-order transition underlies the structural basis for the assembly of a transcriptionally active PGC-1α/ERRγ complex. Proc Natl Acad Sci U S A 108:18678–18683. https://doi.org/10.1073/pnas.1113813108

Devore EE, Kang JH, Breteler MMB, Grodstein F (2012) Dietary intakes of berries and flavonoids in relation to cognitive decline. Ann Neurol 72:135–143. https://doi.org/10.1002/ana.23594

Dexter DT, Carter CJ, Wells FR et al (1989) Basal lipid peroxidation in substantia nigra is increased in Parkinson's disease. J Neurochem 52:381–389. https://doi.org/10.1111/j.1471-4159.1989.tb09133.x

Di Pierro F, Bressan A, Ranaldi D et al (2015) Potential role of bioavailable curcumin in weight loss and omental adipose tissue decrease: preliminary data of a randomized, controlled trial in

overweight people with metabolic syndrome. Preliminary study. Eur Rev Med Pharmacol Sci 19:4195–4202
Díaz-Ruiz A, Guzmán-Ruiz R, Moreno NR et al (2015) Proteasome dysfunction associated to oxidative stress and proteotoxicity in adipocytes compromises insulin sensitivity in human obesity. Antioxid Redox Signal 23:597–612. https://doi.org/10.1089/ars.2014.5939
Dinkova-Kostova AT, Talalay P (2008) Direct and indirect antioxidant properties of inducers of cytoprotective proteins. Mol Nutr Food Res 52(Suppl 1):S128–S138. https://doi.org/10.1002/mnfr.200700195
Dinu M, Pagliai G, Casini A, Sofi F (2018) Mediterranean diet and multiple health outcomes: an umbrella review of meta-analyses of observational studies and randomised trials. Eur J Clin Nutr 72:30–43. https://doi.org/10.1038/ejcn.2017.58
DiSilvestro RA, Joseph E, Zhao S, Bomser J (2012) Diverse effects of a low dose supplement of lipidated curcumin in healthy middle aged people. Nutr J 11:79. https://doi.org/10.1186/1475-2891-11-79
Dkhar P, Sharma R (2010) Effect of dimethylsulphoxide and curcumin on protein carbonyls and reactive oxygen species of cerebral hemispheres of mice as a function of age. Int J Dev Neurosci Off J Int Soc Dev Neurosci 28:351–357. https://doi.org/10.1016/j.ijdevneu.2010.04.005
Dkhar P, Sharma R (2013) Attenuation of age-related increase of protein carbonylation in the liver of mice by melatonin and curcumin. Mol Cell Biochem 380:153–160. https://doi.org/10.1007/s11010-013-1668-9
Domitrovic R (2011) The molecular basis for the pharmacological activity of anthocyans. Curr Med Chem 18:4454–4469. https://doi.org/10.2174/092986711797287601
Dong S, Zeng Q, Mitchell ES et al (2012) Curcumin enhances neurogenesis and cognition in aged rats: implications for transcriptional interactions related to growth and synaptic plasticity. PLoS One 7:e31211. https://doi.org/10.1371/journal.pone.0031211
Doré S (2005) Unique properties of polyphenol stilbenes in the brain: more than direct antioxidant actions; gene/protein regulatory activity. Neurosignals 14:61–70. https://doi.org/10.1159/000085386
Dorling JL, van Vliet S, Huffman KM et al (2021) Effects of caloric restriction on human physiological, psychological, and behavioral outcomes: highlights from CALERIE phase 2. Nutr Rev 79:98–113. https://doi.org/10.1093/nutrit/nuaa085
Dower JI, Geleijnse JM, Gijsbers L et al (2015) Effects of the pure flavonoids epicatechin and quercetin on vascular function and cardiometabolic health: a randomized, double-blind, placebo-controlled, crossover trial. Am J Clin Nutr 101:914–921. https://doi.org/10.3945/ajcn.114.098590
Doytchinova I, Atanasova M, Salamanova E et al (2020) Curcumin inhibits the primary nucleation of amyloid-beta peptide: a molecular dynamics study. Biomolecules 10. https://doi.org/10.3390/biom10091323
Druso JE, Fischbach C (2018) Biophysical properties of extracellular matrix: linking obesity and cancer. Trends Cancer 4:271–273. https://doi.org/10.1016/j.trecan.2018.02.001
Duan W, Guo Z, Jiang H et al (2003) Dietary restriction normalizes glucose metabolism and BDNF levels, slows disease progression, and increases survival in huntingtin mutant mice. Proc Natl Acad Sci U S A 100:2911–2916. https://doi.org/10.1073/pnas.0536856100
Duan W, Mattson MP (1999) Dietary restriction and 2-deoxyglucose administration improve behavioral outcome and reduce degeneration of dopaminergic neurons in models of Parkinson's disease. J Neurosci Res 57:195–206. https://doi.org/10.1002/(SICI)1097-4547(19990715)57:2<195::AID-JNR5>3.0.CO;2-P
Dubuc GR, Phinney SD, Stern JS, Havel PJ (1998) Changes of serum leptin and endocrine and metabolic parameters after 7 days of energy restriction in men and women. Metabolism 47:429–434. https://doi.org/10.1016/s0026-0495(98)90055-5
Duncan RE, Ahmadian M, Jaworski K et al (2007) Regulation of lipolysis in adipocytes. Annu Rev Nutr 27:79–101. https://doi.org/10.1146/annurev.nutr.27.061406.093734

Dungan CM, Peck BD, Walton RG et al (2020) *In vivo* analysis of γH2AX+ cells in skeletal muscle from aged and obese humans. FASEB J 34:7018–7035. https://doi.org/10.1096/fj.202000111RR

Eckel RH, Grundy SM, Zimmet PZ (2005) The metabolic syndrome. Lancet (London) 365:1415–1428. https://doi.org/10.1016/S0140-6736(05)66378-7

Eckert GP, Schiborr C, Hagl S et al (2013) Curcumin prevents mitochondrial dysfunction in the brain of the senescence-accelerated mouse-prone 8. Neurochem Int 62:595–602. https://doi.org/10.1016/j.neuint.2013.02.014

Egert S, Bosy-Westphal A, Seiberl J et al (2009) Quercetin reduces systolic blood pressure and plasma oxidised low-density lipoprotein concentrations in overweight subjects with a high-cardiovascular disease risk phenotype: a double-blinded, placebo-controlled cross-over study. Br J Nutr 102:1065–1074. https://doi.org/10.1017/S0007114509359127

El'darov CM, Vays VB, Vangeli IM et al (2015) Morphometric examination of mitochondrial ultrastructure in aging cardiomyocytes. Biochemistry (Mosc) 80:604–609. https://doi.org/10.1134/S0006297915050132

Elias MF, Elias PK, Sullivan LM et al (2003) Lower cognitive function in the presence of obesity and hypertension: the Framingham heart study. Int J Obes Relat Metab Disord J Int Assoc Study Obes 27:260–268. https://doi.org/10.1038/sj.ijo.802225

Ellgaard L, Helenius A (2003) Quality control in the endoplasmic reticulum. Nat Rev Mol Cell Biol 4:181–191. https://doi.org/10.1038/nrm1052

Ellulu MS, Patimah I, Khaza'ai H et al (2017) Obesity and inflammation: the linking mechanism and the complications. Arch Med Sci 13:851–863. https://doi.org/10.5114/aoms.2016.58928

Emery AC, Eiden LE (2012) Signaling through the neuropeptide GPCR PAC_1 induces neuritogenesis via a single linear cAMP- and ERK-dependent pathway using a novel cAMP sensor. FASEB J 26:3199–3211. https://doi.org/10.1096/fj.11-203042

Engin A (2017) The definition and prevalence of obesity and metabolic syndrome. Adv Exp Med Biol 960:1–17. https://doi.org/10.1007/978-3-319-48382-5_1

Enserink JM, Christensen AE, de Rooij J et al (2002) A novel Epac-specific cAMP analogue demonstrates independent regulation of Rap1 and ERK. Nat Cell Biol 4:901–906. https://doi.org/10.1038/ncb874

Erickson KI, Prakash RS, Voss MW et al (2010) Brain-derived neurotrophic factor is associated with age-related decline in hippocampal volume. J Neurosci Off J Soc Neurosci 30:5368–5375. https://doi.org/10.1523/JNEUROSCI.6251-09.2010

Ermolaeva M, Neri F, Ori A, Rudolph KL (2018) Cellular and epigenetic drivers of stem cell ageing. Nat Rev Mol Cell Biol 19:594–610. https://doi.org/10.1038/s41580-018-0020-3

Esatbeyoglu T, Huebbe P, Ernst IMA et al (2012) Curcumin – from molecule to biological function. Angew Chem Int Ed Engl 51:5308–5332. https://doi.org/10.1002/anie.201107724

Esposito K, Marfella R, Ciotola M et al (2004) Effect of a mediterranean-style diet on endothelial dysfunction and markers of vascular inflammation in the metabolic syndrome: a randomized trial. JAMA 292:1440–1446. https://doi.org/10.1001/jama.292.12.1440

Estrov Z, Shishodia S, Faderl S et al (2003) Resveratrol blocks interleukin-1beta-induced activation of the nuclear transcription factor NF-kappaB, inhibits proliferation, causes S-phase arrest, and induces apoptosis of acute myeloid leukemia cells. Blood 102:987–995. https://doi.org/10.1182/blood-2002-11-3550

Evans JL, Maddux BA, Goldfine ID (2005) The molecular basis for oxidative stress-induced insulin resistance. Antioxid Redox Signal 7:1040–1052. https://doi.org/10.1089/ars.2005.7.1040

Exner N, Lutz AK, Haass C, Winklhofer KF (2012) Mitochondrial dysfunction in Parkinson's disease: molecular mechanisms and pathophysiological consequences. EMBO J 31:3038–3062. https://doi.org/10.1038/emboj.2012.170

Fain JN (2006) Release of interleukins and other inflammatory cytokines by human adipose tissue is enhanced in obesity and primarily due to the nonfat cells. Vitam Horm 74:443–477. https://doi.org/10.1016/S0083-6729(06)74018-3

Fan X, Hussien R, Brooks GA (2010) H2O2-induced mitochondrial fragmentation in C2C12 myocytes. Free Radic Biol Med 49:1646–1654. https://doi.org/10.1016/j.freeradbiomed.2010.08.024

Fantuzzi G (2005) Adipose tissue, adipokines, and inflammation. J Allergy Clin Immunol 115:911–919.; quiz 920. https://doi.org/10.1016/j.jaci.2005.02.023

Fariello RM, Pariz JR, Spaine DM et al (2012) Association between obesity and alteration of sperm DNA integrity and mitochondrial activity. BJU Int 110:863–867. https://doi.org/10.1111/j.1464-410X.2011.10813.x

Féart C, Samieri C, Rondeau V et al (2009) Adherence to a Mediterranean diet, cognitive decline, and risk of dementia. JAMA 302:638–648. https://doi.org/10.1001/jama.2009.1146

Feng K, Ge Y, Chen Z et al (2019) Curcumin inhibits the PERK-eIF2α-CHOP pathway through promoting SIRT1 expression in oxidative stress-induced rat chondrocytes and ameliorates osteoarthritis progression in a rat model. Oxid Med Cell Longev 2019:8574386. https://doi.org/10.1155/2019/8574386

Fernández-Sánchez A, Madrigal-Santillán E, Bautista M et al (2011) Inflammation, oxidative stress, and obesity. Int J Mol Sci 12:3117–3132. https://doi.org/10.3390/ijms12053117

Ferrucci L, Fabbri E (2018) Inflammageing: chronic inflammation in ageing, cardiovascular disease, and frailty. Nat Rev Cardiol 15:505–522. https://doi.org/10.1038/s41569-018-0064-2

Finkbeiner S (2000) CREB couples neurotrophin signals to survival messages. Neuron 25:11–14. https://doi.org/10.1016/s0896-6273(00)80866-1

Finkbeiner S, Tavazoie SF, Maloratsky A et al (1997) CREB: a major mediator of neuronal neurotrophin responses. Neuron 19:1031–1047. https://doi.org/10.1016/s0896-6273(00)80395-5

Finkel T, Holbrook NJ (2000) Oxidants, oxidative stress and the biology of ageing. Nature 408:239–247. https://doi.org/10.1038/35041687

Flanagan EW, Most J, Mey JT, Redman LM (2020) Calorie restriction and aging in humans. Annu Rev Nutr 40:105–133. https://doi.org/10.1146/annurev-nutr-122319-034601

Floreani M, Napoli E, Quintieri L, Palatini P (2003) Oral administration of trans-resveratrol to guinea pigs increases cardiac DT-diaphorase and catalase activities, and protects isolated atria from menadione toxicity. Life Sci 72:2741–2750. https://doi.org/10.1016/s0024-3205(03)00179-6

Flurkey K, Astle CM, Harrison DE (2010) Life extension by diet restriction and N-acetyl-L-cysteine in genetically heterogeneous mice. J Gerontol A Biol Sci Med Sci 65:1275–1284. https://doi.org/10.1093/gerona/glq155

Flynn JM, O'Leary MN, Zambataro CA et al (2013) Late-life rapamycin treatment reverses age-related heart dysfunction. Aging Cell 12:851–862. https://doi.org/10.1111/acel.12109

Fontana L, Ghezzi L, Cross AH, Piccio L (2021) Effects of dietary restriction on neuroinflammation in neurodegenerative diseases. J Exp Med 218. https://doi.org/10.1084/jem.20190086

Ford ES, Mokdad AH (2001) Fruit and vegetable consumption and diabetes mellitus incidence among U.S. adults. Prev Med (Baltim) 32:33–39. https://doi.org/10.1006/pmed.2000.0772

Fordyce DE, Wehner JM (1993) Effects of aging on spatial learning and hippocampal protein kinase C in mice. Neurobiol Aging 14:309–317. https://doi.org/10.1016/0197-4580(93)90116-s

Formica JV, Regelson W (1995) Review of the biology of Quercetin and related bioflavonoids. Food Chem Toxicol 33:1061–1080. https://doi.org/10.1016/0278-6915(95)00077-1

Forster MJ, Morris P, Sohal RS (2003) Genotype and age influence the effect of caloric intake on mortality in mice. FASEB J 17:690–692. https://doi.org/10.1096/fj.02-0533fje

Foscolou A, Critselis E, Panagiotakos D (2018) Olive oil consumption and human health: a narrative review. Maturitas 118:60–66. https://doi.org/10.1016/j.maturitas.2018.10.013

Franceschi C (2007) Inflammaging as a major characteristic of old people: can it be prevented or cured? Nutr Rev 65:S173–S176. https://doi.org/10.1111/j.1753-4887.2007.tb00358.x

Franceschi C, Campisi J (2014) Chronic inflammation (inflammaging) and its potential contribution to age-associated diseases. J Gerontol A Biol Sci Med Sci 69(Suppl 1):S4–S9. https://doi.org/10.1093/gerona/glu057

Franceschi C, Garagnani P, Parini P et al (2018) Inflammaging: a new immune-metabolic viewpoint for age-related diseases. Nat Rev Endocrinol 14:576–590. https://doi.org/10.1038/s41574-018-0059-4

Frautschy SA, Hu W, Kim P et al (2001) Phenolic anti-inflammatory antioxidant reversal of Abeta-induced cognitive deficits and neuropathology. Neurobiol Aging 22:993–1005. https://doi.org/10.1016/s0197-4580(01)00300-1

Frayn KN, Karpe F, Fielding BA et al (2003) Integrative physiology of human adipose tissue. Int J Obes Relat Metab Disord 27:875–888. https://doi.org/10.1038/sj.ijo.0802326

Fujisawa S, Kadoma Y (2006) Comparative study of the alkyl and peroxy radical scavenging activities of polyphenols. Chemosphere 62:71–79. https://doi.org/10.1016/j.chemosphere.2005.04.006

Fukumura D, Incio J, Shankaraiah RC, Jain RK (2016) Obesity and cancer: an angiogenic and inflammatory link. Microcirculation 23:191–206. https://doi.org/10.1111/micc.12270

Fuloria S, Mehta J, Chandel A et al (2022) A comprehensive review on the therapeutic potential of Curcuma longa Linn. in relation to its major active constituent curcumin. Front Pharmacol 13:820806. https://doi.org/10.3389/fphar.2022.820806

Furukawa S, Fujita T, Shimabukuro M et al (2004) Increased oxidative stress in obesity and its impact on metabolic syndrome. J Clin Invest 114:1752–1761. https://doi.org/10.1172/JCI21625

Gabel K, Hoddy KK, Varady KA (2019) Safety of 8-h time restricted feeding in adults with obesity. Appl Physiol Nutr Metab 44:107–109. https://doi.org/10.1139/apnm-2018-0389

Ganjali S, Sahebkar A, Mahdipour E et al (2014) Investigation of the effects of curcumin on serum cytokines in obese individuals: a randomized controlled trial. ScientificWorldJournal 2014:898361. https://doi.org/10.1155/2014/898361

Gardner JP, Li S, Srinivasan SR et al (2005) Rise in insulin resistance is associated with escalated telomere attrition. Circulation 111:2171–2177. https://doi.org/10.1161/01.CIR.0000163550.70487.0B

Garschall K, Dellago H, Gáliková M et al (2017) Ubiquitous overexpression of the DNA repair factor *dPrp19* reduces DNA damage and extends *Drosophila* life span. NPJ Aging Mech Dis 3:5. https://doi.org/10.1038/s41514-017-0005-z

Gavia-García G, Rosado-Pérez J, Arista-Ugalde TL et al (2021) Telomere length and oxidative stress and its relation with metabolic syndrome components in the aging. Biology (Basel) 10. https://doi.org/10.3390/biology10040253

Geng L, Liu Z, Zhang W et al (2019) Chemical screen identifies a geroprotective role of quercetin in premature aging. Protein Cell 10:417–435. https://doi.org/10.1007/s13238-018-0567-y

Gesta S, Tseng Y-H, Kahn CR (2007) Developmental origin of fat: tracking obesity to its source. Cell 131:242–256. https://doi.org/10.1016/j.cell.2007.10.004

Giasson BI, Duda JE, Murray IV et al (2000) Oxidative damage linked to neurodegeneration by selective alpha-synuclein nitration in synucleinopathy lesions. Science 290:985–989. https://doi.org/10.1126/science.290.5493.985

Giri RK, Rajagopal V, Kalra VK (2004) Curcumin, the active constituent of turmeric, inhibits amyloid peptide-induced cytochemokine gene expression and CCR5-mediated chemotaxis of THP-1 monocytes by modulating early growth response-1 transcription factor. J Neurochem 91:1199–1210. https://doi.org/10.1111/j.1471-4159.2004.02800.x

Goel A, Jhurani S, Aggarwal BB (2008) Multi-targeted therapy by curcumin: how spicy is it? Mol Nutr Food Res 52:1010–1030. https://doi.org/10.1002/mnfr.200700354

Golbidi S, Daiber A, Korac B et al (2017) Health benefits of fasting and caloric restriction. Curr Diab Rep 17:123. https://doi.org/10.1007/s11892-017-0951-7

Gomes BAQ, Silva JPB, Romeiro CFR et al (2018) Neuroprotective mechanisms of resveratrol in Alzheimer's disease: role of SIRT1. Oxid Med Cell Longev 2018:8152373. https://doi.org/10.1155/2018/8152373

Gonos ES, Kapetanou M, Sereikaite J et al (2018) Origin and pathophysiology of protein carbonylation, nitration and chlorination in age-related brain diseases and aging. Aging (Albany NY) 10:868–901. https://doi.org/10.18632/aging.101450

Good CK, Holschuh N, Albertson AM, Eldridge AL (2008) Whole grain consumption and body mass index in adult women: an analysis of NHANES 1999-2000 and the USDA pyramid servings database. J Am Coll Nutr 27:80–87. https://doi.org/10.1080/07315724.2008.10719678

Gordon K, Blondin DP, Friesen BJ et al (2019) Seven days of cold acclimation substantially reduces shivering intensity and increases nonshivering thermogenesis in adult humans. J Appl Physiol 126:1598–1606. https://doi.org/10.1152/japplphysiol.01133.2018

Grabowska W, Sikora E, Bielak-Zmijewska A (2017) Sirtuins, a promising target in slowing down the ageing process. Biogerontology 18:447–476. https://doi.org/10.1007/s10522-017-9685-9

Granado-Serrano AB, Martín MA, Haegeman G et al (2010) Epicatechin induces NF-kappaB, activator protein-1 (AP-1) and nuclear transcription factor erythroid 2p45-related factor-2 (Nrf2) via phosphatidylinositol-3-kinase/protein kinase B (PI3K/AKT) and extracellular regulated kinase (ERK) signalling in HepG2 c. Br J Nutr 103:168–179. https://doi.org/10.1017/S0007114509991747

Grant RW, Dixit VD (2015) Adipose tissue as an immunological organ. Obesity (Silver Spring) 23:512–518. https://doi.org/10.1002/oby.21003

Gregor MF, Hotamisligil GS (2007) Thematic review series: Adipocyte Biology. Adipocyte stress: the endoplasmic reticulum and metabolic disease. J Lipid Res 48:1905–1914. https://doi.org/10.1194/jlr.R700007-JLR200

Gregosa A, Vinuesa Á, Todero MF et al (2019) Periodic dietary restriction ameliorates amyloid pathology and cognitive impairment in PDAPP-J20 mice: potential implication of glial autophagy. Neurobiol Dis 132:104542. https://doi.org/10.1016/j.nbd.2019.104542

Griffin MJ (2022) On the immunometabolic role of NF-κB in adipocytes. Immunometabolism 4. https://doi.org/10.20900/immunometab20220003

Griffioen KJ, Rothman SM, Ladenheim B et al (2013) Dietary energy intake modifies brainstem autonomic dysfunction caused by mutant α-synuclein. Neurobiol Aging 34:928–935. https://doi.org/10.1016/j.neurobiolaging.2012.07.008

Gu J-W, Young E, Patterson SG et al (2011) Postmenopausal obesity promotes tumor angiogenesis and breast cancer progression in mice. Cancer Biol Ther 11:910–917. https://doi.org/10.4161/cbt.11.10.15473

Guasch-Ferré M, Willett WC (2021) The Mediterranean diet and health: a comprehensive overview. J Intern Med 290:549–566. https://doi.org/10.1111/joim.13333

Guerre-Millo M (2004) Adipose tissue and adipokines: for better or worse. Diabetes Metab 30:13–19. https://doi.org/10.1016/s1262-3636(07)70084-8

Guerrero-Romero F, Rodriguez-Moran M (2002) Low serum magnesium levels and metabolic syndrome. Acta Diabetol 39:209–213. https://doi.org/10.1007/s005920200036

Gülçin İ (2010) Antioxidant properties of resveratrol: a structure–activity insight. Innov food Sci Emerg Technol 11:210–218. https://doi.org/10.1016/j.ifset.2009.07.002

Guo T, Zhang D, Zeng Y et al (2020) Molecular and cellular mechanisms underlying the pathogenesis of Alzheimer's disease. Mol Neurodegener 15:40. https://doi.org/10.1186/s13024-020-00391-7

Gupta SC, Sung B, Kim JH et al (2013) Multitargeting by turmeric, the golden spice: From kitchen to clinic. Mol Nutr Food Res 57:1510–1528. https://doi.org/10.1002/mnfr.201100741

Gupta SK, Trivedi D, Srivastava S et al (2003) Lycopene attenuates oxidative stress induced experimental cataract development: an in *vitro* and *in vivo* study. Nutrition 19:794–799. https://doi.org/10.1016/s0899-9007(03)00140-0

Haczeyni F, Bell-Anderson KS, Farrell GC (2018) Causes and mechanisms of adipocyte enlargement and adipose expansion. Obes Rev Off J Int Assoc Study Obes 19:406–420. https://doi.org/10.1111/obr.12646

Hagl S, Kocher A, Schiborr C et al (2015) Curcumin micelles improve mitochondrial function in neuronal PC12 cells and brains of NMRI mice – impact on bioavailability. Neurochem Int 89:234–242. https://doi.org/10.1016/j.neuint.2015.07.026

Halagappa VKM, Guo Z, Pearson M et al (2007) Intermittent fasting and caloric restriction ameliorate age-related behavioral deficits in the triple-transgenic mouse model of Alzheimer's disease. Neurobiol Dis 26:212–220. https://doi.org/10.1016/j.nbd.2006.12.019

Halliwell B, Gutteridge JM (1990) Role of free radicals and catalytic metal ions in human disease: an overview. Methods Enzymol 186:1–85. https://doi.org/10.1016/0076-6879(90)86093-b

Hamaguchi T, Ono K, Yamada M (2010) REVIEW: Curcumin and Alzheimer's disease. CNS Neurosci Ther 16:285–297. https://doi.org/10.1111/j.1755-5949.2010.00147.x

Han Y-M, Ramprasath T, Zou M-H (2020) β-hydroxybutyrate and its metabolic effects on age-associated pathology. Exp Mol Med 52:548–555

Haque AM, Hashimoto M, Katakura M et al (2006) Long-term administration of green tea catechins improves spatial cognition learning ability in rats. J Nutr 136:1043–1047. https://doi.org/10.1093/jn/136.4.1043

Haring B, Wu C, Coker LH et al (2016) Hypertension, dietary sodium, and cognitive decline: results from the women's health initiative memory study. Am J Hypertens 29:202–216. https://doi.org/10.1093/ajh/hpv081

Harman D (2001) Aging: overview. Ann N Y Acad Sci 928:1–21. https://doi.org/10.1111/j.1749-6632.2001.tb05631.x

Harman D (1956) Aging: a theory based on free radical and radiation chemistry. J Gerontol 11:298–300. https://doi.org/10.1093/geronj/11.3.298

Harman D (1972) The biologic clock: the mitochondria? J Am Geriatr Soc 20:145–147. https://doi.org/10.1111/j.1532-5415.1972.tb00787.x

Harper S (2014) Economic and social implications of aging societies. Science 346:587–591. https://doi.org/10.1126/science.1254405

Harrison DE, Strong R, Sharp ZD et al (2009) Rapamycin fed late in life extends lifespan in genetically heterogeneous mice. Nature 460:392–395. https://doi.org/10.1038/nature08221

Harwood M, Danielewska-Nikiel B, Borzelleca JF et al (2007) A critical review of the data related to the safety of quercetin and lack of evidence of *in vivo* toxicity, including lack of genotoxic/carcinogenic properties. Food Chem Toxicol 45:2179–2205. https://doi.org/10.1016/j.fct.2007.05.015

Hasty P (2005) The impact of DNA damage, genetic mutation and cellular responses on cancer prevention, longevity and aging: observations in humans and mice. Mech Ageing Dev 126:71–77. https://doi.org/10.1016/j.mad.2004.09.036

Hasty P, Campisi J, Hoeijmakers J et al (2003) Aging and genome maintenance: lessons from the mouse? Science 299:1355–1359. https://doi.org/10.1126/science.1079161

Hatcher H, Planalp R, Cho J et al (2008) Curcumin: from ancient medicine to current clinical trials. Cell Mol Life Sci 65:1631–1652. https://doi.org/10.1007/s00018-008-7452-4

Hatcher HC, Singh RN, Torti FM, Torti SV (2009) Synthetic and natural iron chelators: therapeutic potential and clinical use. Future Med Chem 1:1643–1670. https://doi.org/10.4155/fmc.09.121

Hausman DB, DiGirolamo M, Bartness TJ et al (2001) The biology of white adipocyte proliferation. Obes Rev Off J Int Assoc Study Obes 2:239–254. https://doi.org/10.1046/j.1467-789x.2001.00042.x

Hausman GJ, Richardson RL (2004) Adipose tissue angiogenesis. J Anim Sci 82:925–934. https://doi.org/10.2527/2004.823925x

Haycock PC, Heydon EE, Kaptoge S et al (2014) Leucocyte telomere length and risk of cardiovascular disease: systematic review and meta-analysis. BMJ 349:g4227. https://doi.org/10.1136/bmj.g4227

Hayflick L (1965) The limited in vitro lifetime of human diploid cell strains. Exp Cell Res 37:614–636. https://doi.org/10.1016/0014-4827(65)90211-9

Haynes CM, Ron D (2010) The mitochondrial UPR – protecting organelle protein homeostasis. J Cell Sci 123:3849–3855. https://doi.org/10.1242/jcs.075119

Heebkaew N, Rujanapun N, Kunhorm P et al (2019) Curcumin induces neural differentiation of human pluripotent embryonal carcinoma cells through the activation of autophagy. Biomed Res Int 2019:4378710. https://doi.org/10.1155/2019/4378710

Heilbronn LK, Ravussin E (2003) Calorie restriction and aging: review of the literature and implications for studies in humans. Am J Clin Nutr 78:361–369. https://doi.org/10.1093/ajcn/78.3.361

Heilbronn LK, Smith SR, Martin CK et al (2005) Alternate-day fasting in nonobese subjects: effects on body weight, body composition, and energy metabolism. Am J Clin Nutr 81:69–73. https://doi.org/10.1093/ajcn/81.1.69

Heneka MT, O'Banion MK, Terwel D, Kummer MP (2010) Neuroinflammatory processes in Alzheimer's disease. J Neural Transm 117:919–947. https://doi.org/10.1007/s00702-010-0438-z

Hernandez-Segura A, Nehme J, Demaria M (2018) Hallmarks of cellular senescence. Trends Cell Biol 28:436–453. https://doi.org/10.1016/j.tcb.2018.02.001

Herriot AM, Whitcroft S, Jeanes Y (2008) An retrospective audit of patients with polycystic ovary syndrome: the effects of a reduced glycaemic load diet. J Hum Nutr Diet 21:337–345. https://doi.org/10.1111/j.1365-277X.2008.00890.x

Hill JO, Wyatt HR, Peters JC (2012) Energy balance and obesity. Circulation 126:126–132. https://doi.org/10.1161/CIRCULATIONAHA.111.087213

Hoeijmakers JHJ (2009) DNA damage, aging, and cancer. N Engl J Med 361:1475–1485. https://doi.org/10.1056/NEJMra0804615

Højlund K, Mogensen M, Sahlin K, Beck-Nielsen H (2008) Mitochondrial dysfunction in type 2 diabetes and obesity. Endocrinol Metab Clin North Am 37:713–731, x. https://doi.org/10.1016/j.ecl.2008.06.006

Holmes-McNary M, Baldwin ASJ (2000) Chemopreventive properties of trans-resveratrol are associated with inhibition of activation of the IkappaB kinase. Cancer Res 60:3477–3483

Horvath S (2013) DNA methylation age of human tissues and cell types. Genome Biol 14:R115. https://doi.org/10.1186/gb-2013-14-10-r115

Horvath S, Erhart W, Brosch M et al (2014) Obesity accelerates epigenetic aging of human liver. Proc Natl Acad Sci U S A 111:15538–15543. https://doi.org/10.1073/pnas.1412759111

Horvath S, Raj K (2018) DNA methylation-based biomarkers and the epigenetic clock theory of ageing. Nat Rev Genet 19:371–384. https://doi.org/10.1038/s41576-018-0004-3

Hosking DE, Eramudugolla R, Cherbuin N, Anstey KJ (2019) MIND not Mediterranean diet related to 12-year incidence of cognitive impairment in an Australian longitudinal cohort study. Alzheimers Dement 15:581–589. https://doi.org/10.1016/j.jalz.2018.12.011

Hotamisligil GS, Arner P, Caro JF et al (1995) Increased adipose tissue expression of tumor necrosis factor-alpha in human obesity and insulin resistance. J Clin Invest 95:2409–2415. https://doi.org/10.1172/JCI117936

Howitz KT, Bitterman KJ, Cohen HY et al (2003) Small molecule activators of sirtuins extend *Saccharomyces cerevisiae* lifespan. Nature 425:191–196. https://doi.org/10.1038/nature01965.1

Huang CL, Sumpio BE (2008) Olive oil, the mediterranean diet, and cardiovascular health. J Am Coll Surg 207:407–416. https://doi.org/10.1016/j.jamcollsurg.2008.02.018

Huang EJ, Reichardt LF (2003) Trk receptors: roles in neuronal signal transduction. Annu Rev Biochem 72:609–642. https://doi.org/10.1146/annurev.biochem.72.121801.161629

Huang MT, Lysz T, Ferraro T et al (1991) Inhibitory effects of curcumin on in vitro lipoxygenase and cyclooxygenase activities in mouse epidermis. Cancer Res 51:813–819

Huang X-F, Li H-Q, Shi L et al (2008) Synthesis of resveratrol analogues, and evaluation of their cytotoxic and xanthine oxidase inhibitory activities. Chem Biodivers 5:636–642. https://doi.org/10.1002/cbdv.200890059

Hursel R, Westerterp-Plantenga MS (2010) Thermogenic ingredients and body weight regulation. Int J Obes (Lond) 34:659–669. https://doi.org/10.1038/ijo.2009.299

Ide K, Yamada H, Takuma N et al (2016) Effects of green tea consumption on cognitive dysfunction in an elderly population: a randomized placebo-controlled study. Nutr J 15:49. https://doi.org/10.1186/s12937-016-0168-7

Ikeda H, Tauchi H, Sato T (1985) Fine structural analysis of lipofuscin in various tissues of rats of different ages. Mech Ageing Dev 33:77–93. https://doi.org/10.1016/0047-6374(85)90110-1

Ikeda K, Maretich P, Kajimura S (2018) The common and distinct features of brown and beige adipocytes. Trends Endocrinol Metab 29:191–200. https://doi.org/10.1016/j.tem.2018.01.001

Imai S, Armstrong CM, Kaeberlein M, Guarente L (2000) Transcriptional silencing and longevity protein Sir2 is an NAD-dependent histone deacetylase. Nature 403:795–800. https://doi.org/10.1038/35001622

Imessaoudene A, Merzouk H, Berroukeche F et al (2016) Beneficial effects of quercetin-iron complexes on serum and tissue lipids and redox status in obese rats. J Nutr Biochem 29:107–115. https://doi.org/10.1016/j.jnutbio.2015.11.011

Intra J, Kuo S-M (2007) Physiological levels of tea catechins increase cellular lipid antioxidant activity of vitamin C and vitamin E in human intestinal caco-2 cells. Chem Biol Interact 169:91–99. https://doi.org/10.1016/j.cbi.2007.05.007

Ishrat T, Hoda MN, Khan MB et al (2009) Amelioration of cognitive deficits and neurodegeneration by curcumin in rat model of sporadic dementia of Alzheimer's type (SDAT). Eur Neuropsychopharmacol J Eur Coll Neuropsychopharmacol 19:636–647. https://doi.org/10.1016/j.euroneuro.2009.02.002

Iso H, Date C, Wakai K et al (2006) The relationship between green tea and total caffeine intake and risk for self-reported type 2 diabetes among Japanese adults. Ann Intern Med 144:554–562. https://doi.org/10.7326/0003-4819-144-8-200604180-00005

Izquierdo V, Palomera-Ávalos V, López-Ruiz S et al (2019) Maternal resveratrol supplementation prevents cognitive decline in senescent mice offspring. Int J Mol Sci 20. https://doi.org/10.3390/ijms20051134

Jacobsen MJ, Mentzel CMJ, Olesen AS et al (2016) Altered methylation profile of lymphocytes is concordant with perturbation of lipids metabolism and inflammatory response in obesity. J Diabetes Res 2016:8539057. https://doi.org/10.1155/2016/8539057

Jagust W, Harvey D, Mungas D, Haan M (2005) Central obesity and the aging brain. Arch Neurol 62:1545–1548. https://doi.org/10.1001/archneur.62.10.1545

Jamshed H, Sultan FAT, Iqbal R, Gilani AH (2015) Dietary almonds increase serum HDL cholesterol in coronary artery disease patients in a randomized controlled trial. J Nutr 145:2287–2292. https://doi.org/10.3945/jn.114.207944

Janssen I, Fortier A, Hudson R, Ross R (2002) Effects of an energy-restrictive diet with or without exercise on abdominal fat, intermuscular fat, and metabolic risk factors in obese women. Diabetes Care 25:431–438. https://doi.org/10.2337/diacare.25.3.431

Jeon OH, Kim C, Laberge R-M et al (2017) Local clearance of senescent cells attenuates the development of post-traumatic osteoarthritis and creates a pro-regenerative environment. Nat Med 23:775–781. https://doi.org/10.1038/nm.4324

Ji LL, Kang C (2015) Role of PGC-1α in sarcopenia: etiology and potential intervention – a mini-review. Gerontology 61:139–148. https://doi.org/10.1159/000365947

Jia N, Han K, Kong J-J et al (2013) (-)-Epigallocatechin-3-gallate alleviates spatial memory impairment in APP/PS1 mice by restoring IRS-1 signaling defects in the hippocampus. Mol Cell Biochem 380:211–218. https://doi.org/10.1007/s11010-013-1675-x

Jiang JC, Jaruga E, Repnevskaya MV, Jazwinski SM (2000) An intervention resembling caloric restriction prolongs life span and retards aging in yeast. FASEB J 14:2135–2137. https://doi.org/10.1096/fj.00-0242fje

Jiao N, Baker SS, Nugent CA et al (2018) Gut microbiome may contribute to insulin resistance and systemic inflammation in obese rodents: a meta-analysis. Physiol Genomics 50:244–254. https://doi.org/10.1152/physiolgenomics.00114.2017

Jimenez-Del-Rio M, Guzman-Martinez C, Velez-Pardo C (2010) The effects of polyphenols on survival and locomotor activity in *Drosophila melanogaster* exposed to iron and paraquat. Neurochem Res 35:227–238. https://doi.org/10.1007/s11064-009-0046-1

Jin F, Wu Q, Lu Y-F et al (2008) Neuroprotective effect of resveratrol on 6-OHDA-induced Parkinson's disease in rats. Eur J Pharmacol 600:78–82. https://doi.org/10.1016/j.ejphar.2008.10.005

Johnson SC, Rabinovitch PS, Kaeberlein M (2013a) mTOR is a key modulator of ageing and age-related disease. Nature 493:338–345. https://doi.org/10.1038/nature11861

Johnson SC, Yanos ME, Kayser E-B et al (2013b) mTOR inhibition alleviates mitochondrial disease in a mouse model of Leigh syndrome. Science 342:1524–1528. https://doi.org/10.1126/science.1244360

Johnson TE, Henderson S, Murakami S et al (2002) Longevity genes in the nematode *Caenorhabditis elegans* also mediate increased resistance to stress and prevent disease. J Inherit Metab Dis 25:197–206. https://doi.org/10.1023/A:1015677828407

Jones E, Hughes RE (1982) Quercetin, flavonoids and the life-span of mice. Exp Gerontol 17:213–217. https://doi.org/10.1016/0531-5565(82)90027-4

Joseph JA, Shukitt-Hale B, Denisova NA et al (1999) Reversals of age-related declines in neuronal signal transduction, cognitive, and motor behavioral deficits with blueberry, spinach, or strawberry dietary supplementation. J Neurosci 19:8114–8121. https://doi.org/10.1523/JNEUROSCI.19-18-08114.1999

Jovaisaite V, Mouchiroud L, Auwerx J (2014) The mitochondrial unfolded protein response, a conserved stress response pathway with implications in health and disease. J Exp Biol 217:137–143. https://doi.org/10.1242/jeb.090738

Juan ME, Wenzel U, Daniel H, Planas JM (2008) Resveratrol induces apoptosis through ROS-dependent mitochondria pathway in HT-29 human colorectal carcinoma cells. J Agric Food Chem 56:4813–4818. https://doi.org/10.1021/jf800175a

Jurk D, Wilson C, Passos JF et al (2014) Chronic inflammation induces telomere dysfunction and accelerates ageing in mice. Nat Commun 2:4172. https://doi.org/10.1038/ncomms5172

Kaeberlein TL, Smith ED, Tsuchiya M et al (2006) Lifespan extension in *Caenorhabditis elegans* by complete removal of food. Aging Cell 5:487–494. https://doi.org/10.1111/j.1474-9726.2006.00238.x

Kajimura S, Spiegelman BM, Seale P (2015) Brown and beige fat: physiological roles beyond heat generation. Cell Metab 22:546–559. https://doi.org/10.1016/j.cmet.2015.09.007

Kalia LV, Lang AE (2015) Parkinson's disease. Lancet (London) 386:896–912. https://doi.org/10.1016/S0140-6736(14)61393-3

Kamil A, Chen C-YO (2012) Health benefits of almonds beyond cholesterol reduction. J Agric Food Chem 60:6694–6702. https://doi.org/10.1021/jf2044795

Kampkötter A, Nkwonkam CG, Zurawski RF et al (2007) Investigations of protective effects of the flavonoids quercetin and rutin on stress resistance in the model organism *Caenorhabditis elegans*. Toxicology 234:113–123. https://doi.org/10.1016/j.tox.2007.02.006

Kampkötter A, Timpel C, Zurawski RF et al (2008) Increase of stress resistance and lifespan of *Caenorhabditis elegans* by quercetin. Comp Biochem Physiol B Biochem Mol Biol 149:314–323. https://doi.org/10.1016/j.cbpb.2007.10.004

Kanda H, Tateya S, Tamori Y et al (2006) MCP-1 contributes to macrophage infiltration into adipose tissue, insulin resistance, and hepatic steatosis in obesity. J Clin Invest 116:1494–1505. https://doi.org/10.1172/JCI26498

Kang JH, Ascherio A, Grodstein F (2005) Fruit and vegetable consumption and cognitive decline in aging women. Ann Neurol 57:713–720. https://doi.org/10.1002/ana.20476

Kao WHL, Folsom AR, Nieto FJ et al (1999) Serum and dietary magnesium and the risk for type 2 diabetes mellitus: the Atherosclerosis Risk in Communities Study. Arch Intern Med 159:2151–2159. https://doi.org/10.1001/archinte.159.18.2151

Kao YH, Hiipakka RA, Liao S (2000) Modulation of endocrine systems and food intake by green tea epigallocatechin gallate. Endocrinology 141:980–987. https://doi.org/10.1210/endo.141.3.7368

Kapahi P, Zid BM, Harper T et al (2004) Regulation of lifespan in *Drosophila* by modulation of genes in the TOR signaling pathway. Curr Biol 14:885–890. https://doi.org/10.1016/j.cub.2004.03.059

Karkeni E, Astier J, Tourniaire F et al (2016) Obesity-associated inflammation induces microRNA-155 expression in adipocytes and adipose tissue: outcome on adipocyte function. J Clin Endocrinol Metab 101:1615–1626. https://doi.org/10.1210/jc.2015-3410

Karppi J, Laukkanen JA, Sivenius J et al (2012) Serum lycopene decreases the risk of stroke in men: a population-based follow-up study. Neurology 79:1540–1547. https://doi.org/10.1212/WNL.0b013e31826e26a6

Kashiwaya Y, Takeshima T, Mori N et al (2000) d-β-Hydroxybutyrate protects neurons in models of Alzheimer's and Parkinson's disease. Proc Natl Acad Sci 97:5440–5444. https://doi.org/10.1073/pnas.97.10.5440

Kealy RD, Lawler DF, Ballam JM et al (2002) Effects of diet restriction on life span and age-related changes in dogs. J Am Vet Med Assoc 220:1315–1320. https://doi.org/10.2460/javma.2002.220.1315

Keaney JFJ, Larson MG, Vasan RS et al (2003) Obesity and systemic oxidative stress: clinical correlates of oxidative stress in the Framingham Study. Arterioscler Thromb Vasc Biol 23:434–439. https://doi.org/10.1161/01.ATV.0000058402.34138.11

Keller MP, Attie AD (2010) Physiological insights gained from gene expression analysis in obesity and diabetes. Annu Rev Nutr 30:341–364. https://doi.org/10.1146/annurev.nutr.012809.104747

Kennedy BK, Berger SL, Brunet A et al (2014) Geroscience: linking aging to chronic disease. Cell 159:709–713. https://doi.org/10.1016/j.cell.2014.10.039

Kenyon C (2005) The plasticity of aging: insights from long-lived mutants. Cell 120:449–460. https://doi.org/10.1016/j.cell.2005.02.002

Keogh A, Richardson M, Ruygrok P et al (2004) Sirolimus in de novo heart transplant recipients reduces acute rejection and prevents coronary artery disease at 2 years: a randomized clinical trial. Circulation 110:2694–2700. https://doi.org/10.1161/01.CIR.0000136812.90177.94

Kershaw EE, Flier JS (2004) Adipose tissue as an endocrine organ. J Clin Endocrinol Metab 89:2548–2556. https://doi.org/10.1210/jc.2004-0395

Kersten S (2001) Mechanisms of nutritional and hormonal regulation of lipogenesis. EMBO Rep 2:282–286. https://doi.org/10.1093/embo-reports/kve071

Khajavi M, Lupski JR (2008) Balancing between adaptive and maladaptive cellular stress responses in peripheral neuropathy. Neuron 57:329–330. https://doi.org/10.1016/j.neuron.2008.01.017

Khamzina L, Veilleux A, Bergeron S, Marette A (2005) Increased activation of the mammalian target of rapamycin pathway in liver and skeletal muscle of obese rats: possible involvement in obesity-linked insulin resistance. Endocrinology 146:1473–1481. https://doi.org/10.1210/en.2004-0921

Khatri DK, Juvekar AR (2016) Neuroprotective effect of curcumin as evinced by abrogation of rotenone-induced motor deficits, oxidative and mitochondrial dysfunctions in mouse model of Parkinson's disease. Pharmacol Biochem Behav 150–151:39–47. https://doi.org/10.1016/j.pbb.2016.09.002

Khorshidi M, Moini A, Alipoor E et al (2018) The effects of quercetin supplementation on metabolic and hormonal parameters as well as plasma concentration and gene expression of resistin in overweight or obese women with polycystic ovary syndrome. Phytother Res 32:2282–2289. https://doi.org/10.1002/ptr.6166

Kim D, Nguyen MD, Dobbin MM et al (2007) SIRT1 deacetylase protects against neurodegeneration in models for Alzheimer's disease and amyotrophic lateral sclerosis. EMBO J 26:3169–3179. https://doi.org/10.1038/sj.emboj.7601758

Kim E, Ansell CM, Dudycha JL (2014a) Resveratrol and food effects on lifespan and reproduction in the model crustacean Daphnia. J Exp Zool A Ecol Genet Physiol 321:48–56. https://doi.org/10.1002/jez.1836

Kim H-S, Quon MJ, Kim J-A (2014b) New insights into the mechanisms of polyphenols beyond antioxidant properties; lessons from the green tea polyphenol, epigallocatechin 3-gallate. Redox Biol 2:187–195. https://doi.org/10.1016/j.redox.2013.12.022

Kim J-Y, Hong J-H, Jung HK et al (2012) Grape skin and loquat leaf extracts and acai puree have potent anti-atherosclerotic and anti-diabetic activity *in vitro* and *in vivo* in hypercholesterolemic zebrafish. Int J Mol Med 30:606–614. https://doi.org/10.3892/ijmm.2012.1045

Kim J, Kim J, Huang Z et al (2019) Theracurmin ameliorates cognitive dysfunctions in 5XFAD mice by improving synaptic function and mitigating oxidative stress. Biomol Ther (Seoul) 27: 327–335. https://doi.org/10.4062/biomolther.2019.046

Kim JE, Kim AR, Chung HY et al (2003) In vitro peroxynitrite scavenging activity of diarylheptanoids from Curcuma longa. Phytother Res 17:481–484. https://doi.org/10.1002/ptr.1179

Kim JW, Lim SC, Lee MY et al (2010) Inhibition of neointimal formation by trans-resveratrol: role of phosphatidyl inositol 3-kinase-dependent Nrf2 activation in heme oxygenase-1 induction. Mol Nutr Food Res 54:1497–1505. https://doi.org/10.1002/mnfr.201000016

Kingwell BA, Chapman MJ, Kontush A, Miller NE (2014) HDL-targeted therapies: progress, failures and future. Nat Rev Drug Discov 13:445–464. https://doi.org/10.1038/nrd4279

Kirkwood TBL (2005) Understanding the odd science of aging. Cell 120:437–447. https://doi.org/10.1016/j.cell.2005.01.027

Kirkwood TBL, Melov S (2011) On the programmed/non-programmed nature of ageing within the life history. Curr Biol 21:R701–R707. https://doi.org/10.1016/j.cub.2011.07.020

Kitani K, Osawa T, Yokozawa T (2007) The effects of tetrahydrocurcumin and green tea polyphenol on the survival of male C57BL/6 mice. Biogerontology 8:567–573. https://doi.org/10.1007/s10522-007-9100-z

Klempel MC, Kroeger CM, Bhutani S et al (2012) Intermittent fasting combined with calorie restriction is effective for weight loss and cardio-protection in obese women. Nutr J 11:98. https://doi.org/10.1186/1475-2891-11-98

Kobayashi A, Kang M-I, Okawa H et al (2004) Oxidative stress sensor Keap1 functions as an adaptor for Cul3-based E3 ligase to regulate proteasomal degradation of Nrf2. Mol Cell Biol 24: 7130–7139. https://doi.org/10.1128/MCB.24.16.7130-7139.2004

Köbe T, Witte AV, Schnelle A et al (2017) Impact of resveratrol on glucose control, hippocampal structure and connectivity, and memory performance in patients with mild cognitive impairment. Front Neurosci 11:105. https://doi.org/10.3389/fnins.2017.00105

Koch M, Jensen MK (2016) Association of the MIND diet with cognition and risk of Alzheimer's disease. Curr Opin Lipidol 27:303–304. https://doi.org/10.1097/MOL.0000000000000304

Kodali M, Parihar VK, Hattiangady B et al (2015) Resveratrol prevents age-related memory and mood dysfunction with increased hippocampal neurogenesis and microvasculature, and reduced glial activation. Sci Rep 5:8075. https://doi.org/10.1038/srep08075

Kode A, Rajendrasozhan S, Caito S et al (2008) Resveratrol induces glutathione synthesis by activation of Nrf2 and protects against cigarette smoke-mediated oxidative stress in human lung epithelial cells. Am J Physiol Lung Cell Mol Physiol 294:L478–L488. https://doi.org/10.1152/ajplung.00361.2007

Koh EH, Park J-Y, Park H-S et al (2007) Essential role of mitochondrial function in adiponectin synthesis in adipocytes. Diabetes 56:2973–2981. https://doi.org/10.2337/db07-0510

Kolb R, Kluz P, Tan ZW et al (2019) Obesity-associated inflammation promotes angiogenesis and breast cancer via angiopoietin-like 4. Oncogene 38:2351–2363. https://doi.org/10.1038/s41388-018-0592-6

Kolb R, Phan L, Borcherding N et al (2016) Obesity-associated NLRC4 inflammasome activation drives breast cancer progression. Nat Commun 7:1–12. https://doi.org/10.1038/ncomms13007

Kolosova NG, Muraleva NA, Zhdankina AA et al (2012) Prevention of age-related macular degeneration-like retinopathy by rapamycin in rats. Am J Pathol 181:472–477. https://doi.org/10.1016/j.ajpath.2012.04.018

Kopelman PG (2000) Obesity as a medical problem. Nature 404:635–643. https://doi.org/10.1038/35007508

Kounatidis I, Chtarbanova S, Cao Y et al (2017) NF-κB immunity in the brain determines fly lifespan in healthy aging and age-related neurodegeneration. Cell Rep 19:836–848. https://doi.org/10.1016/j.celrep.2017.04.007

Kratz M, Coats BR, Hisert KB et al (2014) Metabolic dysfunction drives a mechanistically distinct proinflammatory phenotype in adipose tissue macrophages. Cell Metab 20:614–625. https://doi.org/10.1016/j.cmet.2014.08.010

Krikorian R, Nash TA, Shidler MD et al (2010a) Concord grape juice supplementation improves memory function in older adults with mild cognitive impairment. Br J Nutr 103:730–734. https://doi.org/10.1017/S0007114509992364

Krikorian R, Shidler MD, Nash TA et al (2010b) Blueberry supplementation improves memory in older adults. J Agric Food Chem 58:3996–4000. https://doi.org/10.1021/jf9029332

Kris-Etherton P, Eckel RH, Howard BV et al (2001) AHA Science Advisory: Lyon Diet Heart Study. Benefits of a Mediterranean-style, National Cholesterol Education Program/American Heart Association Step I Dietary Pattern on Cardiovascular Disease. Circulation 103:1823–1825. https://doi.org/10.1161/01.cir.103.13.1823

Kuilman T, Michaloglou C, Mooi WJ, Peeper DS (2010) The essence of senescence. Genes Dev 24:2463–2479. https://doi.org/10.1101/gad.1971610

Kuilman T, Peeper DS (2009) Senescence-messaging secretome: SMS-ing cellular stress. Nat Rev Cancer 9:81–94. https://doi.org/10.1038/nrc2560

Kumar D, Dwivedi DK, Lahkar M, Jangra A (2019) Hepatoprotective potential of 7,8-Dihydroxyflavone against alcohol and high-fat diet induced liver toxicity via attenuation of oxido-nitrosative stress and NF-κB activation. Pharmacol Rep 71:1235–1243. https://doi.org/10.1016/j.pharep.2019.07.002

Kumar S, Lombard DB (2015) Mitochondrial sirtuins and their relationships with metabolic disease and cancer. Antioxid Redox Signal 22:1060–1077. https://doi.org/10.1089/ars.2014.6213

Kusminski CM, Scherer PE (2012) Mitochondrial dysfunction in white adipose tissue. Trends Endocrinol Metab 23:435–443. https://doi.org/10.1016/j.tem.2012.06.004

Kuszel L, Trzeciak T, Richter M, Czarny-Ratajczak M (2015) Osteoarthritis and telomere shortening. J Appl Genet 56:169–176. https://doi.org/10.1007/s13353-014-0251-8

Labbadia J, Morimoto RI (2014) Proteostasis and longevity: when does aging really begin? F1000Prime Rep 6:7. https://doi.org/10.12703/P6-7

Labbadia J, Morimoto RI (2015) The biology of proteostasis in aging and disease. Annu Rev Biochem 84:435–464. https://doi.org/10.1146/annurev-biochem-060614-033955

Lagger C, Ursu E, Equey A et al (2021) scAgeCom: a murine atlas of age-related changes in intercellular communication inferred with the package scDiffCom. bioRxiv 2021.08.13.456238. https://doi.org/10.1101/2021.08.13.456238

Lagouge M, Argmann C, Gerhart-Hines Z et al (2006) Resveratrol improves mitochondrial function and protects against metabolic disease by activating SIRT1 and PGC-1alpha. Cell 127:1109–1122. https://doi.org/10.1016/j.cell.2006.11.013

Lairon D, Arnault N, Bertrais S et al (2005) Dietary fiber intake and risk factors for cardiovascular disease in French adults. Am J Clin Nutr 82:1185–1194. https://doi.org/10.1093/ajcn/82.6.1185

Lamming DW, Sabatini DM (2013) A central role for mTOR in lipid homeostasis. Cell Metab 18:465–469. https://doi.org/10.1016/j.cmet.2013.08.002

Lanza IR, Nair KS (2010) Mitochondrial function as a determinant of life span. Pflugers Arch 459:277–289. https://doi.org/10.1007/s00424-009-0724-5

Lanza IR, Zabielski P, Klaus KA et al (2012) Chronic caloric restriction preserves mitochondrial function in senescence without increasing mitochondrial biogenesis. Cell Metab 16:777–788. https://doi.org/10.1016/j.cmet.2012.11.003

Laplante M, Sabatini DM (2012) mTOR signaling in growth control and disease. Cell 149:274–293. https://doi.org/10.1016/j.cell.2012.03.017

Larsen PL (1993) Aging and resistance to oxidative damage in *Caenorhabditis elegans*. Proc Natl Acad Sci U S A 90:8905–8909. https://doi.org/10.1073/pnas.90.19.8905

Lau FC, Bielinski DF, Joseph JA (2007) Inhibitory effects of blueberry extract on the production of inflammatory mediators in lipopolysaccharide-activated BV2 microglia. J Neurosci Res 85: 1010–1017. https://doi.org/10.1002/jnr.21205

Leduc-Gaudet J-P, Picard M, St-Jean Pelletier F et al (2015) Mitochondrial morphology is altered in atrophied skeletal muscle of aged mice. Oncotarget 6:17923–17937. https://doi.org/10.18632/oncotarget.4235

Lee CJ, Sears CL, Maruthur N (2020) Gut microbiome and its role in obesity and insulin resistance. Ann N Y Acad Sci 1461:37–52. https://doi.org/10.1111/nyas.14107

Lee GD, Wilson MA, Zhu M et al (2006) Dietary deprivation extends lifespan in *Caenorhabditis elegans*. Aging Cell 5:515–524. https://doi.org/10.1111/j.1474-9726.2006.00241.x

Lee HJ, Yang SJ (2017) Aging-related correlation between serum sirtuin 1 activities and basal metabolic rate in women, but not in men. Clin Nutr Res 6:18–26. https://doi.org/10.7762/cnr.2017.6.1.18

Lee J-G, Yon J-M, Lin C et al (2012) Combined treatment with capsaicin and resveratrol enhances neuroprotection against glutamate-induced toxicity in mouse cerebral cortical neurons. Food Chem Toxicol 50:3877–3885. https://doi.org/10.1016/j.fct.2012.08.040

Lee K-S, Lee B-S, Semnani S et al (2010) Curcumin extends life span, improves health span, and modulates the expression of age-associated aging genes in *Drosophila melanogaster*. Rejuvenation Res 13:561–570. https://doi.org/10.1089/rej.2010.1031

Lee M, Martin H, Firpo MA, Demerath EW (2011) Inverse association between adiposity and telomere length: the Fels Longitudinal Study. Am J Hum Biol 23:100–106. https://doi.org/10.1002/ajhb.21109

Lee YH, Nair S, Rousseau E et al (2005) Microarray profiling of isolated abdominal subcutaneous adipocytes from obese vs non-obese Pima Indians: increased expression of inflammation-related genes. Diabetologia 48:1776–1783. https://doi.org/10.1007/s00125-005-1867-3

Lehtonen H-M, Suomela J-P, Tahvonen R et al (2011) Different berries and berry fractions have various but slightly positive effects on the associated variables of metabolic diseases on overweight and obese women. Eur J Clin Nutr 65:394–401. https://doi.org/10.1038/ejcn.2010.268

Leonard SS, Xia C, Jiang B-H et al (2003) Resveratrol scavenges reactive oxygen species and effects radical-induced cellular responses. Biochem Biophys Res Commun 309:1017–1026. https://doi.org/10.1016/j.bbrc.2003.08.105

Leung CW, Fung TT, McEvoy CT et al (2018) Diet quality indices and leukocyte telomere length among Healthy US adults: data from the National Health and Nutrition Examination Survey, 1999–2002. Am J Epidemiol 187:2192–2201. https://doi.org/10.1093/aje/kwy124

Levin J, Maaß S, Schuberth M et al (2019) Safety and efficacy of epigallocatechin gallate in multiple system atrophy (PROMESA): a randomised, double-blind, placebo-controlled trial. Lancet Neurol 18:724–735. https://doi.org/10.1016/S1474-4422(19)30141-3

Levine ME, Lu AT, Quach A et al (2018) An epigenetic biomarker of aging for lifespan and healthspan. Aging (Albany NY) 10:573–591. https://doi.org/10.18632/aging.101414

Levy MZ, Allsopp RC, Futcher AB et al (1992) Telomere end-replication problem and cell aging. J Mol Biol 225:951–960. https://doi.org/10.1016/0022-2836(92)90096-3

Leyva-Soto A, Alejandra Chavez-Santoscoy R, Porras O et al (2021) Epicatechin and quercetin exhibit in vitro antioxidant effect, improve biochemical parameters related to metabolic syndrome, and decrease cellular genotoxicity in humans. Food Res Int 142:110101. https://doi.org/10.1016/j.foodres.2020.110101

Li C, Zhang W-J, Frei B (2016a) Quercetin inhibits LPS-induced adhesion molecule expression and oxidant production in human aortic endothelial cells by p38-mediated Nrf2 activation and antioxidant enzyme induction. Redox Biol 9:104–113. https://doi.org/10.1016/j.redox.2016.06.006

Li F, Gao C, Yan P et al (2018) EGCG reduces obesity and white adipose tissue gain partly through AMPK activation in mice. Front Pharmacol 9:1366. https://doi.org/10.3389/fphar.2018.01366

Li H, Qi Y, Jasper H (2016b) Preventing age-related decline of gut compartmentalization limits microbiota dysbiosis and extends lifespan. Cell Host Microbe 19:240–253. https://doi.org/10.1016/j.chom.2016.01.008

Li RW, Douglas TD, Maiyoh GK et al (2006) Green tea leaf extract improves lipid and glucose homeostasis in a fructose-fed insulin-resistant hamster model. J Ethnopharmacol 104:24–31. https://doi.org/10.1016/j.jep.2005.08.045

Li S, Bouzar C, Cottet-Rousselle C et al (2016c) Resveratrol inhibits lipogenesis of 3T3-L1 and SGBS cells by inhibition of insulin signaling and mitochondrial mass increase. Biochim Biophys Acta 1857:643–652. https://doi.org/10.1016/j.bbabio.2016.03.009

Liao VH-C, Yu C-W, Chu Y-J et al (2011) Curcumin-mediated lifespan extension in *Caenorhabditis elegans*. Mech Ageing Dev 132:480–487. https://doi.org/10.1016/j.mad.2011.07.008

Liese AD, Roach AK, Sparks KC et al (2003) Whole-grain intake and insulin sensitivity: the Insulin Resistance Atherosclerosis Study. Am J Clin Nutr 78:965–971. https://doi.org/10.1093/ajcn/78.5.965

Lim GP, Chu T, Yang F et al (2001) The curry spice curcumin reduces oxidative damage and amyloid pathology in an Alzheimer transgenic mouse. J Neurosci Off J Soc Neurosci 21:8370–8377. https://doi.org/10.1523/JNEUROSCI.21-21-08370.2001

Lin B-H, Morrison RM (2002) Higher fruit consumption linked with lower body mass index. Food Rev 25:28–32. https://doi.org/10.22004/ag.econ.234628

Lin B-W, Gong C-C, Song H-F, Cui Y-Y (2017) Effects of anthocyanins on the prevention and treatment of cancer. Br J Pharmacol 174:1226–1243. https://doi.org/10.1111/bph.13627

Lin D, Chun T-H, Kang L (2016) Adipose extracellular matrix remodelling in obesity and insulin resistance. Biochem Pharmacol 119:8–16. https://doi.org/10.1016/j.bcp.2016.05.005

Lin MT, Beal MF (2006) Mitochondrial dysfunction and oxidative stress in neurodegenerative diseases. Nature 443:787–795. https://doi.org/10.1038/nature05292

Lindinger A, Peterli R, Peters T et al (2010) Mitochondrial DNA content in human omental adipose tissue. Obes Surg 20:84–92. https://doi.org/10.1007/s11695-009-9987-3

Ling L, Mulligan JA, Ouyang Y et al (2020) Obesity-associated adipose stromal cells promote breast cancer invasion through direct cell contact and ECM remodeling. Adv Funct Mater 30. https://doi.org/10.1002/adfm.201910650

Liu B, Page AJ, Hatzinikolas G et al (2019a) Intermittent fasting improves glucose tolerance and promotes adipose tissue remodeling in male mice fed a high-fat diet. Endocrinology 160:169–180. https://doi.org/10.1210/en.2018-00701

Liu B, Page AJ, Hutchison AT et al (2019b) Intermittent fasting increases energy expenditure and promotes adipose tissue browning in mice. Nutrition 66:38–43. https://doi.org/10.1016/j.nut.2019.03.015

Liu C-Y, Huang C-J, Huang L-H et al (2014a) Effects of green tea extract on insulin resistance and glucagon-like peptide 1 in patients with type 2 diabetes and lipid abnormalities: a randomized, double-blinded, and placebo-controlled trial. PLoS One 9:e91163. https://doi.org/10.1371/journal.pone.0091163

Liu D, Wang Z, Gao Z et al (2014b) Effects of curcumin on learning and memory deficits, BDNF, and ERK protein expression in rats exposed to chronic unpredictable stress. Behav Brain Res 271:116–121. https://doi.org/10.1016/j.bbr.2014.05.068

Liu GY, Sabatini DM (2020) mTOR at the nexus of nutrition, growth, ageing and disease. Nat Rev Mol Cell Biol 21:183–203. https://doi.org/10.1038/s41580-019-0199-y

Liu H, Li R, Li W et al (2015) Maternally inherited diabetes is associated with a homoplasmic T10003C mutation in the mitochondrial tRNA(Gly) gene. Mitochondrion 21:49–57. https://doi.org/10.1016/j.mito.2015.01.004

Liu Q, Zhang J (2014) Lipid metabolism in Alzheimer's disease. Neurosci Bull 30:331–345. https://doi.org/10.1007/s12264-013-1410-3

Liu T, Zhang L, Joo D, Sun S-C (2017a) NF-κB signaling in inflammation. Signal Transduct Target Ther 2:17023. https://doi.org/10.1038/sigtrans.2017.23

Liu Y, Chen X, Li J (2017b) Resveratrol protects against oxidized low-density lipoprotein-induced human umbilical vein endothelial cell apoptosis via inhibition of mitochondrial-derived oxidative stress. Mol Med Rep 15:2457–2464. https://doi.org/10.3892/mmr.2017.6304

Liu Y, Fiskum G, Schubert D (2002) Generation of reactive oxygen species by the mitochondrial electron transport chain. J Neurochem 80:780–787. https://doi.org/10.1046/j.0022-3042.2002.00744.x

Lloberas N, Cruzado JM, Franquesa M et al (2006) Mammalian target of rapamycin pathway blockade slows progression of diabetic kidney disease in rats. J Am Soc Nephrol 17:1395–1404. https://doi.org/10.1681/ASN.2005050549

Logue J, Sattar N, Freeman D (2019) Obesity. In: Touyz RM, Delles C (eds) Textbook of vascular medicine. Springer International Publishing, Cham, pp 419–427

Long LH, Clement MV, Halliwell B (2000) Artifacts in cell culture: rapid generation of hydrogen peroxide on addition of (-)-epigallocatechin, (-)-epigallocatechin gallate, (+)-catechin, and quercetin to commonly used cell culture media. Biochem Biophys Res Commun 273:50–53. https://doi.org/10.1006/bbrc.2000.2895

Longo VD, Anderson RM (2022) Nutrition, longevity and disease: from molecular mechanisms to interventions. Cell 185:1455–1470. https://doi.org/10.1016/j.cell.2022.04.002

Lopes HF, Martin KL, Nashar K et al (2003) DASH diet lowers blood pressure and lipid-induced oxidative stress in obesity. Hypertension (Dallas, TX 1979) 41:422–430. https://doi.org/10.1161/01.HYP.0000053450.19998.11

López-Lluch G, Navas P (2016) Calorie restriction as an intervention in ageing. J Physiol 594:2043–2060. https://doi.org/10.1113/JP270543

López-López G, Moreno L, Cogolludo A et al (2004) Nitric oxide (NO) scavenging and NO protecting effects of quercetin and their biological significance in vascular smooth muscle. Mol Pharmacol 65:851–859. https://doi.org/10.1124/mol.65.4.851

López-Otín C, Blasco MA, Partridge L et al (2013) The hallmarks of aging. Cell 153:1194–1217. https://doi.org/10.1016/j.cell.2013.05.039

Lopez T, Schriner SE, Okoro M et al (2014) Green tea polyphenols extend the lifespan of male *Drosophila melanogaster* while impairing reproductive fitness. J Med Food 17:1314–1321. https://doi.org/10.1089/jmf.2013.0190

Lu K-T, Ko M-C, Chen B-Y et al (2008) Neuroprotective effects of resveratrol on MPTP-induced neuron loss mediated by free radical scavenging. J Agric Food Chem 56:6910–6913. https://doi.org/10.1021/jf8007212

Luchsinger JA, Tang M-X, Shea S, Mayeux R (2002) Caloric intake and the risk of Alzheimer disease. Arch Neurol 59:1258–1263. https://doi.org/10.1001/archneur.59.8.1258

Luo C, Wu X-G (2011) Lycopene enhances antioxidant enzyme activities and immunity function in N-methyl-N'-nitro-N-nitrosoguanidine-induced gastric cancer rats. Int J Mol Sci 12:3340–3351. https://doi.org/10.3390/ijms12053340

Luo L, Liu M (2016) Adipose tissue in control of metabolism. J Endocrinol 231:R77–R99. https://doi.org/10.1530/JOE-16-0211

Ma J, Folsom AR, Melnick SL et al (1995) Associations of serum and dietary magnesium with cardiovascular disease, hypertension, diabetes, insulin, and carotid arterial wall thickness: the ARIC study. J Clin Epidemiol 48:927–940. https://doi.org/10.1016/0895-4356(94)00200-a

MacRae SL, Croken MM, Calder RB et al (2015) DNA repair in species with extreme lifespan differences. Aging (Albany NY) 7:1171–1184. https://doi.org/10.18632/aging.100866

Madhira SL, Challa SS, Chalasani M et al (2012) Promise(s) of mesenchymal stem cells as an in vitro model system to depict pre-diabetic/diabetic milieu in WNIN/GR-Ob mutant rats. PLoS One 7:e48061. https://doi.org/10.1371/journal.pone.0048061

Madhira SL, Nappanveethl G, Kodavalla V, Venkatesan V (2011) Comparison of adipocyte-specific gene expression from WNIN/Ob mutant obese rats, lean control, and parental control. Mol Cell Biochem 357:217–225. https://doi.org/10.1007/s11010-011-0892-4

Mair W, Goymer P, Pletcher SD, Partridge L (2003) Demography of dietary restriction and death in *Drosophila*. Science 301:1731–1733. https://doi.org/10.1126/science.1086016

Maiti P, Dunbar GL (2018) Use of curcumin, a natural polyphenol for targeting molecular pathways in treating age-related neurodegenerative diseases. Int J Mol Sci 19. https://doi.org/10.3390/ijms19061637

Majeed Y, Halabi N, Madani AY et al (2021) SIRT1 promotes lipid metabolism and mitochondrial biogenesis in adipocytes and coordinates adipogenesis by targeting key enzymatic pathways. Sci Rep 11:8177. https://doi.org/10.1038/s41598-021-87759-x

Maki KC, Reeves MS, Farmer M et al (2009) Green tea catechin consumption enhances exercise-induced abdominal fat loss in overweight and obese adults. J Nutr 139:264–270. https://doi.org/10.3945/jn.108.098293

Malhotra JD, Kaufman RJ (2007) Endoplasmic reticulum stress and oxidative stress: a vicious cycle or a double-edged sword? Antioxid Redox Signal 9:2277–2293. https://doi.org/10.1089/ars.2007.1782

Mamun MA, Faruk M, Rahman MM et al (2019) High carbohydrate high fat diet induced hepatic steatosis and dyslipidemia were ameliorated by Psidium guajava leaf powder supplementation in rats. Evid Based Complement Alternat Med 2019:1897237. https://doi.org/10.1155/2019/1897237

Mandel SA, Weinreb O, Amit T, Youdim MBH (2012) Molecular mechanisms of the neuroprotective/neurorescue action of multi-target green tea polyphenols. Front Biosci (Schol Ed) 4:581–598. https://doi.org/10.2741/S286

Manna P, Jain SK (2015) Obesity, oxidative stress, adipose tissue dysfunction, and the associated health risks: causes and therapeutic strategies. Metab Syndr Relat Disord 13:423–444. https://doi.org/10.1089/met.2015.0095

Manna SK, Mukhopadhyay A, Aggarwal BB (2000) Resveratrol suppresses TNF-induced activation of nuclear transcription factors NF-kappa B, activator protein-1, and apoptosis: potential role of reactive oxygen intermediates and lipid peroxidation. J Immunol 164:6509–6519. https://doi.org/10.4049/jimmunol.164.12.6509

Manning BD, Toker A (2017) AKT/PKB signaling: navigating the network. Cell 169:381–405. https://doi.org/10.1016/j.cell.2017.04.001

Marchesi JR, Adams DH, Fava F et al (2016) The gut microbiota and host health: a new clinical frontier. Gut 65:330–339. https://doi.org/10.1136/gutjnl-2015-309990

Mariani S, di Giorgio MR, Martini P et al (2018) Inverse association of circulating SIRT1 and adiposity: a study on underweight, normal weight, and obese patients. Front Endocrinol (Lausanne) 9:449. https://doi.org/10.3389/fendo.2018.00449

Martin GM, Oshima J (2000) Lessons from human progeroid syndromes. Nature 408:263–266. https://doi.org/10.1038/35041705

Martínez-Lapiscina EH, Clavero P, Toledo E et al (2013) Mediterranean diet improves cognition: the PREDIMED-NAVARRA randomised trial. J Neurol Neurosurg Psychiatry 84:1318–1325. https://doi.org/10.1136/jnnp-2012-304792

Martínez de Toda I, Vida C, Sanz San Miguel L, De la Fuente M (2019) Function, oxidative, and inflammatory stress parameters in immune cells as predictive markers of lifespan throughout aging. Oxid Med Cell Longev 2019. https://doi.org/10.1155/2019/4574276

Masoro EJ (2006) Role of hormesis in life extension by caloric restriction. Dose Response 5:163–173. https://doi.org/10.2203/dose-response.06-005.Masoro

Massie HR, Aiello VR, Doherty TJ (1984) Dietary vitamin C improves the survival of mice. Gerontology 30:371–375. https://doi.org/10.1159/000212659

Masuda T, Maekawa T, Hidaka K et al (2001) Chemical studies on antioxidant mechanism of curcumin: analysis of oxidative coupling products from curcumin and linoleate. J Agric Food Chem 49:2539–2547. https://doi.org/10.1021/jf001442x

Maswood N, Young J, Tilmont E et al (2004) Caloric restriction increases neurotrophic factor levels and attenuates neurochemical and behavioral deficits in a primate model of Parkinson's disease. Proc Natl Acad Sci U S A 101:18171–18176. https://doi.org/10.1073/pnas.0405831102

Mattson MP, Longo VD, Harvie M (2017) Impact of intermittent fasting on health and disease processes. Ageing Res Rev 39:46–58. https://doi.org/10.1016/j.arr.2016.10.005
Mattson MP, Magnus T (2006) Ageing and neuronal vulnerability. Nat Rev Neurosci 7:278–294. https://doi.org/10.1038/nrn1886
Mazon JN, de Mello AH, Ferreira GK, Rezin GT (2017) The impact of obesity on neurodegenerative diseases. Life Sci 182:22–28. https://doi.org/10.1016/j.lfs.2017.06.002
Mazza E, Fava A, Ferro Y et al (2018) Effect of the replacement of dietary vegetable oils with a low dose of extravirgin olive oil in the Mediterranean Diet on cognitive functions in the elderly. J Transl Med 16:10. https://doi.org/10.1186/s12967-018-1386-x
McGeer EG, McGeer PL (1998) The importance of inflammatory mechanisms in Alzheimer disease. Exp Gerontol 33:371–378. https://doi.org/10.1016/s0531-5565(98)00013-8
McGregor RA, Kwon E-Y, Shin S-K et al (2013) Time-course microarrays reveal modulation of developmental, lipid metabolism and immune gene networks in intrascapular brown adipose tissue during the development of diet-induced obesity. Int J Obes (Lond) 37:1524–1531. https://doi.org/10.1038/ijo.2013.52
Mecocci P, Tinarelli C, Schulz RJ, Polidori MC (2014) Nutraceuticals in cognitive impairment and Alzheimer's disease. Front Pharmacol 5:147. https://doi.org/10.3389/fphar.2014.00147
Medawar PB (1952) An unsolved problem of biology
Mendonça LM, Dos Santos GC, Antonucci GA et al (2009) Evaluation of the cytotoxicity and genotoxicity of curcumin in PC12 cells. Mutat Res 675:29–34. https://doi.org/10.1016/j.mrgentox.2009.02.003
Meng T, Qin W, Liu B (2020) SIRT1 antagonizes oxidative stress in diabetic vascular complication. Front Endocrinol (Lausanne) 11:568861. https://doi.org/10.3389/fendo.2020.568861
Mercken EM, Carboneau BA, Krzysik-Walker SM, de Cabo R (2012) Of mice and men: the benefits of caloric restriction, exercise, and mimetics. Ageing Res Rev 11:390–398. https://doi.org/10.1016/j.arr.2011.11.005
Merkwirth C, Jovaisaite V, Durieux J et al (2016) Two conserved histone demethylases regulate mitochondrial stress-induced longevity. Cell 165:1209–1223. https://doi.org/10.1016/j.cell.2016.04.012
Metcalfe-Roach A, Yu AC, Golz E et al (2021) MIND and mediterranean diets associated with later onset of Parkinson's disease. Mov Disord 36:977–984. https://doi.org/10.1002/mds.28464
Meydani M, Hasan ST (2010) Dietary polyphenols and obesity. Nutrients 2:737–751. https://doi.org/10.3390/nu2070737
Mezghani N, Mkaouar-Rebai E, Mnif M et al (2010) The heteroplasmic m.14709T>C mutation in the tRNA(Glu) gene in two Tunisian families with mitochondrial diabetes. J Diabetes Complications 24:270–277. https://doi.org/10.1016/j.jdiacomp.2009.11.002
Mi Y, Qi G, Fan R et al (2017) EGCG ameliorates high-fat- and high-fructose-induced cognitive defects by regulating the IRS/AKT and ERK/CREB/BDNF signaling pathways in the CNS. FASEB J 31:4998–5011. https://doi.org/10.1096/fj.201700400RR
Miao Y, Zhao S, Gao Y et al (2016) Curcumin pretreatment attenuates inflammation and mitochondrial dysfunction in experimental stroke: the possible role of Sirt1 signaling. Brain Res Bull 121:9–15. https://doi.org/10.1016/j.brainresbull.2015.11.019
Michel PP, Hirsch EC, Hunot S (2016) Understanding dopaminergic cell death pathways in Parkinson disease. Neuron 90:675–691. https://doi.org/10.1016/j.neuron.2016.03.038
Mikula M, Majewska A, Ledwon JK et al (2014) Obesity increases histone H3 lysine 9 and 18 acetylation at Tnfa and Ccl2 genes in mouse liver. Int J Mol Med 34:1647–1654. https://doi.org/10.3892/ijmm.2014.1958
Milbury PE, Kalt W (2010) Xenobiotic metabolism and berry flavonoid transport across the blood-brain barrier. J Agric Food Chem 58:3950–3956. https://doi.org/10.1021/jf903529m
Miller RA, Harrison DE, Astle CM et al (2011) Rapamycin, but not resveratrol or simvastatin, extends life span of genetically heterogeneous mice. J Gerontol Ser A 66:191–201. https://doi.org/10.1093/gerona/glq178

Minamino T, Orimo M, Shimizu I et al (2009) A crucial role for adipose tissue p53 in the regulation of insulin resistance. Nat Med 15:1082–1087. https://doi.org/10.1038/nm.2014

Miquel J (1991) An integrated theory of aging as the result of mitochondrial-DNA mutation in differentiated cells. Arch Gerontol Geriatr 12:99–117. https://doi.org/10.1016/0167-4943(91)90022-i

Mira L, Fernandez MT, Santos M et al (2002) Interactions of flavonoids with iron and copper ions: a mechanism for their antioxidant activity. Free Radic Res 36:1199–1208. https://doi.org/10.1080/1071576021000016463

Mohamed-Ali V, Pinkney JH, Coppack SW (1998) Adipose tissue as an endocrine and paracrine organ. Int J Obes Relat Metab Disord J Int Assoc Study Obes 22:1145–1158. https://doi.org/10.1038/sj.ijo.0800770

Mohammadi A, Sahebkar A, Iranshahi M et al (2013) Effects of supplementation with curcuminoids on dyslipidemia in obese patients: a randomized crossover trial. Phytother Res 27:374–379. https://doi.org/10.1002/ptr.4715

Mohammadpour S, Ghorbaninejad P, Janbozorgi N, Shab-Bidar S (2020) Associations between adherence to MIND diet and metabolic syndrome and general and abdominal obesity: a cross-sectional study. Diabetol Metab Syndr 12:101. https://doi.org/10.1186/s13098-020-00611-6

Mohanty I, Joshi S, Trivedi D et al (2002) Lycopene prevents sugar-induced morphological changes and modulates antioxidant status of human lens epithelial cells. Br J Nutr 88:347–354. https://doi.org/10.1079/BJN2002659

Montine TJ, Neely MD, Quinn JF et al (2002) Lipid peroxidation in aging brain and Alzheimer's disease. Free Radic Biol Med 33:620–626. https://doi.org/10.1016/s0891-5849(02)00807-9

Montonen J, Knekt P, Härkänen T et al (2005) Dietary patterns and the incidence of type 2 diabetes. Am J Epidemiol 161:219–227. https://doi.org/10.1093/aje/kwi039

Moore TL, Bowley BGE, Shultz PL et al (2018) Oral curcumin supplementation improves fine motor function in the middle-aged rhesus monkey. Somatosens Mot Res 35:1–10. https://doi.org/10.1080/08990220.2018.1432481

Moreno-Ulloa A, Nogueira L, Rodriguez A et al (2015) Recovery of indicators of mitochondrial biogenesis, oxidative stress, and aging with (-)-epicatechin in senile mice. J Gerontol A Biol Sci Med Sci 70:1370–1378. https://doi.org/10.1093/gerona/glu131

Morimoto RI (2008) Proteotoxic stress and inducible chaperone networks in neurodegenerative disease and aging. Genes Dev 22:1427–1438. https://doi.org/10.1101/gad.1657108

Morimoto RI, Cuervo AM (2014) Proteostasis and the aging proteome in health and disease. J Gerontol A Biol Sci Med Sci 69(Suppl 1):S33–S38. https://doi.org/10.1093/gerona/glu049

Morris MC, Tangney CC, Wang Y et al (2015a) MIND diet associated with reduced incidence of Alzheimer's disease. Alzheimers Dement 11:1007–1014. https://doi.org/10.1016/j.jalz.2014.11.009

Morris MC, Tangney CC, Wang Y et al (2015b) MIND diet slows cognitive decline with aging. Alzheimers Dement 11:1015–1022. https://doi.org/10.1016/j.jalz.2015.04.011

Morris MC, Wang Y, Barnes LL et al (2018) Nutrients and bioactives in green leafy vegetables and cognitive decline: prospective study. Neurology 90:e214–e222. https://doi.org/10.1212/WNL.0000000000004815

Morrison SJ, Spradling AC (2008) Stem cells and niches: mechanisms that promote stem cell maintenance throughout life. Cell 132:598–611. https://doi.org/10.1016/j.cell.2008.01.038

Mosconi L, Pupi A, De Leon MJ (2008) Brain glucose hypometabolism and oxidative stress in preclinical Alzheimer's disease. Ann N Y Acad Sci 1147:180–195. https://doi.org/10.1196/annals.1427.007

Moskalev A, Shaposhnikov M (2011) Pharmacological inhibition of NF-κB prolongs lifespan of *Drosophila melanogaster*. Aging (Albany NY) 3:391–394. https://doi.org/10.18632/aging.100314

Most J, Tosti V, Redman LM, Fontana L (2017) Calorie restriction in humans: an update. Ageing Res Rev 39:36–45. https://doi.org/10.1016/j.arr.2016.08.005

Motta MC, Divecha N, Lemieux M et al (2004) Mammalian SIRT1 represses forkhead transcription factors. Cell 116:551–563. https://doi.org/10.1016/s0092-8674(04)00126-6

Muller FL, Lustgarten MS, Jang Y et al (2007) Trends in oxidative aging theories. Free Radic Biol Med 43:477–503. https://doi.org/10.1016/j.freeradbiomed.2007.03.034

Muñoz-Espín D, Serrano M (2014) Cellular senescence: from physiology to pathology. Nat Rev Mol Cell Biol 15:482–496. https://doi.org/10.1038/nrm3823

Murase T, Misawa K, Haramizu S, Hase T (2009) Catechin-induced activation of the LKB1/AMP-activated protein kinase pathway. Biochem Pharmacol 78:78–84. https://doi.org/10.1016/j.bcp.2009.03.021

Murase T, Nagasawa A, Suzuki J et al (2002) Beneficial effects of tea catechins on diet-induced obesity: stimulation of lipid catabolism in the liver. Int J Obes Relat Metab Disord J Int Assoc Study Obes 26:1459–1464. https://doi.org/10.1038/sj.ijo.0802141

Musa-Veloso K, Paulionis L, Poon T, Lee HY (2016) The effects of almond consumption on fasting blood lipid levels: a systematic review and meta-analysis of randomised controlled trials. J Nutr Sci 5:e34. https://doi.org/10.1017/jns.2016.19

Mylnikov SV, Kokko HI, Kärenlampi SO et al (2005) Rubus fruit juices affect lipid peroxidation in a *Drosophila melanogaster* model *in vivo*. J Agric Food Chem 53:7728–7733. https://doi.org/10.1021/jf051303l

Nabavi SF, Nabavi SM, Mirzaei M, Moghaddam AH (2012) Protective effect of quercetin against sodium fluoride induced oxidative stress in rat's heart. Food Funct 3:437–441. https://doi.org/10.1039/c2fo10264a

Nair S, Lee YH, Rousseau E et al (2005) Increased expression of inflammation-related genes in cultured preadipocytes/stromal vascular cells from obese compared with non-obese Pima Indians. Diabetologia 48:1784–1788. https://doi.org/10.1007/s00125-005-1868-2

Nam SM, Choi JH, Yoo DY et al (2014) Effects of curcumin (Curcuma longa) on learning and spatial memory as well as cell proliferation and neuroblast differentiation in adult and aged mice by upregulating brain-derived neurotrophic factor and CREB signaling. J Med Food 17:641–649. https://doi.org/10.1089/jmf.2013.2965

National Heart Lung and Blood Institute (2022) DASH eating plan. https://www.nhlbi.nih.gov/education/dash-eating-plan

Navarro CL, Cau P, Lévy N (2006) Molecular bases of progeroid syndromes. Hum Mol Genet 15 Spec No:R151–R161. https://doi.org/10.1093/hmg/ddl214

Nedergaard J, Bengtsson T, Cannon B (2007) Unexpected evidence for active brown adipose tissue in adult humans. Am J Physiol Metab 293:E444–E452. https://doi.org/10.1152/ajpendo.00691.2006

Nedergaard J, Cannon B (2014) The browning of white adipose tissue: some burning issues. Cell Metab 20:396–407. https://doi.org/10.1016/j.cmet.2014.07.005

Nguyen JCD, Killcross AS, Jenkins TA (2014) Obesity and cognitive decline: role of inflammation and vascular changes. Front Neurosci 8:375. https://doi.org/10.3389/fnins.2014.00375

Niccoli T, Partridge L (2012) Ageing as a risk factor for disease. Curr Biol 22:R741–R752. https://doi.org/10.1016/j.cub.2012.07.024

Niedernhofer LJ, Gurkar AU, Wang Y et al (2018) Nuclear genomic instability and aging. Annu Rev Biochem 87:295–322. https://doi.org/10.1146/annurev-biochem-062917-012239

Nikolac Perkovic M, Pivac N (2019) Genetic markers of Alzheimer's disease. Adv Exp Med Biol 1192:27–52. https://doi.org/10.1007/978-981-32-9721-0_3

Nishihira J, Nishimura M, Kurimoto M et al (2021) The effect of 24-week continuous intake of quercetin-rich onion on age-related cognitive decline in healthy elderly people: a randomized, double-blind, placebo-controlled, parallel-group comparative clinical trial. J Clin Biochem Nutr 69:203–215. https://doi.org/10.3164/jcbn.21-17

Nishimura M, Muro T, Kobori M, Nishihira J (2019) Effect of daily ingestion of quercetin-rich onion powder for 12 weeks on visceral fat: a randomised, double-blind, placebo-controlled, parallel-group study. Nutrients 12. https://doi.org/10.3390/nu12010091

Nisoli E, Clementi E, Carruba MO, Moncada S (2007) Defective mitochondrial biogenesis: a hallmark of the high cardiovascular risk in the metabolic syndrome? Circ Res 100:795–806. https://doi.org/10.1161/01.RES.0000259591.97107.6c

Niu Y, Na L, Feng R et al (2013) The phytochemical, EGCG, extends lifespan by reducing liver and kidney function damage and improving age-associated inflammation and oxidative stress in healthy rats. Aging Cell 12:1041–1049. https://doi.org/10.1111/acel.12133

Nordfjall K, Eliasson M, Stegmayr B et al (2008) Telomere length is associated with obesity parameters but with a gender difference. Obesity (Silver Spring) 16:2682–2689. https://doi.org/10.1038/oby.2008.413

Nuss JE, Choksi KB, DeFord JH, Papaconstantinou J (2008) Decreased enzyme activities of chaperones PDI and BiP in aged mouse livers. Biochem Biophys Res Commun 365:355–361. https://doi.org/10.1016/j.bbrc.2007.10.194

Nussbaum RL, Ellis CE (2003) Alzheimer's disease and Parkinson's disease. N Engl J Med 348:1356–1364. https://doi.org/10.1056/NEJM2003ra020003

Ogden CL, Carroll MD, Curtin LR et al (2006) Prevalence of overweight and obesity in the United States, 1999-2004. JAMA 295:1549–1555. https://doi.org/10.1001/jama.295.13.1549

Okamoto T (2005) Safety of quercetin for clinical application (Review). Int J Mol Med 16:275–278. https://doi.org/10.3892/ijmm.16.2.275

Oñate B, Vilahur G, Camino-López S et al (2013) Stem cells isolated from adipose tissue of obese patients show changes in their transcriptomic profile that indicate loss in stemcellness and increased commitment to an adipocyte-like phenotype. BMC Genomics 14:625. https://doi.org/10.1186/1471-2164-14-625

Oñate B, Vilahur G, Ferrer-Lorente R et al (2012) The subcutaneous adipose tissue reservoir of functionally active stem cells is reduced in obese patients. FASEB J 26:4327–4336. https://doi.org/10.1096/fj.12-207217

Ooi TC, Meramat A, Rajab NF et al (2020) Intermittent fasting enhanced the cognitive function in older adults with mild cognitive impairment by inducing biochemical and metabolic changes: a 3-year progressive study. Nutrients 12. https://doi.org/10.3390/nu12092644

Ortsäter H, Grankvist N, Wolfram S et al (2012) Diet supplementation with green tea extract epigallocatechin gallate prevents progression to glucose intolerance in db/db mice. Nutr Metab (Lond) 9:11. https://doi.org/10.1186/1743-7075-9-11

Overman A, Chuang C-C, McIntosh M (2011) Quercetin attenuates inflammation in human macrophages and adipocytes exposed to macrophage-conditioned media. Int J Obes (Lond) 35:1165–1172. https://doi.org/10.1038/ijo.2010.272

Pal S, Tyler JK (2016) Epigenetics and aging. Sci Adv 2:e1600584. https://doi.org/10.1126/sciadv.1600584

Pan H, Guo J, Su Z (2014) Advances in understanding the interrelations between leptin resistance and obesity. Physiol Behav 130:157–169. https://doi.org/10.1016/j.physbeh.2014.04.003

Panagiotakos DB, Chrysohoou C, Pitsavos C, Stefanadis C (2006) Association between the prevalence of obesity and adherence to the Mediterranean diet: the ATTICA study. Nutrition 22:449–456. https://doi.org/10.1016/j.nut.2005.11.004

Panchal SK, Poudyal H, Brown L (2012) Quercetin ameliorates cardiovascular, hepatic, and metabolic changes in diet-induced metabolic syndrome in rats. J Nutr 142:1026–1032. https://doi.org/10.3945/jn.111.157263

Paradies G, Ruggiero FM (1991) Effect of aging on the activity of the phosphate carrier and on the lipid composition in rat liver mitochondria. Arch Biochem Biophys 284:332–337. https://doi.org/10.1016/0003-9861(91)90304-2

Parihar P, Solanki I, Mansuri ML, Parihar MS (2015) Mitochondrial sirtuins: emerging roles in metabolic regulations, energy homeostasis and diseases. Exp Gerontol 61:130–141. https://doi.org/10.1016/j.exger.2014.12.004

Park S, Choi M, Lee M (2021) Effects of anthocyanin supplementation on reduction of obesity criteria: a systematic review and meta-analysis of randomized controlled trials. Nutrients 13. https://doi.org/10.3390/nu13062121

Patel NV, Gordon MN, Connor KE et al (2005) Caloric restriction attenuates Abeta-deposition in Alzheimer transgenic models. Neurobiol Aging 26:995–1000. https://doi.org/10.1016/j.neurobiolaging.2004.09.014

Patterson RE, Sears DD (2017) Metabolic effects of intermittent fasting. Annu Rev Nutr 37:371–393. https://doi.org/10.1146/annurev-nutr-071816-064634

Pearson KJ, Baur JA, Lewis KN et al (2008) Resveratrol delays age-related deterioration and mimics transcriptional aspects of dietary restriction without extending life span. Cell Metab 8:157–168. https://doi.org/10.1016/j.cmet.2008.06.011

Pérez LM, Bernal A, San Martín N, Gálvez BG (2013) Obese-derived ASCs show impaired migration and angiogenesis properties. Arch Physiol Biochem 119:195–201. https://doi.org/10.3109/13813455.2013.784339

Pérez LM, Pareja-Galeano H, Sanchis-Gomar F et al (2016) "Adipaging": ageing and obesity share biological hallmarks related to a dysfunctional adipose tissue. J Physiol 594:3187–3207. https://doi.org/10.1113/JP271691

Pérez VI, Buffenstein R, Masamsetti V et al (2009) Protein stability and resistance to oxidative stress are determinants of longevity in the longest-living rodent, the naked mole-rat. Proc Natl Acad Sci U S A 106:3059–3064. https://doi.org/10.1073/pnas.0809620106

Perron NR, Brumaghim JL (2009) A review of the antioxidant mechanisms of polyphenol compounds related to iron binding. Cell Biochem Biophys 53:75–100. https://doi.org/10.1007/s12013-009-9043-x

Petersen KF, Dufour S, Befroy D et al (2004) Impaired mitochondrial activity in the insulin-resistant offspring of patients with type 2 diabetes. N Engl J Med 350:664–671. https://doi.org/10.1056/NEJMoa031314

Petersen RC (2004) Mild cognitive impairment as a diagnostic entity. J Intern Med 256:183–194. https://doi.org/10.1111/j.1365-2796.2004.01388.x

Petersson SD, Philippou E (2016) Mediterranean diet, cognitive function, and dementia: a systematic review of the evidence. Adv Nutr 7:889–904. https://doi.org/10.3945/an.116.012138

Pi-Sunyer X (2009) The medical risks of obesity. Postgrad Med 121:21–33. https://doi.org/10.3810/pgm.2009.11.2074

Piecuch J, Mertas A, Nowowiejska-Wiewiora A et al (2019) The relationship between the rheological behavior of RBCs and angiogenesis in the morbidly obese. Clin Hemorheol Microcirc 71:95–102. https://doi.org/10.3233/CH-180420

Pietsch K, Saul N, Chakrabarti S et al (2011) Hormetins, antioxidants and prooxidants: defining quercetin-, caffeic acid- and rosmarinic acid-mediated life extension in *C. elegans*. Biogerontology 12:329–347. https://doi.org/10.1007/s10522-011-9334-7

Pietsch K, Saul N, Menzel R et al (2009) Quercetin mediated lifespan extension in *Caenorhabditis elegans* is modulated by *age-1*, *daf-2*, *sek-1* and *unc-43*. Biogerontology 10:565–578. https://doi.org/10.1007/s10522-008-9199-6

Pietsch K, Saul N, Swain SC et al (2012) Meta-analysis of global transcriptomics suggests that conserved genetic pathways are responsible for quercetin and tannic acid mediated longevity in *C. elegans*. Front Genet 3:48. https://doi.org/10.3389/fgene.2012.00048

Pihl E, Zilmer K, Kullisaar T et al (2006) Atherogenic inflammatory and oxidative stress markers in relation to overweight values in male former athletes. Int J Obes (Lond) 30:141–146. https://doi.org/10.1038/sj.ijo.0803068

Pinkus R, Weiner LM, Daniel V (1996) Role of oxidants and antioxidants in the induction of AP-1, NF-kappaB, and glutathione S-transferase gene expression. J Biol Chem 271:13422–13429. https://doi.org/10.1074/jbc.271.23.13422

Pintana H, Sripetchwandee J, Supakul L et al (2014) Garlic extract attenuates brain mitochondrial dysfunction and cognitive deficit in obese-insulin resistant rats. Appl Physiol Nutr Metab 39:1373–1379. https://doi.org/10.1139/apnm-2014-0255

Pinti MV, Fink GK, Hathaway QA et al (2019) Mitochondrial dysfunction in type 2 diabetes mellitus: an organ-based analysis. Am J Physiol Endocrinol Metab 316:E268–E285. https://doi.org/10.1152/ajpendo.00314.2018

Pipatpiboon N, Pratchayasakul W, Chattipakorn N, Chattipakorn SC (2012) PPARγ agonist improves neuronal insulin receptor function in hippocampus and brain mitochondria function in rats with insulin resistance induced by long term high-fat diets. Endocrinology 153:329–338. https://doi.org/10.1210/en.2011-1502

Pizzino G, Irrera N, Cucinotta M et al (2017) Oxidative stress: harms and benefits for human health. Oxid Med Cell Longev 2017:8416763. https://doi.org/10.1155/2017/8416763

Polito CA, Cai Z-Y, Shi Y-L et al (2018) Association of tea consumption with risk of Alzheimer's disease and anti-beta-amyloid effects of tea. Nutrients 10. https://doi.org/10.3390/nu10050655

Porquet D, Griñán-Ferré C, Ferrer I et al (2014) Neuroprotective role of trans-resveratrol in a murine model of familial Alzheimer's disease. J Alzheimers Dis 42:1209–1220. https://doi.org/10.3233/JAD-140444

Potenza MA, Marasciulo FL, Tarquinio M et al (2007) EGCG, a green tea polyphenol, improves endothelial function and insulin sensitivity, reduces blood pressure, and protects against myocardial I/R injury in SHR. Am J Physiol Endocrinol Metab 292:E1378–E1387. https://doi.org/10.1152/ajpendo.00698.2006

Poulose SM, Fisher DR, Larson J et al (2012) Anthocyanin-rich açai (Euterpe oleracea Mart.) fruit pulp fractions attenuate inflammatory stress signaling in mouse brain BV-2 microglial cells. J Agric Food Chem 60:1084–1093. https://doi.org/10.1021/jf203989k

Poulsen MM, Vestergaard PF, Clasen BF et al (2013) High-dose resveratrol supplementation in obese men: an investigator-initiated, randomized, placebo-controlled clinical trial of substrate metabolism, insulin sensitivity, and body composition. Diabetes 62:1186–1195. https://doi.org/10.2337/db12-0975

Powers ET, Morimoto RI, Dillin A et al (2009) Biological and chemical approaches to diseases of proteostasis deficiency. Annu Rev Biochem 78:959–991. https://doi.org/10.1146/annurev.biochem.052308.114844

Powers RW, Kaeberlein M, Caldwell SD et al (2006) Extension of chronological life span in yeast by decreased TOR pathway signaling. Genes Dev 20:174–184. https://doi.org/10.1101/gad.1381406

Prasad S, Gupta SC, Tyagi AK, Aggarwal BB (2014) Curcumin, a component of golden spice: from bedside to bench and back. Biotechnol Adv 32:1053–1064. https://doi.org/10.1016/j.biotechadv.2014.04.004

Pratchayasakul W, Sa-Nguanmoo P, Sivasinprasasn S et al (2015) Obesity accelerates cognitive decline by aggravating mitochondrial dysfunction, insulin resistance and synaptic dysfunction under estrogen-deprived conditions. Horm Behav 72:68–77. https://doi.org/10.1016/j.yhbeh.2015.04.023

Prior RL, Wu X, Gu L et al (2008) Whole berries versus berry anthocyanins: interactions with dietary fat levels in the C57BL/6J mouse model of obesity. J Agric Food Chem 56:647–653. https://doi.org/10.1021/jf071993o

Prior RL, Wu X, Gu L et al (2009) Purified berry anthocyanins but not whole berries normalize lipid parameters in mice fed an obesogenic high fat diet. Mol Nutr Food Res 53:1406–1418. https://doi.org/10.1002/mnfr.200900026

Proshkina E, Lashmanova E, Dobrovolskaya E et al (2016) Geroprotective and radioprotective activity of quercetin, (-)-epicatechin, and ibuprofen in *Drosophila melanogaster*. Front Pharmacol 7:505. https://doi.org/10.3389/fphar.2016.00505

Qi Y, Guo L, Jiang Y et al (2020) Brain delivery of quercetin-loaded exosomes improved cognitive function in AD mice by inhibiting phosphorylated tau-mediated neurofibrillary tangles. Drug Deliv 27:745–755. https://doi.org/10.1080/10717544.2020.1762262

Qin W, Chachich M, Lane M et al (2006) Calorie restriction attenuates Alzheimer's disease type brain amyloidosis in Squirrel monkeys (*Saimiri sciureus*). J Alzheimers Dis 10:417–422. https://doi.org/10.3233/jad-2006-10411

Quincozes-Santos A, Bobermin LD, Tramontina AC et al (2014) Oxidative stress mediated by NMDA, AMPA/KA channels in acute hippocampal slices: neuroprotective effect of resveratrol. Toxicol In Vitro 28:544–551. https://doi.org/10.1016/j.tiv.2013.12.021

Quintana DD, Garcia JA, Anantula Y et al (2020) Amyloid-β causes mitochondrial dysfunction via a Ca2+-driven upregulation of oxidative phosphorylation and superoxide production in cerebrovascular endothelial cells. J Alzheimers Dis 75:119–138. https://doi.org/10.3233/JAD-190964

Rahman MM, Ichiyanagi T, Komiyama T et al (2008) Effects of anthocyanins on psychological stress-induced oxidative stress and neurotransmitter status. J Agric Food Chem 56:7545–7550. https://doi.org/10.1021/jf800930s

Rainey-Smith SR, Brown BM, Sohrabi HR et al (2016) Curcumin and cognition: a randomised, placebo-controlled, double-blind study of community-dwelling older adults. Br J Nutr 115: 2106–2113. https://doi.org/10.1017/S0007114516001203

Raman M, Chen W, Cobb MH (2007) Differential regulation and properties of MAPKs. Oncogene 26:3100–3112. https://doi.org/10.1038/sj.onc.1210392

Ramos FJ, Chen SC, Garelick MG et al (2012) Rapamycin reverses elevated mTORC1 signaling in lamin A/C-deficient mice, rescues cardiac and skeletal muscle function, and extends survival. Sci Transl Med 4:144ra103. https://doi.org/10.1126/scitranslmed.3003802

Rauf A, Imran M, Khan IA et al (2018) Anticancer potential of quercetin: a comprehensive review. Phytother Res 32:2109–2130. https://doi.org/10.1002/ptr.6155

Razay G, Vreugdenhil A, Wilcock G (2007) The metabolic syndrome and Alzheimer disease. Arch Neurol 64:93–96. https://doi.org/10.1001/archneur.64.1.93

Receno CN, Liang C, Korol DL et al (2019) Effects of prolonged dietary curcumin exposure on skeletal muscle biochemical and functional responses of aged male rats. Int J Mol Sci 20. https://doi.org/10.3390/ijms20051178

Reddy AC, Lokesh BR (1994) Effect of dietary turmeric (*Curcuma longa*) on iron-induced lipid peroxidation in the rat liver. Food Chem Toxicol 32:279–283. https://doi.org/10.1016/0278-6915(94)90201-1

Reddy PH, Manczak M, Yin X et al (2016) Protective effects of a natural product, curcumin, against amyloid β induced mitochondrial and synaptic toxicities in Alzheimer's disease. J Investig Med Off Publ Am Fed Clin Res 64:1220–1234. https://doi.org/10.1136/jim-2016-000240

Regmi SG, Rolland SG, Conradt B (2014) Age-dependent changes in mitochondrial morphology and volume are not predictors of lifespan. Aging (Albany NY) 6:118–130. https://doi.org/10.18632/aging.100639

Reis JF, Monteiro VVS, de Souza GR et al (2016) Action mechanism and cardiovascular effect of anthocyanins: a systematic review of animal and human studies. J Transl Med 14:315. https://doi.org/10.1186/s12967-016-1076-5

Ren R, Ocampo A, Liu G-H, Izpisua Belmonte JC (2017) Regulation of stem cell aging by metabolism and epigenetics. Cell Metab 26:460–474. https://doi.org/10.1016/j.cmet.2017.07.019

Rezai-Zadeh K, Arendash GW, Hou H et al (2008) Green tea epigallocatechin-3-gallate (EGCG) reduces beta-amyloid mediated cognitive impairment and modulates tau pathology in Alzheimer transgenic mice. Brain Res 1214:177–187. https://doi.org/10.1016/j.brainres.2008.02.107

Rice-Evans CA, Miller NJ, Bolwell PG et al (1995) The relative antioxidant activities of plant-derived polyphenolic flavonoids. Free Radic Res 22:375–383. https://doi.org/10.3109/10715769509145649

Ringman JM, Frautschy SA, Teng E et al (2012) Oral curcumin for Alzheimer's disease: tolerability and efficacy in a 24-week randomized, double blind, placebo-controlled study. Alzheimers Res Ther 4:43. https://doi.org/10.1186/alzrt146

Robb EL, Winkelmolen L, Visanji N et al (2008) Dietary resveratrol administration increases MnSOD expression and activity in mouse brain. Biochem Biophys Res Commun 372:254–259. https://doi.org/10.1016/j.bbrc.2008.05.028

Rocha N, Bulger DA, Frontini A et al (2017) Human biallelic MFN2 mutations induce mitochondrial dysfunction, upper body adipose hyperplasia, and suppression of leptin expression. Elife 6. https://doi.org/10.7554/eLife.23813

Rodriguez KA, Edrey YH, Osmulski P et al (2012) Altered composition of liver proteasome assemblies contributes to enhanced proteasome activity in the exceptionally long-lived naked mole-rat. PLoS One 7:e35890. https://doi.org/10.1371/journal.pone.0035890

Roh H-T, Cho S-Y, So W-Y (2017) Obesity promotes oxidative stress and exacerbates blood-brain barrier disruption after high-intensity exercise. J Sport Heal Sci 6:225–230. https://doi.org/10.1016/j.jshs.2016.06.005

Rosen ED, Spiegelman BM (2014) What we talk about when we talk about fat. Cell 156:20–44. https://doi.org/10.1016/j.cell.2013.12.012

Roth LW, Polotsky AJ (2012) Can we live longer by eating less? A review of caloric restriction and longevity. Maturitas 71:315–319. https://doi.org/10.1016/j.maturitas.2011.12.017

Rubio-Perez JM, Morillas-Ruiz JM (2012) A review: inflammatory process in Alzheimer's disease, role of cytokines. ScientificWorldJournal 2012:756357. https://doi.org/10.1100/2012/756357

Rudolph KL, Chang S, Lee HW et al (1999) Longevity, stress response, and cancer in aging telomerase-deficient mice. Cell 96:701–712. https://doi.org/10.1016/s0092-8674(00)80580-2

Runas KA, Malmstadt N (2015) Low levels of lipid oxidation radically increase the passive permeability of lipid bilayers. Soft Matter 11:499–505. https://doi.org/10.1039/c4sm01478b

Rupnick MA, Panigrahy D, Zhang C-Y et al (2002) Adipose tissue mass can be regulated through the vasculature. Proc Natl Acad Sci U S A 99:10730–10735. https://doi.org/10.1073/pnas.162349799

Russell SJ, Kahn CR (2007) Endocrine regulation of ageing. Nat Rev Mol Cell Biol 8:681–691. https://doi.org/10.1038/nrm2234

Sa-Nguanmoo P, Tanajak P, Kerdphoo S et al (2016) FGF21 improves cognition by restored synaptic plasticity, dendritic spine density, brain mitochondrial function and cell apoptosis in obese-insulin resistant male rats. Horm Behav 85:86–95. https://doi.org/10.1016/j.yhbeh.2016.08.006

Saad MJA, Santos A, Prada PO (2016) Linking gut microbiota and inflammation to obesity and insulin resistance. Physiology (Bethesda) 31:283–293. https://doi.org/10.1152/physiol.00041.2015

Sabogal-Guáqueta AM, Muñoz-Manco JI, Ramírez-Pineda JR et al (2015) The flavonoid quercetin ameliorates Alzheimer's disease pathology and protects cognitive and emotional function in aged triple transgenic Alzheimer's disease model mice. Neuropharmacology 93:134–145. https://doi.org/10.1016/j.neuropharm.2015.01.027

Saely CH, Geiger K, Drexel H (2012) Brown versus white adipose tissue: a mini-review. Gerontology 58:15–23. https://doi.org/10.1159/000321319

Sala de Oyanguren FJ, Rainey NE, Moustapha A et al (2020) Highlighting curcumin-induced crosstalk between autophagy and apoptosis as supported by its specific subcellular localization. Cells 9. https://doi.org/10.3390/cells9020361

Salehi B, Machin L, Monzote L et al (2020) Therapeutic potential of quercetin: new insights and perspectives for human health. ACS Omega 5:11849–11872. https://doi.org/10.1021/acsomega.0c01818

Salehi B, Mishra AP, Nigam M et al (2018) Resveratrol: a double-edged sword in health benefits. Biomedicines 6. https://doi.org/10.3390/biomedicines6030091

Salmon AB, Leonard S, Masamsetti V et al (2009) The long lifespan of two bat species is correlated with resistance to protein oxidation and enhanced protein homeostasis. FASEB J 23:2317–2326. https://doi.org/10.1096/fj.08-122523

Santos-Parker JR, Lubieniecki KL, Rossman MJ et al (2018) Curcumin supplementation and motor-cognitive function in healthy middle-aged and older adults. Nutr Heal Aging 4:323–333. https://doi.org/10.3233/NHA-170029

Santos AL, Sinha S (2021) Obesity and aging: molecular mechanisms and therapeutic approaches. Ageing Res Rev 67:101268. https://doi.org/10.1016/j.arr.2021.101268

Santos AL, Sinha S, Lindner AB (2018) The good, the bad and the ugly of ROS: new insights on aging and aging-related diseases from eukaryotic and prokaryotic model organisms. Oxid Med Cell Longev 2018. https://doi.org/10.1155/2018/1941285

Saraf-Bank S, Ahmadi A, Paknahad Z et al (2019) Effects of curcumin on cardiovascular risk factors in obese and overweight adolescent girls: a randomized clinical trial. Sao Paulo Med J 137:414–422. https://doi.org/10.1590/1516-3180.2018.0454120419

Sargeant LA, Khaw KT, Bingham S et al (2001) Fruit and vegetable intake and population glycosylated haemoglobin levels: the EPIC-Norfolk Study. Eur J Clin Nutr 55:342–348. https://doi.org/10.1038/sj.ejcn.1601162

Saris N-EL, Mervaala E, Karppanen H et al (2000) Magnesium: an update on physiological, clinical and analytical aspects. Clin Chim Acta 294:1–26. https://doi.org/10.1016/s0009-8981(99)00258-2

Sarraf P, Parohan M, Javanbakht MH et al (2019) Short-term curcumin supplementation enhances serum brain-derived neurotrophic factor in adult men and women: a systematic review and dose-response meta-analysis of randomized controlled trials. Nutr Res 69:1–8. https://doi.org/10.1016/j.nutres.2019.05.001

Saul N, Pietsch K, Menzel R, Steinberg CEW (2008) Quercetin-mediated longevity in *Caenorhabditis elegans*: is DAF-16 involved? Mech Ageing Dev 129:611–613. https://doi.org/10.1016/j.mad.2008.07.001

Saura-Calixto F, Goñi I (2009) Definition of the Mediterranean diet based on bioactive compounds. Crit Rev Food Sci Nutr 49:145–152. https://doi.org/10.1080/10408390701764732

Sawada M, Carlson JC (1987) Changes in superoxide radical and lipid peroxide formation in the brain, heart and liver during the lifetime of the rat. Mech Ageing Dev 41:125–137. https://doi.org/10.1016/0047-6374(87)90057-1

Saxton RA, Sabatini DM (2017) mTOR signaling in growth, metabolism, and disease. Cell 169:361–371. https://doi.org/10.1016/j.cell.2017.03.035

Sbierski-Kind J, Grenkowitz S, Schlickeiser S et al (2022) Effects of caloric restriction on the gut microbiome are linked with immune senescence. Microbiome 10:57. https://doi.org/10.1186/s40168-022-01249-4

Scarpato R, Verola C, Fabiani B et al (2011) Nuclear damage in peripheral lymphocytes of obese and overweight Italian children as evaluated by the gamma-H2AX focus assay and micronucleus test. FASEB J 25:685–693. https://doi.org/10.1096/fj.10-168427

Schäffler A, Schölmerich J (2010) Innate immunity and adipose tissue biology. Trends Immunol 31:228–235. https://doi.org/10.1016/j.it.2010.03.001

Schapira AH, Cooper JM, Dexter D et al (1990) Mitochondrial complex I deficiency in Parkinson's disease. J Neurochem 54:823–827. https://doi.org/10.1111/j.1471-4159.1990.tb02325.x

Schapira AHV (2008) Mitochondria in the aetiology and pathogenesis of Parkinson's disease. Lancet Neurol 7:97–109. https://doi.org/10.1016/S1474-4422(07)70327-7

Schapira AHV, Chaudhuri KR, Jenner P (2017) Non-motor features of Parkinson disease. Nat Rev Neurosci 18:435–450. https://doi.org/10.1038/nrn.2017.62

Scheller Madrid A, Rode L, Nordestgaard BG, Bojesen SE (2016) Short telomere length and ischemic heart disease: observational and genetic studies in 290 022 individuals. Clin Chem 62:1140–1149. https://doi.org/10.1373/clinchem.2016.258566

Schimmer O, Häfele F, Krüger A (1988) The mutagenic potencies of plant extracts containing quercetin in Salmonella typhimurium TA98 and TA100. Mutat Res 206:201–208. https://doi.org/10.1016/0165-1218(88)90161-9

Schmatz R, Bitencourt MR, Patias LD et al (2017) Evaluation of the biochemical, inflammatory and oxidative profile of obese patients given clinical treatment and bariatric surgery. Clin Chim Acta 465:72–79. https://doi.org/10.1016/j.cca.2016.12.012

Schröder H (2007) Protective mechanisms of the Mediterranean diet in obesity and type 2 diabetes. J Nutr Biochem 18:149–160. https://doi.org/10.1016/j.jnutbio.2006.05.006

Schröder H, Marrugat J, Vila J et al (2004) Adherence to the traditional mediterranean diet is inversely associated with body mass index and obesity in a spanish population. J Nutr 134:3355–3361. https://doi.org/10.1093/jn/134.12.3355

Schröder M, Kaufman RJ (2005) The mammalian unfolded protein response. Annu Rev Biochem 74:739–789. https://doi.org/10.1146/annurev.biochem.73.011303.074134

Sebori R, Kuno A, Hosoda R et al (2018) Resveratrol decreases oxidative stress by restoring mitophagy and improves the pathophysiology of dystrophin-deficient mdx mice. Oxid Med Cell Longev 2018:9179270. https://doi.org/10.1155/2018/9179270

Secord AA, Gehrig PA (2012) Obesity: too big a problem to ignore. Gynecol Oncol 126:274–276. https://doi.org/10.1016/j.ygyno.2012.04.033

Seo BR, Bhardwaj P, Choi S et al (2015) Obesity-dependent changes in interstitial ECM mechanics promote breast tumorigenesis. Sci Transl Med 7:301ra130. https://doi.org/10.1126/scitranslmed.3010467

Seo K, Choi E, Lee D et al (2013) Heat shock factor 1 mediates the longevity conferred by inhibition of TOR and insulin/IGF-1 signaling pathways in *C. elegans*. Aging Cell 12:1073–1081. https://doi.org/10.1111/acel.12140

Serafini MM, Catanzaro M, Fagiani F et al (2019) Modulation of Keap1/Nrf2/ARE signaling pathway by curcuma- and garlic-derived hybrids. Front Pharmacol 10:1597. https://doi.org/10.3389/fphar.2019.01597

Serrano-Pozo A, Frosch MP, Masliah E, Hyman BT (2011) Neuropathological alterations in Alzheimer disease. Cold Spring Harb Perspect Med 1:a006189. https://doi.org/10.1101/cshperspect.a006189

Sesso HD, Wang L, Ridker PM, Buring JE (2012) Tomato-based food products are related to clinically modest improvements in selected coronary biomarkers in women. J Nutr 142:326–333. https://doi.org/10.3945/jn.111.150631

Seubert P, Oltersdorf T, Lee MG et al (1993) Secretion of beta-amyloid precursor protein cleaved at the amino terminus of the beta-amyloid peptide. Nature 361:260–263. https://doi.org/10.1038/361260a0

Shaito A, Posadino AM, Younes N et al (2020) Potential adverse effects of resveratrol: a literature review. Int J Mol Sci 21. https://doi.org/10.3390/ijms21062084

Shaposhnikov M, Proshkina E, Shilova L et al (2015) Lifespan and stress resistance in *Drosophila* with overexpressed DNA repair genes. Sci Rep 5:15299. https://doi.org/10.1038/srep15299

Shaposhnikov MV, Moskalev AA, Plyusnina EN (2011) Effect of PARP-1 overexpression and pharmacological inhibition of NF-kB on the lifespan of *Drosophila melanogaster*. Adv Gerontol 24:405–419

Sharma M, Gupta YK (2002) Chronic treatment with trans resveratrol prevents intracerebroventricular streptozotocin induced cognitive impairment and oxidative stress in rats. Life Sci 71:2489–2498. https://doi.org/10.1016/s0024-3205(02)02083-0

Sharma N, Nehru B (2018) Curcumin affords neuroprotection and inhibits α-synuclein aggregation in lipopolysaccharide-induced Parkinson's disease model. Inflammopharmacology 26:349–360. https://doi.org/10.1007/s10787-017-0402-8

Sharp LZ, Shinoda K, Ohno H et al (2012) Human BAT possesses molecular signatures that resemble beige/brite cells. PLoS One 7:e49452. https://doi.org/10.1371/journal.pone.0049452

Shaw RJ (2008) mTOR signaling: RAG GTPases transmit the amino acid signal. Trends Biochem Sci 33:565–568. https://doi.org/10.1016/j.tibs.2008.09.005

Shay JW, Wright WE (2006) Telomerase therapeutics for cancer: challenges and new directions. Nat Rev Drug Discov 5:577–584. https://doi.org/10.1038/nrd2081

Shi C, Zhang M, Tong M et al (2015) miR-148a is associated with obesity and modulates adipocyte differentiation of mesenchymal stem cells through Wnt signaling. Sci Rep 5:9930. https://doi.org/10.1038/srep09930

Shi Y, Williamson G (2016) Quercetin lowers plasma uric acid in pre-hyperuricaemic males: a randomised, double-blinded, placebo-controlled, cross-over trial. Br J Nutr 115:800–806. https://doi.org/10.1017/S0007114515005310

Shields HJ, Traa A, Van Raamsdonk JM (2021) Beneficial and detrimental effects of reactive oxygen species on lifespan: a comprehensive review of comparative and experimental studies. Front cell Dev Biol 9:628157. https://doi.org/10.3389/fcell.2021.628157

Shin BK, Kang S, Kim DS, Park S (2018) Intermittent fasting protects against the deterioration of cognitive function, energy metabolism and dyslipidemia in Alzheimer's disease-induced

estrogen deficient rats. Exp Biol Med (Maywood) 243:334–343. https://doi.org/10.1177/1535370217751610
Shin JW, Chun K-S, Kim D-H et al (2020) Curcumin induces stabilization of Nrf2 protein through Keap1 cysteine modification. Biochem Pharmacol 173:113820. https://doi.org/10.1016/j.bcp.2020.113820
Short KR, Bigelow ML, Kahl J et al (2005) Decline in skeletal muscle mitochondrial function with aging in humans. Proc Natl Acad Sci U S A 102:5618–5623. https://doi.org/10.1073/pnas.0501559102
Shytle RD, Bickford PC, Rezai-zadeh K et al (2009) Optimized turmeric extracts have potent anti-amyloidogenic effects. Curr Alzheimer Res 6:564–571. https://doi.org/10.2174/156720509790147115
Si H, Fu Z, Babu PVA et al (2011) Dietary epicatechin promotes survival of obese diabetic mice and *Drosophila melanogaster*. J Nutr 141:1095–1100. https://doi.org/10.3945/jn.110.134270
Sieber OM, Heinimann K, Tomlinson IPM (2003) Genomic instability–the engine of tumorigenesis? Nat Rev Cancer 3:701–708. https://doi.org/10.1038/nrc1170
Singh BN, Shankar S, Srivastava RK (2011) Green tea catechin, epigallocatechin-3-gallate (EGCG): mechanisms, perspectives and clinical applications. Biochem Pharmacol 82:1807–1821. https://doi.org/10.1016/j.bcp.2011.07.093
Skvortsova K, Iovino N, Bogdanović O (2018) Functions and mechanisms of epigenetic inheritance in animals. Nat Rev Mol Cell Biol 19:774–790. https://doi.org/10.1038/s41580-018-0074-2
Small GW, Siddarth P, Li Z et al (2018) Memory and brain amyloid and tau effects of a bioavailable form of curcumin in non-demented adults: a double-blind, placebo-controlled 18-month trial. Am J Geriatr psychiatry Off J Am Assoc Geriatr Psychiatry 26:266–277. https://doi.org/10.1016/j.jagp.2017.10.010
Smith PJ, Blumenthal JA, Babyak MA et al (2010) Effects of the dietary approaches to stop hypertension diet, exercise, and caloric restriction on neurocognition in overweight adults with high blood pressure. Hypertension 55:1331–1338. https://doi.org/10.1161/HYPERTENSIONAHA.109.146795
Soare A, Weiss EP, Pozzilli P (2014) Benefits of caloric restriction for cardiometabolic health, including type 2 diabetes mellitus risk. Diabetes Metab Res Rev 30(Suppl 1):41–47. https://doi.org/10.1002/dmrr.2517
Soh J-W, Marowsky N, Nichols TJ et al (2013) Curcumin is an early-acting stage-specific inducer of extended functional longevity in *Drosophila*. Exp Gerontol 48:229–239. https://doi.org/10.1016/j.exger.2012.09.007
Sohal RS, Dubey A (1994) Mitochondrial oxidative damage, hydrogen peroxide release, and aging. Free Radic Biol Med 16:621–626. https://doi.org/10.1016/0891-5849(94)90062-0
Sohal RS, Sohal BH (1991) Hydrogen peroxide release by mitochondria increases during aging. Mech Ageing Dev 57:187–202. https://doi.org/10.1016/0047-6374(91)90034-w
Solinas G, Becattini B (2017) JNK at the crossroad of obesity, insulin resistance, and cell stress response. Mol Metab 6:174–184. https://doi.org/10.1016/j.molmet.2016.12.001
Soltani S, Shirani F, Chitsazi MJ, Salehi-Abargouei A (2016) The effect of dietary approaches to stop hypertension (DASH) diet on weight and body composition in adults: a systematic review and meta-analysis of randomized controlled clinical trials. Obes Rev 17:442–454. https://doi.org/10.1111/obr.12391
Son TG, Camandola S, Mattson MP (2008) Hormetic dietary phytochemicals. Neuromolecular Med 10:236–246. https://doi.org/10.1007/s12017-008-8037-y
Song H-C, Chen Y, Chen Y et al (2020) GSK-3β inhibition by curcumin mitigates amyloidogenesis via TFEB activation and anti-oxidative activity in human neuroblastoma cells. Free Radic Res 54:918–930. https://doi.org/10.1080/10715762.2020.1791843
Soro-Arnaiz I, Li QOY, Torres-Capelli M et al (2016) Role of mitochondrial complex IV in age-dependent obesity. Cell Rep 16:2991–3002. https://doi.org/10.1016/j.celrep.2016.08.041

Spadaro O, Youm Y, Shchukina I et al (2022) Caloric restriction in humans reveals immunometabolic regulators of health span. Science 375:671–677. https://doi.org/10.1126/science.abg7292

Spillantini MG, Schmidt ML, Lee VM et al (1997) Alpha-synuclein in Lewy bodies. Nature 388: 839–840. https://doi.org/10.1038/42166

Spindler SR, Mote PL, Flegal JM, Teter B (2013) Influence on longevity of blueberry, cinnamon, green and black tea, pomegranate, sesame, curcumin, morin, pycnogenol, quercetin, and taxifolin fed iso-calorically to long-lived, F1 hybrid mice. Rejuvenation Res 16:143–151. https://doi.org/10.1089/rej.2012.1386

Spinelli KJ, Osterberg VR, Meshul CK et al (2015) Curcumin treatment improves motor behavior in α-synuclein transgenic mice. PLoS One 10:e0128510. https://doi.org/10.1371/journal.pone.0128510

Sreejayan, Rao MN (1994) Curcuminoids as potent inhibitors of lipid peroxidation. J Pharm Pharmacol 46:1013–1016. https://doi.org/10.1111/j.2042-7158.1994.tb03258.x

Sreejayan, Rao MN (1997) Nitric oxide scavenging by curcuminoids. J Pharm Pharmacol 49:105–107. https://doi.org/10.1111/j.2042-7158.1997.tb06761.x

Srinivasan M, Rajendra Prasad N, Menon VP (2006) Protective effect of curcumin on gamma-radiation induced DNA damage and lipid peroxidation in cultured human lymphocytes. Mutat Res 611:96–103. https://doi.org/10.1016/j.mrgentox.2006.07.002

Stadtman ER (2001) Protein oxidation in aging and age-related diseases. Ann N Y Acad Sci 928: 22–38. https://doi.org/10.1111/j.1749-6632.2001.tb05632.x

Strathearn KE, Yousef GG, Grace MH et al (2014) Neuroprotective effects of anthocyanin- and proanthocyanidin-rich extracts in cellular models of Parkinson's disease. Brain Res 1555:60–77. https://doi.org/10.1016/j.brainres.2014.01.047

Strikwerda AJ, Dommershuijsen LJ, Ikram MK, Voortman T (2021) Diet quality and risk of Parkinson's disease: the Rotterdam study. Nutrients 13. https://doi.org/10.3390/nu13113970

Strimpakos AS, Sharma RA (2008) Curcumin: preventive and therapeutic properties in laboratory studies and clinical trials. Antioxid Redox Signal 10:511–545. https://doi.org/10.1089/ars.2007.1769

Strong R, Miller RA, Astle CM et al (2013) Evaluation of resveratrol, green tea extract, curcumin, oxaloacetic acid, and medium-chain triglyceride oil on life span of genetically heterogeneous mice. J Gerontol A Biol Sci Med Sci 68:6–16. https://doi.org/10.1093/gerona/gls070

Stull AJ, Cash KC, Johnson WD et al (2010) Bioactives in blueberries improve insulin sensitivity in obese, insulin-resistant men and women. J Nutr 140:1764–1768. https://doi.org/10.3945/jn.110.125336

Suganami T, Nishida J, Ogawa Y (2005) A paracrine loop between adipocytes and macrophages aggravates inflammatory changes: role of free fatty acids and tumor necrosis factor alpha. Arterioscler Thromb Vasc Biol 25:2062–2068. https://doi.org/10.1161/01.ATV.0000183883.72263.13

Sun AY, Wang Q, Simonyi A, Sun GY (2008) Botanical phenolics and brain health. Neuromolecular Med 10:259–274. https://doi.org/10.1007/s12017-008-8052-z

Sun CY, Qi SS, Zhou P et al (2013) Neurobiological and pharmacological validity of curcumin in ameliorating memory performance of senescence-accelerated mice. Pharmacol Biochem Behav 105:76–82. https://doi.org/10.1016/j.pbb.2013.02.002

Sun N, Youle RJ, Finkel T (2016a) The mitochondrial basis of aging. Mol Cell 61:654–666. https://doi.org/10.1016/j.molcel.2016.01.028

Sun Z, Zhan L, Liang L et al (2016b) ZiBu PiYin recipe prevents diabetes-associated cognitive decline in rats: possible involvement of ameliorating mitochondrial dysfunction, insulin resistance pathway and histopathological changes. BMC Complement Altern Med 16:200. https://doi.org/10.1186/s12906-016-1177-y

Surh YJ, Chun KS, Cha HH et al (2001) Molecular mechanisms underlying chemopreventive activities of anti-inflammatory phytochemicals: down-regulation of COX-2 and iNOS through

suppression of NF-kappa B activation. Mutat Res 480–481:243–268. https://doi.org/10.1016/s0027-5107(01)00183-x

Suzuki S, Takada T, Sugawara Y et al (1991) Quercetin induces recombinational mutations in cultured cells as detected by DNA fingerprinting. Jpn J Cancer Res 82:1061–1064. https://doi.org/10.1111/j.1349-7006.1991.tb01757.x

Swindell WR (2012) Dietary restriction in rats and mice: a meta-analysis and review of the evidence for genotype-dependent effects on lifespan. Ageing Res Rev 11:254–270. https://doi.org/10.1016/j.arr.2011.12.006

Szegezdi E, Logue SE, Gorman AM, Samali A (2006) Mediators of endoplasmic reticulum stress-induced apoptosis. EMBO Rep 7:880–885. https://doi.org/10.1038/sj.embor.7400779

Szendroedi J, Phielix E, Roden M (2011) The role of mitochondria in insulin resistance and type 2 diabetes mellitus. Nat Rev Endocrinol 8:92–103. https://doi.org/10.1038/nrendo.2011.138

Tabatabaei Mirakabad FS, Khoramgah MS, Tahmasebinia F et al (2020) The effect of low-level laser therapy and curcumin on the expression of LC3, ATG10 and BAX/BCL2 ratio in PC12 cells induced by 6-hydroxide dopamine. J Lasers Med Sci 11:299–304. https://doi.org/10.34172/jlms.2020.50

Tabrizi R, Tamtaji OR, Mirhosseini N et al (2020) The effects of quercetin supplementation on lipid profiles and inflammatory markers among patients with metabolic syndrome and related disorders: a systematic review and meta-analysis of randomized controlled trials. Crit Rev Food Sci Nutr 60:1855–1868. https://doi.org/10.1080/10408398.2019.1604491

Taguchi A, White MF (2008) Insulin-like signaling, nutrient homeostasis, and life span. Annu Rev Physiol 70:191–212. https://doi.org/10.1146/annurev.physiol.70.113006.100533

Tambara AL, de Los Santos Moraes L, Dal Forno AH et al (2018) Purple pitanga fruit (*Eugenia uniflora* L.) protects against oxidative stress and increase the lifespan in *Caenorhabditis elegans* via the DAF-16/FOXO pathway. Food Chem Toxicol 120:639–650. https://doi.org/10.1016/j.fct.2018.07.057

Tangney CC, Li H, Wang Y et al (2014) Relation of DASH- and Mediterranean-like dietary patterns to cognitive decline in older persons. Neurology 83:1410–1416. https://doi.org/10.1212/WNL.0000000000000884

Taylor RC, Dillin A (2011) Aging as an event of proteostasis collapse. Cold Spring Harb Perspect Biol 3. https://doi.org/10.1101/cshperspect.a004440

Tchkonia T, Morbeck DE, von Zglinicki T et al (2010) Fat tissue, aging, and cellular senescence. Aging Cell 9:667–684. https://doi.org/10.1111/j.1474-9726.2010.00608.x

Tencerova M, Figeac F, Ditzel N et al (2018) High-fat diet-induced obesity promotes expansion of bone marrow adipose tissue and impairs skeletal stem cell functions in mice. J bone Miner Res Off J Am Soc Bone Miner Res 33:1154–1165. https://doi.org/10.1002/jbmr.3408

Terao J (2009) Dietary flavonoids as antioxidants. Forum Nutr 61:87–94. https://doi.org/10.1159/000212741

Thrasivoulou C, Soubeyre V, Ridha H et al (2006) Reactive oxygen species, dietary restriction and neurotrophic factors in age-related loss of myenteric neurons. Aging Cell 5:247–257. https://doi.org/10.1111/j.1474-9726.2006.00214.x

Threapleton DE, Greenwood DC, Evans CEL et al (2013) Dietary fibre intake and risk of cardiovascular disease: systematic review and meta-analysis. BMJ 347:f6879. https://doi.org/10.1136/bmj.f6879

Tian J, Geiss C, Zarse K et al (2021) Green tea catechins EGCG and ECG enhance the fitness and lifespan of *Caenorhabditis elegans* by complex I inhibition. Aging (Albany NY) 13:22629–22648. https://doi.org/10.18632/aging.203597

Tiwari SK, Agarwal S, Seth B et al (2014) Curcumin-loaded nanoparticles potently induce adult neurogenesis and reverse cognitive deficits in Alzheimer's disease model via canonical Wnt/β-catenin pathway. ACS Nano 8:76–103. https://doi.org/10.1021/nn405077y

Tönnies E, Trushina E (2017) Oxidative stress, synaptic dysfunction, and Alzheimer's disease. J Alzheimers Dis 57:1105–1121. https://doi.org/10.3233/JAD-161088

Tower J (2015) Mitochondrial maintenance failure in aging and role of sexual dimorphism. Arch Biochem Biophys 576:17–31. https://doi.org/10.1016/j.abb.2014.10.008

Trayhurn P, Wood IS (2004) Adipokines: inflammation and the pleiotropic role of white adipose tissue. Br J Nutr 92:347–355. https://doi.org/10.1079/bjn20041213

Treaster SB, Ridgway ID, Richardson CA et al (2014) Superior proteome stability in the longest lived animal. Age (Dordr) 36:9597. https://doi.org/10.1007/s11357-013-9597-9

Tremblay F, Brûlé S, Hee Um S et al (2007) Identification of IRS-1 Ser-1101 as a target of S6K1 in nutrient- and obesity-induced insulin resistance. Proc Natl Acad Sci U S A 104:14056–14061. https://doi.org/10.1073/pnas.0706517104

Trifunovic A, Wredenberg A, Falkenberg M et al (2004) Premature ageing in mice expressing defective mitochondrial DNA polymerase. Nature 429:417–423. https://doi.org/10.1038/nature02517

Trim W, Turner JE, Thompson D (2018) Parallels in immunometabolic adipose tissue dysfunction with ageing and obesity. Front Immunol 9:169. https://doi.org/10.3389/fimmu.2018.00169

Trounce I, Byrne E, Marzuki S (1989) Decline in skeletal muscle mitochondrial respiratory chain function: possible factor in ageing. Lancet (London) 1:637–639. https://doi.org/10.1016/s0140-6736(89)92143-0

Tsai I-C, Hsu C-W, Chang C-H et al (2021) The effect of curcumin differs on individual cognitive domains across different patient populations: a systematic review and meta-analysis. Pharmaceuticals (Basel) 14. https://doi.org/10.3390/ph14121235

Tsai SH, Lin-Shiau SY, Lin JK (1999) Suppression of nitric oxide synthase and the down-regulation of the activation of NFkappaB in macrophages by resveratrol. Br J Pharmacol 126:673–680. https://doi.org/10.1038/sj.bjp.0702357

Tseng H-C, Wang M-H, Chang K-C et al (2020) Protective effect of (-)epigallocatechin-3-gallate on rotenone-induced Parkinsonism-like symptoms in rats. Neurotox Res 37:669–682. https://doi.org/10.1007/s12640-019-00143-6

Tsuda T (2012) Dietary anthocyanin-rich plants: biochemical basis and recent progress in health benefits studies. Mol Nutr Food Res 56:159–170. https://doi.org/10.1002/mnfr.201100526

Tsuji PA, Stephenson KK, Wade KL et al (2013) Structure-activity analysis of flavonoids: direct and indirect antioxidant, and antiinflammatory potencies and toxicities. Nutr Cancer 65:1014–1025. https://doi.org/10.1080/01635581.2013.809127

Tufekci KU, Civi Bayin E, Genc S, Genc K (2011) The Nrf2/ARE pathway: a promising target to counteract mitochondrial dysfunction in Parkinson's disease. Parkinsons Dis 2011:314082. https://doi.org/10.4061/2011/314082

Turner RS, Thomas RG, Craft S et al (2015) A randomized, double-blind, placebo-controlled trial of resveratrol for Alzheimer disease. Neurology 85:1383–1391. https://doi.org/10.1212/WNL.0000000000002035

Tyedmers J, Mogk A, Bukau B (2010) Cellular strategies for controlling protein aggregation. Nat Rev Mol Cell Biol 11:777–788. https://doi.org/10.1038/nrm2993

Tysnes O-B, Storstein A (2017) Epidemiology of Parkinson's disease. J Neural Transm 124:901–905. https://doi.org/10.1007/s00702-017-1686-y

Tzanetakou IP, Katsilambros NL, Benetos A et al (2012) "Is obesity linked to aging?": adipose tissue and the role of telomeres. Ageing Res Rev 11:220–229. https://doi.org/10.1016/j.arr.2011.12.003

Ukil A, Maity S, Karmakar S et al (2003) Curcumin, the major component of food flavour turmeric, reduces mucosal injury in trinitrobenzene sulphonic acid-induced colitis. Br J Pharmacol 139:209–218. https://doi.org/10.1038/sj.bjp.0705241

Ulum B, Teker HT, Sarikaya A et al (2018) Bone marrow mesenchymal stem cell donors with a high body mass index display elevated endoplasmic reticulum stress and are functionally impaired. J Cell Physiol 233:8429–8436. https://doi.org/10.1002/jcp.26804

Um SH, Frigerio F, Watanabe M et al (2004) Absence of S6K1 protects against age-and diet-induced obesity while enhancing insulin sensitivity. Nature 431:200–205. https://doi.org/10.1038/nature02866

Unamuno X, Gómez-Ambrosi J, Rodríguez A et al (2018) Adipokine dysregulation and adipose tissue inflammation in human obesity. Eur J Clin Invest 48:e12997. https://doi.org/10.1111/eci.12997

Ungvari Z, Bagi Z, Feher A et al (2010) Resveratrol confers endothelial protection via activation of the antioxidant transcription factor Nrf2. Am J Physiol Heart Circ Physiol 299:H18–H24. https://doi.org/10.1152/ajpheart.00260.2010

Valdes AM, Andrew T, Gardner JP et al (2005) Obesity, cigarette smoking, and telomere length in women. Lancet (London) 366:662–664. https://doi.org/10.1016/S0140-6736(05)66630-5

Valenza A, Bonfanti C, Pasini ME, Bellosta P (2018) Anthocyanins function as anti-inflammatory agents in a *Drosophila* model for adipose tissue macrophage infiltration. Biomed Res Int 2018:6413172. https://doi.org/10.1155/2018/6413172

Valenzano DR, Terzibasi E, Genade T et al (2006) Resveratrol prolongs lifespan and retards the onset of age-related markers in a short-lived vertebrate. Curr Biol 16:296–300. https://doi.org/10.1016/j.cub.2005.12.038

Valls-Pedret C, Sala-Vila A, Serra-Mir M et al (2015) Mediterranean diet and age-related cognitive decline: a randomized clinical trial. JAMA Intern Med 175:1094–1103. https://doi.org/10.1001/jamainternmed.2015.1668

van den Brink AC, Brouwer-Brolsma EM, Berendsen AAM, van de Rest O (2019) The Mediterranean, Dietary Approaches to Stop Hypertension (DASH), and Mediterranean-DASH Intervention for Neurodegenerative Delay (MIND) diets are associated with less cognitive decline and a lower risk of Alzheimer's disease-a review. Adv Nutr 10:1040–1065. https://doi.org/10.1093/advances/nmz054

van der Horst A, Tertoolen LGJ, de Vries-Smits LMM et al (2004) FOXO4 is acetylated upon peroxide stress and deacetylated by the longevity protein hSir2(SIRT1). J Biol Chem 279:28873–28879. https://doi.org/10.1074/jbc.M401138200

van Dijk SJ, Feskens EJM, Bos MB et al (2009) A saturated fatty acid-rich diet induces an obesity-linked proinflammatory gene expression profile in adipose tissue of subjects at risk of metabolic syndrome. Am J Clin Nutr 90:1656–1664. https://doi.org/10.3945/ajcn.2009.27792

Van Gaal LF, Vertommen J, De Leeuw IH (1998) The in vitro oxidizability of lipoprotein particles in obese and non-obese subjects. Atherosclerosis 137(Suppl):S39–S44. https://doi.org/10.1016/s0021-9150(97)00316-x

van Velthoven CTJ, Rando TA (2019) Stem cell quiescence: dynamism, restraint, and cellular idling. Cell Stem Cell 24:213–225. https://doi.org/10.1016/j.stem.2019.01.001

Vanfleteren JR (1993) Oxidative stress and ageing in *Caenorhabditis elegans*. Biochem J 292:605–608. https://doi.org/10.1042/bj2920605

Varady KA, Bhutani S, Church EC, Klempel MC (2009) Short-term modified alternate-day fasting: a novel dietary strategy for weight loss and cardioprotection in obese adults. Am J Clin Nutr 90:1138–1143. https://doi.org/10.3945/ajcn.2009.28380

Varady KA, Bhutani S, Klempel MC et al (2013) Alternate day fasting for weight loss in normal weight and overweight subjects: a randomized controlled trial. Nutr J 12:146. https://doi.org/10.1186/1475-2891-12-146

Varady KA, Cienfuegos S, Ezpeleta M, Gabel K (2021) Cardiometabolic benefits of intermittent fasting. Annu Rev Nutr 41:333–361. https://doi.org/10.1146/annurev-nutr-052020-041327

Vellai T, Takacs-Vellai K, Zhang Y et al (2003) Genetics: influence of TOR kinase on lifespan in *C. elegans*. Nature 426:620. https://doi.org/10.1038/426620a

Vilchez D, Saez I, Dillin A (2014) The role of protein clearance mechanisms in organismal ageing and age-related diseases. Nat Commun 5:5659. https://doi.org/10.1038/ncomms6659

Vinayagam R, Xu B (2015) Antidiabetic properties of dietary flavonoids: a cellular mechanism review. Nutr Metab (Lond) 12:60. https://doi.org/10.1186/s12986-015-0057-7

Virtanen KA, Lidell ME, Orava J et al (2009) Functional brown adipose tissue in healthy adults. N Engl J Med 360:1518–1525. https://doi.org/10.1056/NEJMoa0808949

Visser M, Bouter LM, McQuillan GM et al (1999) Elevated C-reactive protein levels in overweight and obese adults. JAMA 282:2131–2135. https://doi.org/10.1001/jama.282.22.2131

Viswanathan M, Kim SK, Berdichevsky A, Guarente L (2005) A role for SIR-2.1 regulation of ER stress response genes in determining *C. elegans* life span. Dev Cell 9:605–615. https://doi.org/10.1016/j.devcel.2005.09.017

Vitali A, Murano I, Zingaretti MC et al (2012) The adipose organ of obesity-prone C57BL/6J mice is composed of mixed white and brown adipocytes. J Lipid Res 53:619–629

von Zglinicki T (2002) Oxidative stress shortens telomeres. Trends Biochem Sci 27:339–344. https://doi.org/10.1016/s0968-0004(02)02110-2

Vuong T, Benhaddou-Andaloussi A, Brault A et al (2009) Antiobesity and antidiabetic effects of biotransformed blueberry juice in KKA(y) mice. Int J Obes (Lond) 33:1166–1173. https://doi.org/10.1038/ijo.2009.149

Wade AT, Elias MF, Murphy KJ (2021) Adherence to a Mediterranean diet is associated with cognitive function in an older non-Mediterranean sample: findings from the Maine-Syracuse Longitudinal Study. Nutr Neurosci 24:542–553. https://doi.org/10.1080/1028415X.2019.1655201

Wagner AE, Piegholdt S, Rabe D et al (2015) Epigallocatechin gallate affects glucose metabolism and increases fitness and lifespan in *Drosophila melanogaster*. Oncotarget 6:30568–30578. https://doi.org/10.18632/oncotarget.5215

Wajchenberg BL (2000) Subcutaneous and visceral adipose tissue: their relation to the metabolic syndrome. Endocr Rev 21:697–738. https://doi.org/10.1210/edrv.21.6.0415

Walker JM, Klakotskaia D, Ajit D et al (2015) Beneficial effects of dietary EGCG and voluntary exercise on behavior in an Alzheimer's disease mouse model. J Alzheimers Dis 44:561–572. https://doi.org/10.3233/JAD-140981

Wallace DC (1992) Mitochondrial genetics: a paradigm for aging and degenerative diseases? Science 256:628–632. https://doi.org/10.1126/science.1533953

Walter P, Ron D (2011) The unfolded protein response: from stress pathway to homeostatic regulation. Science 334:1081–1086. https://doi.org/10.1126/science.1209038

Wang C, Maddick M, Miwa S et al (2010) Adult-onset, short-term dietary restriction reduces cell senescence in mice. Aging (Albany NY) 2:555–566. https://doi.org/10.18632/aging.100196

Wang D, Yan J, Chen J et al (2015) Naringin improves neuronal insulin signaling, brain mitochondrial function, and cognitive function in high-fat diet-induced obese mice. Cell Mol Neurobiol 35:1061–1071. https://doi.org/10.1007/s10571-015-0201-y

Wang H, Liu J, Li T, Liu RH (2018) Blueberry extract promotes longevity and stress tolerance via DAF-16 in *Caenorhabditis elegans*. Food Funct 9:5273–5282. https://doi.org/10.1039/c8fo01680a

Wang J, Ho L, Qin W et al (2005) Caloric restriction attenuates beta-amyloid neuropathology in a mouse model of Alzheimer's disease. FASEB J 19:659–661. https://doi.org/10.1096/fj.04-3182fje

Wang J, Ho L, Zhao Z et al (2006) Moderate consumption of Cabernet Sauvignon attenuates Abeta neuropathology in a mouse model of Alzheimer's disease. FASEB J 20:2313–2320. https://doi.org/10.1096/fj.06-6281com

Wang L, Li YM, Lei L et al (2016) Purple sweet potato anthocyanin attenuates fat-induced mortality in *Drosophila melanogaster*. Exp Gerontol 82:95–103. https://doi.org/10.1016/j.exger.2016.06.006

Wang Y, Beydoun MA, Liang L et al (2008) Will all Americans become overweight or obese? Estimating the progression and cost of the US obesity epidemic. Obesity 16:2323–2330. https://doi.org/10.1038/oby.2008.351

Wang Y, Li M, Xu X et al (2012) Green tea epigallocatechin-3-gallate (EGCG) promotes neural progenitor cell proliferation and sonic hedgehog pathway activation during adult hippocampal neurogenesis. Mol Nutr Food Res 56:1292–1303. https://doi.org/10.1002/mnfr.201200035

Wang Y, Yin H, Wang L et al (2013) Curcumin as a potential treatment for Alzheimer's disease: a study of the effects of curcumin on hippocampal expression of glial fibrillary acidic protein. Am J Chin Med 41:59–70. https://doi.org/10.1142/S0192415X13500055

Webb AE, Brunet A (2014) FOXO transcription factors: key regulators of cellular quality control. Trends Biochem Sci 39:159–169. https://doi.org/10.1016/j.tibs.2014.02.003

Wegman MP, Guo MH, Bennion DM et al (2015) Practicality of intermittent fasting in humans and its effect on oxidative stress and genes related to aging and metabolism. Rejuvenation Res 18: 162–172. https://doi.org/10.1089/rej.2014.1624

Wein S, Schrader E, Rimbach G, Wolffram S (2013) Oral green tea catechins transiently lower plasma glucose concentrations in female *db/db* mice. J Med Food 16:312–317. https://doi.org/10.1089/jmf.2012.0205

Weisberg SP, Leibel R, Tortoriello DV (2008) Dietary curcumin significantly improves obesity-associated inflammation and diabetes in mouse models of diabesity. Endocrinology 149:3549–3558. https://doi.org/10.1210/en.2008-0262

Weisberg SP, McCann D, Desai M et al (2003) Obesity is associated with macrophage accumulation in adipose tissue. J Clin Invest 112:1796–1808. https://doi.org/10.1172/JCI19246

Welle S, Bhatt K, Thornton CA (2000) High-abundance mRNAs in human muscle: comparison between young and old. J Appl Physiol 89:297–304. https://doi.org/10.1152/jappl.2000.89.1.297

Wellen KE, Hotamisligil GS (2005) Inflammation, stress, and diabetes. J Clin Invest 115:1111–1119. https://doi.org/10.1172/JCI25102

Welton S, Minty R, O'Driscoll T et al (2020) Intermittent fasting and weight loss: systematic review. Can Fam Physician 66:117–125. PMC7021351

Wensink MJ, Cohen AA (2021) The Danaid theory of aging. Front Cell Dev Biol 9:671208. https://doi.org/10.3389/fcell.2021.671208

Whitmer RA, Gunderson EP, Quesenberry CPJ et al (2007) Body mass index in midlife and risk of Alzheimer disease and vascular dementia. Curr Alzheimer Res 4:103–109. https://doi.org/10.2174/156720507780362047

WHO (2021a) Obesity and overweight. https://www.who.int/news-room/fact-sheets/detail/obesity-and-overweight

WHO (2021b) Dementia. https://www.who.int/news-room/fact-sheets/detail/dementia

Widmer RJ, Flammer AJ, Lerman LO, Lerman A (2015) The Mediterranean diet, its components, and cardiovascular disease. Am J Med 128:229–238. https://doi.org/10.1016/j.amjmed.2014.10.014

Wiewiora M, Mertas A, Gluck M et al (2020) Effect of weight loss surgery on biomarkers of angiogenesis in obese patients. Obes Surg 30:3417–3425. https://doi.org/10.1007/s11695-020-04580-7

Wiewiora M, Mertas A, Nowowiejska-Wiewiora A et al (2019) The effects of venous hemodynamics on angiogenesis in morbid obese. Clin Hemorheol Microcirc 71:347–356. https://doi.org/10.3233/CH-180414

Wightman EL, Reay JL, Haskell CF et al (2014) Effects of resveratrol alone or in combination with piperine on cerebral blood flow parameters and cognitive performance in human subjects: a randomised, double-blind, placebo-controlled, cross-over investigation. Br J Nutr 112:203–213. https://doi.org/10.1017/S0007114514000737

Wilkinson JE, Burmeister L, Brooks SV et al (2012) Rapamycin slows aging in mice. Aging Cell 11:675–682. https://doi.org/10.1111/j.1474-9726.2012.00832.x

Willcox BJ, Willcox DC, Todoriki H et al (2007) Caloric restriction, the traditional Okinawan diet, and healthy aging: the diet of the world's longest-lived people and its potential impact on morbidity and life span. Ann N Y Acad Sci 1114:434–455. https://doi.org/10.1196/annals.1396.037

Willeit P, Raschenberger J, Heydon EE et al (2014) Leucocyte telomere length and risk of type 2 diabetes mellitus: new prospective cohort study and literature-based meta-analysis. PLoS One 9:e112483. https://doi.org/10.1371/journal.pone.0112483

Williams AS, Kang L, Wasserman DH (2015) The extracellular matrix and insulin resistance. Trends Endocrinol Metab 26:357–366. https://doi.org/10.1016/j.tem.2015.05.006

Williams GC (1966) Natural selection, the costs of reproduction, and a refinement of Lack's principle. Am Nat 100:687–690. https://doi.org/10.1086/282461

Wilson RA, Deasy W, Stathis CG et al (2018) Intermittent fasting with or without exercise prevents weight gain and improves lipids in diet-induced obese mice. Nutrients 10. https://doi.org/10.3390/nu10030346

Wing RR (2010) Long-term effects of a lifestyle intervention on weight and cardiovascular risk factors in individuals with type 2 diabetes mellitus: four-year results of the Look AHEAD trial. Arch Intern Med 170:1566–1575. https://doi.org/10.1001/archinternmed.2010.334

Wing RR, Blair EH, Bononi P et al (1994) Caloric restriction per se is a significant factor in improvements in glycemic control and insulin sensitivity during weight loss in obese NIDDM patients. Diabetes Care 17:30–36. https://doi.org/10.2337/diacare.17.1.30

Wisløff U, Najjar SM, Ellingsen O et al (2005) Cardiovascular risk factors emerge after artificial selection for low aerobic capacity. Science 307:418–420. https://doi.org/10.1126/science.1108177

Witte AV, Kerti L, Margulies DS, Flöel A (2014) Effects of resveratrol on memory performance, hippocampal functional connectivity, and glucose metabolism in healthy older adults. J Neurosci Off J Soc Neurosci 34:7862–7870. https://doi.org/10.1523/JNEUROSCI.0385-14.2014

Witte AV, Fobker M, Gellner R et al (2009) Caloric restriction improves memory in elderly humans. Proc Natl Acad Sci U S A 106:1255–1260. https://doi.org/10.1073/pnas.0808587106

Włodarczyk M, Jabłonowska-Lietz B, Olejarz W, Nowicka G (2018) Anthropometric and dietary factors as predictors of DNA damage in obese women. Nutrients 10. https://doi.org/10.3390/nu10050578

Włodarczyk M, Nowicka G (2019) Obesity, DNA damage, and development of obesity-related diseases. Int J Mol Sci 20. https://doi.org/10.3390/ijms20051146

Włodarek D (2019) Role of ketogenic diets in neurodegenerative diseases (Alzheimer's disease and Parkinson's disease). Nutrients 11. https://doi.org/10.3390/nu11010169

Wlodek D, Gonzales M (2003) Decreased energy levels can cause and sustain obesity. J Theor Biol 225:33–44. https://doi.org/10.1016/s0022-5193(03)00218-2

Wolfram S, Raederstorff D, Wang Y et al (2005) TEAVIGO (epigallocatechin gallate) supplementation prevents obesity in rodents by reducing adipose tissue mass. Ann Nutr Metab 49:54–63. https://doi.org/10.1159/000084178

Wong RHX, Berry NM, Coates AM et al (2013) Chronic resveratrol consumption improves brachial flow-mediated dilatation in healthy obese adults. J Hypertens 31:1819–1827. https://doi.org/10.1097/HJH.0b013e328362b9d6

Wong RHX, Howe PRC, Buckley JD et al (2011) Acute resveratrol supplementation improves flow-mediated dilatation in overweight/obese individuals with mildly elevated blood pressure. Nutr Metab Cardiovasc Dis 21:851–856. https://doi.org/10.1016/j.numecd.2010.03.003

Wong RHX, Nealon RS, Scholey A, Howe PRC (2016) Low dose resveratrol improves cerebrovascular function in type 2 diabetes mellitus. Nutr Metab Cardiovasc Dis 26:393–399. https://doi.org/10.1016/j.numecd.2016.03.003

Wong YC, Krainc D (2017) α-Synuclein toxicity in neurodegeneration: mechanism and therapeutic strategies. Nat Med 23:1–13. https://doi.org/10.1038/nm.4269

Wood JG, Rogina B, Lavu S et al (2004) Sirtuin activators mimic caloric restriction and delay ageing in metazoans. Nature 430:686–689. https://doi.org/10.1038/nature02789

Wright ORL, Netzel GA, Sakzewski AR (2013) A randomized, double-blind, placebo-controlled trial of the effect of dried purple carrot on body mass, lipids, blood pressure, body composition, and inflammatory markers in overweight and obese adults: the QUENCH trial. Can J Physiol Pharmacol 91:480–488. https://doi.org/10.1139/cjpp-2012-0349

Wu A, Ying Z, Gomez-Pinilla F (2004) The interplay between oxidative stress and brain-derived neurotrophic factor modulates the outcome of a saturated fat diet on synaptic plasticity and cognition. Eur J Neurosci 19:1699–1707. https://doi.org/10.1111/j.1460-9568.2004.03246.x

Wu J, Boström P, Sparks LM et al (2012a) Beige adipocytes are a distinct type of thermogenic fat cell in mouse and human. Cell 150:366–376. https://doi.org/10.1016/j.cell.2012.05.016

Wu K-J, Hsieh M-T, Wu C-R et al (2012b) Green tea extract ameliorates learning and memory deficits in ischemic rats via its active component polyphenol epigallocatechin-3-gallate by modulation of oxidative stress and neuroinflammation. Evid Based Complement Alternat Med 2012:163106. https://doi.org/10.1155/2012/163106

Wullschleger S, Loewith R, Hall MN (2006) TOR signaling in growth and metabolism. Cell 124: 471–484. https://doi.org/10.1016/j.cell.2006.01.016

Xiao J, Sheng X, Zhang X et al (2016) Curcumin protects against myocardial infarction-induced cardiac fibrosis via SIRT1 activation *in vivo* and *in vitro*. Drug Des Devel Ther 10:1267–1277. https://doi.org/10.2147/DDDT.S104925

Xiong L-G, Chen Y-J, Tong J-W et al (2018) Epigallocatechin-3-gallate promotes healthy lifespan through mitohormesis during early-to-mid adulthood in *Caenorhabditis elegans*. Redox Biol 14:305–315. https://doi.org/10.1016/j.redox.2017.09.019

Xu C, Bailly-Maitre B, Reed JC (2005) Endoplasmic reticulum stress: cell life and death decisions. J Clin Invest 115:2656–2664. https://doi.org/10.1172/JCI26373

Xu M, Palmer AK, Ding H et al (2015) Targeting senescent cells enhances adipogenesis and metabolic function in old age. Elife 4:e12997. https://doi.org/10.7554/eLife.12997

Xu M, Pirtskhalava T, Farr JN et al (2018) Senolytics improve physical function and increase lifespan in old age. Nat Med 24:1246–1256. https://doi.org/10.1038/s41591-018-0092-9

Xu Q, Langley M, Kanthasamy AG, Reddy MB (2017) Epigallocatechin gallate has a neurorescue effect in a mouse model of Parkinson disease. J Nutr 147:1926–1931. https://doi.org/10.3945/jn.117.255034

Xu R, Bai Y, Yang K, Chen G (2020) Effects of green tea consumption on glycemic control: a systematic review and meta-analysis of randomized controlled trials. Nutr Metab (Lond) 17:56. https://doi.org/10.1186/s12986-020-00469-5

Yahagi N, Shimano H, Matsuzaka T et al (2004) p53 involvement in the pathogenesis of fatty liver disease. J Biol Chem 279:20571–20575. https://doi.org/10.1074/jbc.M400884200

Yakes FM, Van Houten B (1997) Mitochondrial DNA damage is more extensive and persists longer than nuclear DNA damage in human cells following oxidative stress. Proc Natl Acad Sci U S A 94:514–519. https://doi.org/10.1073/pnas.94.2.514

Yamamoto M, Kensler TW, Motohashi H (2018) The KEAP1-NRF2 system: a thiol-based sensor-effector apparatus for maintaining redox homeostasis. Physiol Rev 98:1169–1203. https://doi.org/10.1152/physrev.00023.2017

Yamauchi T, Kamon J, Ito Y et al (2003) Cloning of adiponectin receptors that mediate antidiabetic metabolic effects. Nature 423:762–769. https://doi.org/10.1038/nature01705

Yang S, Wang G, Ma ZF et al (2020) Dietary advanced glycation end products-induced cognitive impairment in aged ICR mice: protective role of quercetin. Mol Nutr Food Res 64:1901019. https://doi.org/10.1002/mnfr.201901019

Yang Y, Liu Y, Wang Y et al (2022) Regulation of SIRT1 and its roles in inflammation. Front Immunol 13:831168. https://doi.org/10.3389/fimmu.2022.831168

Yasumoto Y, Hashimoto C, Nakao R et al (2016) Short-term feeding at the wrong time is sufficient to desynchronize peripheral clocks and induce obesity with hyperphagia, physical inactivity and metabolic disorders in mice. Metabolism 65:714–727. https://doi.org/10.1016/j.metabol.2016.02.003

Ye J, Meng X, Yan C, Wang C (2010) Effect of purple sweet potato anthocyanins on beta-amyloid-mediated PC-12 cells death by inhibition of oxidative stress. Neurochem Res 35:357–365. https://doi.org/10.1007/s11064-009-0063-0

Yeh WC, Bierer BE, McKnight SL (1995) Rapamycin inhibits clonal expansion and adipogenic differentiation of 3T3-L1 cells. Proc Natl Acad Sci U S A 92:11086–11090. https://doi.org/10.1073/pnas.92.24.11086

Yilmaz E (2017) Endoplasmic reticulum stress and obesity. Adv Exp Med Biol 960:261–276. https://doi.org/10.1007/978-3-319-48382-5_11

Yin W, Löf M, Pedersen NL et al (2021) Mediterranean dietary pattern at middle age and risk of Parkinson's disease: a Swedish Cohort Study. Mov Disord 36:255–260. https://doi.org/10.1002/mds.28314

Yoon G, Song J (2019) Intermittent fasting: a promising approach for preventing vascular dementia. J Lipid Atheroscler 8:1–7. https://doi.org/10.12997/jla.2019.8.1.1

Yoshino J, Conte C, Fontana L et al (2012) Resveratrol supplementation does not improve metabolic function in nonobese women with normal glucose tolerance. Cell Metab 16:658–664. https://doi.org/10.1016/j.cmet.2012.09.015

Yoshizaki T, Milne JC, Imamura T et al (2009) SIRT1 exerts anti-inflammatory effects and improves insulin sensitivity in adipocytes. Mol Cell Biol 29:1363–1374. https://doi.org/10.1128/MCB.00705-08

Youdim KA, Shukitt-Hale B, MacKinnon S et al (2000) Polyphenolics enhance red blood cell resistance to oxidative stress: *in vitro* and *in vivo*. Biochim Biophys Acta 1523:117–122. https://doi.org/10.1016/s0304-4165(00)00109-4

Yu H, Lin L, Zhang Z et al (2020) Targeting NF-κB pathway for the therapy of diseases: mechanism and clinical study. Signal Transduct Target Ther 5:209. https://doi.org/10.1038/s41392-020-00312-6

Yu SY, Zhang M, Luo J et al (2013) Curcumin ameliorates memory deficits via neuronal nitric oxide synthase in aged mice. Prog Neuropsychopharmacol Biol Psychiatry 45:47–53. https://doi.org/10.1016/j.pnpbp.2013.05.001

Yu Z, Malik VS, Keum N et al (2016) Associations between nut consumption and inflammatory biomarkers. Am J Clin Nutr 104:722–728. https://doi.org/10.3945/ajcn.116.134205

Yuan H, Li Y, Ling F et al (2020) The phytochemical epigallocatechin gallate prolongs the lifespan by improving lipid metabolism, reducing inflammation and oxidative stress in high-fat diet-fed obese rats. Aging Cell 19:e13199. https://doi.org/10.1111/acel.13199

Zaki M, Basha W, El-Bassyouni HT et al (2018a) Evaluation of DNA damage profile in obese women and its association to risk of metabolic syndrome, polycystic ovary syndrome and recurrent preeclampsia. Genes Dis 5:367–373. https://doi.org/10.1016/j.gendis.2018.03.001

Zaki M, Kamal S, Basha WA et al (2018b) Assessment of DNA damage in obese premenopausal women with metabolic syndrome. Gene Rep 10:42–46. https://doi.org/10.1016/j.genrep.2017.10.012

Zang M, Xu S, Maitland-Toolan KA et al (2006) Polyphenols stimulate AMP-activated protein kinase, lower lipids, and inhibit accelerated atherosclerosis in diabetic LDL receptor-deficient mice. Diabetes 55:2180–2191. https://doi.org/10.2337/db05-1188

Zenaro E, Piacentino G, Constantin G (2017) The blood-brain barrier in Alzheimer's disease. Neurobiol Dis 107:41–56. https://doi.org/10.1016/j.nbd.2016.07.007

Zhang DD, Lo S-C, Cross JV et al (2004) Keap1 is a redox-regulated substrate adaptor protein for a Cul3-dependent ubiquitin ligase complex. Mol Cell Biol 24:10941–10953. https://doi.org/10.1128/MCB.24.24.10941-10953.2004

Zhang HH, Huang J, Düvel K et al (2009a) Insulin stimulates adipogenesis through the Akt-TSC2-mTORC1 pathway. PLoS One 4:e6189. https://doi.org/10.1371/journal.pone.0006189

Zhang K, Kaufman RJ (2008) From endoplasmic-reticulum stress to the inflammatory response. Nature 454:455–462. https://doi.org/10.1038/nature07203

Zhang L, Jie G, Zhang J, Zhao B (2009b) Significant longevity-extending effects of EGCG on *Caenorhabditis elegans* under stress. Free Radic Biol Med 46:414–421. https://doi.org/10.1016/j.freeradbiomed.2008.10.041

Zhang Q, Lenardo MJ, Baltimore D (2017) 30 Years of NF-κB: a blossoming of relevance to human pathobiology. Cell 168:37–57. https://doi.org/10.1016/j.cell.2016.12.012

Zhang W, Qu J, Liu G-H, Belmonte JCI (2020) The ageing epigenome and its rejuvenation. Nat Rev Mol Cell Biol 21:137–150. https://doi.org/10.1038/s41580-019-0204-5

Zhang X, Zhang G, Zhang H et al (2008) Hypothalamic IKKbeta/NF-kappaB and ER stress link overnutrition to energy imbalance and obesity. Cell 135:61–73. https://doi.org/10.1016/j.cell.2008.07.043

Zhang Y, Fischer KE, Soto V et al (2015) Obesity-induced oxidative stress, accelerated functional decline with age and increased mortality in mice. Arch Biochem Biophys 576:39–48. https://doi.org/10.1016/j.abb.2014.12.018

Zhou T, Zhu M, Liang Z (2018) (-)-Epigallocatechin-3-gallate modulates peripheral immunity in the MPTP-induced mouse model of Parkinson's disease. Mol Med Rep 17:4883–4888. https://doi.org/10.3892/mmr.2018.8470

Zhou W, Chen L, Hu X et al (2019) Effects and mechanism of epigallocatechin-3-gallate on apoptosis and mTOR/AKT/GSK-3β pathway in substantia nigra neurons in Parkinson rats. Neuroreport 30:60–65. https://doi.org/10.1097/WNR.0000000000001149

Zhu L-N, Mei X, Zhang Z-G et al (2019) Curcumin intervention for cognitive function in different types of people: a systematic review and meta-analysis. Phytother Res 33:524–533. https://doi.org/10.1002/ptr.6257

Zhu Y, Bickford PC, Sanberg P et al (2008) Blueberry opposes beta-amyloid peptide-induced microglial activation via inhibition of p44/42 mitogen-activation protein kinase. Rejuvenation Res 11:891–901. https://doi.org/10.1089/rej.2008.0757

Zhu Y, Chen X, Chen Z et al (2004) Curcumin protects mitochondria from oxidative damage and attenuates apoptosis in cortical neurons. Acta Pharmacol Sin 25:1606–1612

Zoncu R, Efeyan A, Sabatini DM (2011) mTOR: from growth signal integration to cancer, diabetes and ageing. Nat Rev Mol Cell Biol 12:21–35. https://doi.org/10.1038/nrm3025

Zuo L, Prather ER, Stetskiv M et al (2019) Inflammaging and oxidative stress in human diseases: from molecular mechanisms to novel treatments. Int J Mol Sci 20. https://doi.org/10.3390/ijms20184472

Chapter 16
Clinical Ageing

Roxana Surugiu, Daiana Burdusel, Mihai-Andrei Ruscu, Andreea Cercel, Dirk M. Hermann, Israel Fernandez Cadenas, and Aurel Popa-Wagner

Abstract Ageing is generally characterised by the declining ability to respond to stress, increasing homeostatic imbalance, and increased risk of ageing-associated diseases. Mechanistically, the lifelong accumulation of a wide range of molecular and cellular impairments leads to organismal senescence. The aging population poses a severe medical concern due to the burden it places on healthcare systems and the general public as well as the prevalence of diseases and impairments associated with old age. In this chapter, we discuss organ failure during ageing as well as ageing of the hypothalamic–pituitary–adrenal axis and drugs that can regulate it. A much-debated subject is about ageing and regeneration. With age, there is a gradual decline in the regenerative properties of most tissues. The goal of regenerative medicine is to restore cells, tissues, and structures that are lost or damaged after disease, injury, or ageing. The question arises as to whether this is due to the intrinsic ageing of stem cells or, rather, to the impairment of stem-cell function in the aged tissue environment. The risk of having a stroke event doubles each decade after the age of 55. Therefore, it is of great interest to develop neurorestorative therapies for stroke which occurs mostly in elderly people. Initial enthusiasm for stimulating restorative processes in the ischaemic brain with cell-based therapies has meanwhile converted into a more balanced view, recognising impediments related to survival, migration, differentiation, and integration of

R. Surugiu · D. Burdusel · D. M. Hermann · A. Popa-Wagner (✉)
Experimental Research Centre for Normal and Pathological Aging, University of Medicine and Pharmacy of Craiova, Craiova, Romania

University Hospital Essen, University of Duisburg-Essen, Essen, Germany
e-mail: Dirk.Hermann@uk-essen.de; aurel.popa-wagner@geriatrics-healthyageing.com

M.-A. Ruscu
Experimental Research Centre for Normal and Pathological Aging, University of Medicine and Pharmacy of Craiova, Craiova, Romania

A. Cercel · I. F. Cadenas
Stroke Pharmacogenomics and Genetics Group, Sant Pau Hospital Institute of Research, Barcelona, Spain

J. R. Harris, V. I. Korolchuk (eds.), *Biochemistry and Cell Biology of Ageing: Part IV, Clinical Science*, Subcellular Biochemistry 103,
https://doi.org/10.1007/978-3-031-26576-1_16

therapeutic cells in the hostile aged brain environment. Therefore, a current lack of understanding of the fate of transplanted cells means that the safety of cell therapy in stroke patients is still unproven. Another issue associated with ischaemic stroke is that patients at risk for these sequels of stroke are not duly diagnosed and treated due to the lack of reliable biomarkers. However, recently neurovascular unit-derived exosomes in response to Stroke and released into serum are new plasma genetic and proteomic biomarkers associated with ischaemic stroke. The second valid option, which is also more economical, is to invest in prevention.

Keywords Ageing · Obesity · Comorbidities · Dementia · Stroke · Genomics · Rehabilitation

Introduction

Ageing is associated with a decline of locomotor, sensory, and cognitive performance in humans and experimental animals, part of which is due to the lifelong accumulation of damage at the molecular, cellular, and tissular levels. However, these defects alone cannot account for the systematic deterioration and loss of function that characterises the senescent phenotype, making it imperative to pursue a multi-systems, multi-disciplinary, integrative approach to functional senescence. At the molecular level, age-related changes occur gradually, and most such changes are within the error range of current investigational tools.

Ageing, a Systems-Biology Approach

Organismal senescence is the ageing of whole organisms. Ageing is generally characterised by the declining ability to respond to stress, increasing homeostatic imbalance, and increased risk of ageing-associated diseases. Mechanistically, organismal senescence is caused by the gradual, lifelong accumulation of a wide variety of molecular and cellular damage. Various types of errors have been proposed to accumulate with age, either due to an increased rate of production or because of decreased repair or clearance of damage with time. However, no theory sufficiently explains all the changes in the ageing process. Although some changes typically occur with ageing, they occur at different rates and to different extents. With the continuing increase in lifespan, new approaches are required to unravel the complexity of the ageing process and its implications for age-associated diseases. The rapid accumulation of biological data makes it possible to compile detailed schemes of the metabolic networks within a cell. In order to improve our understanding of cells and organisms as physiological, biochemical, and genetic systems, we have to study them as an integrated informational and metabolic system. It is clear that the next step in implementing these databases is to integrate them under a specific biological perspective (Buga et al. 2011).

The Impact of Ageing and Age-Related Comorbidities on Stroke Outcome in Animal Models and Humans

Obesity and Ageing

In today's society, ageing has become one of the biggest concerns in regard to health-associated problems. By 2050, there will be over 2.1 billion people over the age of 60 (Amaya-Montoya et al. 2020). Age-related diseases (ARDs) are hard to identify, having a pathogenesis that remains unclear. ARDs are characterised by ongoing cell death and degradation in the quality or functionality of tissues and organs, increasing sensitivity and vulnerability to specific diseases (Luo et al. 2020). Even though multiple factors contribute to ARDs, some of the basic mechanisms seem to be common, such as oxidative stress, iron build-up, inflammation, cell damage, and malfunction (Xu et al. 2008; Franceschi et al. 2018).

A prospective study examined the potential link between midlife body mass index (BMI) and long-term health effects, concluding that midlife obesity is associated with considerably increased risks of hospitalisation and mortality among people who lived above the age of 65 compared to normal-weight subjects with comparable baseline cardiovascular profiles (Yan et al. 2006).

In a cross-sectional analysis of over 73,000 people between 50 and 76 years, BMI was linked to adverse pathologies in 90% of women and 71% of men (https://doi.org/10.1016/j.ssmph.2020.100547). Increased BMI is also associated with symptoms like chronic pain. One study reported that subjects (mean age 80 years old, adjusted for age and comorbidities) who were obese were twice as likely to complain of recurring pain of at least moderate intensity compared to those who were of normal weight and that subjects who were severely obese were four times more likely to report chronic pain (McCarthy et al. 2008).

Obesity modifies the way that fatty acids, glucose, and amino acids are metabolised, which lowers insulin sensitivity and reduces the body's capacity to respond to changes in energy supply (Johnson et al. 2009). Given that ageing's metabolic impacts include altered mitochondrial function and impaired nutrient-signalling pathways, which control the ratio of insulin to glucagon in regulating blood glucose, there is reason to suspect that obesity directly contributes to the ageing process (Riera and Dillin 2015). Nutrient-signalling pathways might target rapamycin (mTOR) responsible for detecting energy levels in the cell and sirtuin proteins involved in AMPK (5′ adenosine monophosphate-activated protein kinase) activity (Kathleen Berkowitz et al. 2014; Kanfi et al. 2012; Tomimatsu and Narita 2015).

By comparing young and old people in various microarray studies done on different animals and also on humans, the discovery of shared functional genes and biomarkers for ageing and obesity may reveal metabolic links between obesity and ageing (Edwards et al. 2007; Ida et al. 2003).

Both adipose tissue (AT) and adipocytes can be impacted by hypoxia, which is a well-known hallmark of pathological conditions including wound healing or

ischaemic diseases (Trayhurn et al. 2008). According to studies done on human adipocytes, hypoxia causes the production of several adipokines that are linked to inflammation, such as adiponectin, leptin, and interleukin-6. Furthermore, it has been demonstrated that macrophages alter their inflammatory mediator production in response to hypoxia (Trayhurn et al. 2008; Lewis et al. 1999). Since ageing itself is characterised by a reduction in oxygen delivery to tissues, hypoxia, which is considered to be partially responsible for inflammation in obesity, may also have an impact on the ageing process (Valli et al. 2015).

Insulin Resistance and Ageing

Ageing and obesity have numerous links with diabetes. According to a study conducted in America, the prevalence of diabetes increases with age (Fang 2018). Furthermore, there will be at least 592 million diabetes patients worldwide by the year 2035, up from the anticipated 422 million in 2014, and it has been shown that more than half of all diabetics are with an over the normal BMI (Khan et al. 2019). Therefore, there is a solid basis for suspicion that diabetes and obesity are closely related (Khadra et al. 2019). On the other hand, in diabetics, insulin no longer has the ability to mediate cellular glucose uptake and utilisation, which is clinically known as insulin resistance and encourages fat deposits. Additionally, the growing fat mass promotes many cytokines that accelerate the breakdown of muscles. A severe form of insulin resistance is brought on by the loss of muscle mass, which results in less insulin-responsive target tissue (Dominguez and Barbagallo 2007; Baek et al. 2014).

In older and obese individuals, insulin production and effectiveness decrease. The increased synthesis and release of several inflammatory substances, such as TNF-α and IL-6, which are associated with obesity, modify insulin sensitivity by changing some crucial stages in the insulin signalling pathway, which leads to the development of insulin resistance (Mcternan et al. 2006; Shoelson et al. 2007; Matulewicz and Karczewska-Kupczewska 2016). Studies have shown that insulin resistance is necessary for protein anabolism, which directly affects the atrophy of muscle fibres (Nomura et al. 2007). The rate of muscle catabolism is greater in obese people with insulin resistance, as shown in research highlighting that the quality and strength of the leg muscles decline noticeably in older diabetics (Abbatecola et al. 2005). Therefore, low muscle mass and strength are caused by insulin resistance, which leads to obesity over time. Furthermore, mitochondrial dysfunction and insulin resistance are connected to one another (Wang et al. 2020).

Insulin resistance encourages dyslipidaemia, which is characterised by a reduction in high-density lipoprotein cholesterol (HDL-C) concentration and an increase in triglyceride levels (TAG) (Robins et al. 2011). On the other hand, excessive TAG synthesis might lead to cellular and systemic oxidative damage. As a result, these systemic diseases may impede insulin signalling, which in turn may accelerate ageing and encourage atherosclerosis (Dzięgielewska-Gęsiak et al. 2020). In addition, oxidative stress and insulin resistance are both significantly influenced by

ageing (Brewer 2010; da Costaa et al. 2016). One of the key factors in the ageing process, according to damage theories, is the build-up of ROS (reactive oxygen species), which causes accumulative damage to DNA, protein, and lipid molecules (Dzięgielewska-Gęsiak et al. 2020). However, Dzięgielewska-Gęsiak and colleagues found no discernible difference in antioxidant defence between older people with greater body mass who were insulin-resistant and those who were insulin-sensitive. Furthermore, there was an inverse connection between TBARS (Thiobarbituric Acid-Reacting Substances) and HDL-C, but only in the elderly insulin-resistant group. It has been demonstrated that the low molecular weight antioxidants, of which HDL-C is an agent, are altered in older individuals, which may increase oxidative stress and hence contribute to the onset of ageing.

Organ Failure During Ageing

The aged population represents a serious medical concern, not only because of disabilities and age-related diseases (ARDs), but also because of the burden on healthcare systems and the general public. ARDs are caused by the progressive deterioration of tissues and organs through injury of cells, oxidation stress, and iron accumulation (Zhou et al. 2020).

The mitochondrial theory of ageing suggests that reactive oxygen species production causes DNA mutations in mitochondria which leads to decreased functionality and eventually cell death. Additionally, ROS production leads to damaged proteins and lipids in the body, which causes many age-related diseases such as Parkinson's, Huntington's, Alzheimer's, hereditary spastic paraplegia, and more. It is believed that tissues with high energy demands such as the eyes, heart, liver, muscles, and endocrine glands have higher numbers of mitochondria per cell (Brennan and Kantorow 2009).

The sources of ROS can be exogenous (UV light, visible light, ionising radiation, chemotherapeutics, and environmental toxins) or endogenous (activity of peroxisomes, lipoxygenases, NADH oxidase, cytochrome P450, and, of course, mitochondrial respiration) (Brennan et al. 2018).

In the context of Alzheimer's disease (AD), a number of researchers have demonstrated the possibility that this type of oxidative damage could occur before or even precipitate the aggregation of Aβ (β amyloid). Through electron-transfer interactions involving bound redox-active copper and/or iron ions it has, however, been demonstrated that Aβ can generate H_2O_2, a crucial ROS, directly from molecular oxygen. Fenton chemistry quickly converts H_2O_2 into a very aggressive hydroxyl radical. This highly reactive free radical may be responsible for a lot of the early oxidative damage in AD (Allsop et al. 2008).

The ferroptosis is due to elevated intracellular iron concentration, which increases ROS levels and causes lipid peroxidation and cell death. Small molecules that inhibit glutathione biosynthesis or the glutathione-dependent antioxidant enzyme glutathione peroxidase 4 (GPX4) can initiate ferroptosis, a non-apoptotic iron-dependent

cell death characterised by the iron-dependent accumulation of reactive oxygen species and depletion of plasma membrane polyunsaturated fatty acids (PUFAs) (Lee et al. 2020).

The primary site of iron utilisation is the mitochondria, the iron transport across the inner mitochondrial membrane is dependent on the membrane transporter mitoferrin 1/2 (Mfrn1/2), and an imbalance of it can lead to mitochondrial iron accumulation and oxidative injury. Numerous neurological conditions linked to ferroptosis have been found to have Mfrn1/2 damage. During ageing, iron accumulates in a number of organs, like the brain and muscle, causing more oxidative damage and functional decline. Iron levels in the cerebral cortex and cerebellum are highest in preclinical AD patients, who also experience gradual cognitive impairment. This suggests that an imbalance in iron homeostasis is a precursor to neurodegeneration in AD. Abnormal iron accumulation in the substantia nigra, particularly in the RII region, can be used as a biomarker to determine the severity of Parkinson's disease and distinguish it from healthy controls. The level of iron is therefore a biomarker for the potential role of ferroptosis and an important factor for ARDs, because it contributes to organ and tissue cell death, in the pathogenesis and progression of amyotrophic lateral sclerosis, Huntington's disease, cardiomyopathy, and type 1 diabetes.

Numerous small-molecule ferroptosis inducers and inhibitors including erastin, glutamate, liproxstatin-1 (Lip-1), and ferrostatin-1 (Fer-1) have been discovered (Zhou et al. 2020).

Recently, there has been an increased interest in the role that ferroptosis plays in myocardial pathology. Myocardial injury relies heavily on iron homeostasis, and the accumulation of iron in the myocardium results in iron overload cardiomyopathy. In addition, myocardial haemorrhage may play a role in the accumulation of iron in cardiac tissue, leading to excessive production of ROS and the onset of inflammatory processes (Kremastinos and Farmakis 2011; Kobayashi et al. 2018).

The dysregulation of the cytokine network and its homeostasis, also known as "inflamm-ageing", is a common finding in ageing and age-related diseases. Cytokine dysregulation is thought to play a key role in the remodelling of the immune system as people get older (Rea et al. 2018). The major pro-inflammatory cytokines, such as IL-6, TNF-α, and IL-1, play a significant role in many age-related diseases and significantly contribute to the phenomenon of inflammation-ageing in healthy elderly people. IL-1 gene variations are associated with cardiovascular disease and Alzheimer's disease with earlier onset or more severe progression, but not with osteoporosis. Certain IL-1 haplotype carriers produce elevated levels of IL-1. Centenarians have higher levels of IL-18, which have been linked to patients with heart failure, ischaemic heart disease, type 1 diabetes, and Alzheimer's disease. IL-6 modulates the acute phase response, the transition from innate to acquired immunity, metabolic regulation, and the pathogenesis of numerous chronic diseases. By producing IL-1 Rα and soluble tumour necrosis factor receptor p55 (sTNF-R55), it modulates the acute inflammatory response and has both pro- and anti-inflammatory effects.

Normally, interleukin-6 is low in the blood, but it increases with age and in people with signs of frailty and chronic disease, where it correlates with mortality. Sarcopenia and muscle loss are correlated with IL-6, a cardiovascular disease risk factor.

The pro-inflammatory cytokine TNF-α, which increases with age and is linked to age-related disease is another key player in the immune response. When it acts locally in the tissues, it is a pro-inflammatory mediator that can be beneficial, but when it is released systemically, it can be extremely harmful.

In studies of intracellular ageing, tumour necrosis factor-α has been found to increase in elderly people with atherosclerosis, centenarians, and octogenarians, and to be linked to mortality. TNF-α increased the risk of recurrent cardiac events in post-MI (myocardial infarction) patients, and TNF receptors predicted cardiovascular disease in renal patients.

TNF-α mediates metabolic changes, and type 2 diabetes mellitus was associated with lower muscle mass and strength in older groups when TNF-α levels were elevated.

Complex cellular and molecular changes that take place in the cells over time are basically linked to cell senescence, the most important process of ageing. Telomere erosion, changes in protein processing, lifestyle/epigenetics factors, and changes in gene expression are all major biological phenomena. Recent research has shown that miRNAs regulate several pathways that are involved in ageing and cellular senescence. Three miRNAs, miR151a-5p, miR-181a-5p, and miR-1248, were found to be significantly down-regulated in older individuals in recent studies. In addition, their levels decreased in the serum samples of elderly rhesus monkeys (Kumar et al. 2017).

Patients with hyperlipidaemia, hypertension, and diabetes were found to have lower levels of miR-92a, miR-126, miR-130a, miR-222, and miR-370 expression in serum samples from patients with atherosclerosis and pre-atherosclerosis. However, when compared to healthy controls, miR-21, miR-122, miR-130a, and miR-211 were significantly elevated while miR-92a, miR-126, and miR-222 were significantly decreased (Kumar et al. 2017).

Obesity is also a serious risk factor for many metabolic disorders, particularly diabetes, and an age-related health problem. The prevalence of obesity has increased over the past 10 years, particularly in developed countries. Three serum miRNAs—miR-138, miR-15b, and miR-376a—that were found to have potential as predictive biomarkers in obesity were found in a study on 13 patients with type 2 diabetes, 20 obese patients, 16 obese patients with type 2 diabetes, and 20 healthy controls (Pescador et al. 2013).

Recent studies have proposed that miRNA expression is deregulated in obese patients, and miRNAs are the potent regulator of many diseases related to obesity. miR-138 and miR-376a can be used as predictive biomarkers to distinguish obese patients from diabetic patients, obese diabetic patients, and healthy controls. Additionally, diabetic and obese diabetic patients can be distinguished using the combination of miR-503 and miR-138 (Kumar et al. 2017).

Ageing of the HPA Axis and Drugs That Can Regulate It

The primary neuroendocrine response regulating stress adaptation is activation of the hypothalamic–pituitary–adrenal (HPA) axis, which is accompanied by stimulation of parvocellular neurons in the hypothalamic paraventricular nucleus to secrete corticotropin-releasing hormone (CRH) and vasopressin (VP), which in turn enhances pituitary adrenocorticotropic hormone (ACTH) secretion and glucocorticoid secretion from the adrenal cortex. Normal brain function and neuroplasticity depend on the basal synthesis of glucocorticoids as well as brief spikes during stressful situations. While the involvement of the HPA axis is necessary for survival under stress, prolonged exposure to stress hormones can increase the risk of immunological, metabolic, and psychological changes (Aguilera 2011).

In some elderly adults, HPA axis dysfunction may play a role in ageing-related conditions such as depression, cognitive impairment, and Alzheimer's disease. Additionally to neuro-cognitive dysfunction, it has been linked to deteriorating physical performance that could be brought on by sarcopenia (Gupta 2014).

Acute and chronic stress are both experienced by elderly adults in ways that are similar to earlier stages of life. A specific and typically unpredictable life event, as well as the supposed mental or physical difficulty that goes along with it, are the main components of acute stress (for example: giving a speech while being evaluated or criticised) (Dickerson and Kemeny 2004). Due to their physical and mental decline, older persons frequently experience acute stress, which can later turn into chronic stress. A person's reaction to a stressor that lasts for a long time (such as caring) (Miller et al. 2007) or to numerous acute stressors is chronic stress (e.g., negative interpersonal experiences) (Rosnick et al. 2007; Uchino et al. 2001).

At many levels, the HPA axis is influenced by significant morphological and functional changes, in both experimental animals and humans, during the ageing process. Particularly visible are the impaired neuronal cells and the compensatory gliosis at the level of the limbic system, the hippocampus, and the hypothalamus. Due to the suprachiasmatic nucleus function as the essential pacemaker of multiple circadian rhythms, in particular, neuronal damage of this nucleus (Swaab et al. 1985) may be blamed for changes in the circadian temporal structure of the ageing organism. Additionally, the loss of neurons suggests a decrease in glucocorticoid receptors, which results in a dysfunctional control of adrenocortical secretion, particularly in the hypothalamus, hippocampus, and limbic region. In fact, it is well known that the hippocampus plays a role in maintaining the adrenocortical circadian rhythmicity in both humans and animals. It also plays a role in modulating the glucocorticoid feedback control of ACTH secretion and the adrenocortical responses to stressful situations. Therefore, the diminished sensitivity to pain may be caused by hippocampal degenerative changes that occur with physiological ageing and even more so with pathological ageing steroid feedback and for a certain degree of hyperactivity of the HPA axis in elderly subjects (Aguilera 2011).

There have been some subgroups of patients with anxiety and mood disorders who have hyperactivity of the HPA axis. Additionally, several anti-anxiety

medications, such as benzodiazepines, tricyclic antidepressants (TCAs), and selective serotonin reuptake inhibitors (SSRIs), have been studied for their impacts on various HPA axis parts. For instance, TCAs, SSRIs, and benzodiazepines, such as clonazepam and alprazolam, have been explored as anti-anxiety medications. And also, it has been shown that benzodiazepines, such as clonazepam and alprazolam, decrease the activity of corticotrophin-releasing factor (CRF) neurons in the hypothalamus. Effective anti-anxiety medications like TCAs and SSRIs may also work in part by modifying the HPA axis (Tafet and Nemeroff 2020). Other studies demonstrated that TCAs and SSRIs may also induce significant changes in the HPA axis, associated with their therapeutic effects, in addition to their well-known pharmacological effects, including blockade of neurotransmitter uptake and subsequent regulation of various pre- and post-synaptic receptors (Nikisch 2009). Some of these, at least in part, have been linked to the possibility that anti-anxiety medications affect the transcriptional regulation of various molecules involved in the control of the HPA axis, such as glucocorticoid receptors (GRs), mineralocorticoid receptors (MRs), and CRH. In this regard, it has been suggested that an altered GR gene regulation, which could result in decreased GR concentrations in the hippocampus or hypothalamus due to the HPA system's insufficient feedback, could lead to a variety of adverse effects (Barden 1996).

Another study shows that when desipramine or amitriptyline was added to cell cultures originating from the hypothalamus or amygdala, an increase in GR mRNA expression was first seen (Pepin et al. 1989). Studies showed that long-term TCA treatment reduces CRH mRNA expression, but short-term treatment showed different results (Brady et al. 1991). Long-term imipramine administration led to in vivo observations of similar effects. In this regard, it has been demonstrated that long-term administration of this TCA hindered the CRH gene's transcriptional regulation, which led to a decrease in CRH mRNA expression in the hypothalamus (Michelson et al. 1997), which in turn led to a marked decrease in the HPA axis' activity (Heydendael and Jacobson 2008).

In research involving SSRIs, it was shown that long-term fluoxetine treatment raised the expression of GR mRNA in hippocampus neurons (Lai et al. 2003). More recently, in vivo experiments showed that fluoxetine long-term administration may also cause hippocampus GRs to regain their functionality after prolonged stress. Furthermore, even in the absence of altered glucocorticoid secretion, enhanced hippocampus GRs activation, including phosphorylation and subsequent nuclear translocation, was seen after prolonged fluoxetine therapy (Lee et al. 2016). More subsequent research has also revealed that additional alterations in GRs are not necessary for the behavioural efficacy of the SSRI, even though these data suggest that this mechanism should be implicated in the therapeutic impact of fluoxetine (Simard et al. 2018).

Other neurotransmitters, including aminobutyric acid (GABA), the primary inhibitory neurotransmitter in the CNS, also control the HPA axis (Roberts 1976). GABA plays a crucial role in controlling hypothalamic function (Hermann and Cullinan 1997). It has been demonstrated that the medial parvocellular hypothalamic prefrontal cortex (PVN) innervates hypophysiotropic CRH neurons by inhibitory

GABAergic input, either directly from peri-PVN sources or indirectly from various limbic areas. GABAergic projections can travel directly to the PVN from nearby hypothalamic nuclei and the bed nucleus of the stria terminalis (BNST), or they can travel indirectly via the ventral subiculum, the amygdala, and the prefrontal cortex (PFC), especially the anterior cingulate cortex (ACC), prelimbic, and infralimbic areas, from a variety of cortex and limbic structures, including the hippocampus. Local GABAergic projections to the PVN may then be activated or repressed by glutamatergic or GABAergic projections from cortical and limbic areas, which are crucial for adaptive stress responses and, consequently, for the PVN's ability to regulate its own internal environment (Cullinan et al. 2008).

Alprazolam was used to further investigate this regulatory function, and it was shown that the benzodiazepines (BZD) are capable of blocking the HPA axis. This was attributed to the BZD's impact on CRH neurons, which may help explain its therapeutic success (Kalogeras et al. 1990). The vast study of CRH's probable role in the pathogenesis of anxiety disorders (Nemeroff 1992) calls for more investigation into how anti-anxiety medications affect CRH neurotransmission. In this context, it has been demonstrated that alprazolam acute therapy reduced CRH concentrations in the locus coeruleus (LC). Alprazolam's impact was subsequently investigated in vivo, where it was discovered that either acute or long-term dosing reduced CRH concentrations in the LC. The LC plays a crucial role in the pathophysiology of stress and anxiety disorders because it includes CRH receptors and gets a significant amount of CRH innervation (Bloom 1979). Alprazolam's actions on hypothalamic CRH neurons are therefore probably both direct and indirect through the LC. In vivo investigations with lorazepam and clonazepam more recently showed that both BZDs were successful at reversing anxiety-like behaviour, including social avoidance, and these effects were connected with their inhibitory action on the HPA axis, which was mediated via the reduction of CRH activity. Additionally, it has been demonstrated that both BZDs were successful in lowering stress's ability to stimulate CRH mRNA expression in the hypothalamus (Ramirez et al. 2016).

Ageing, Cerebrovascular Diseases and Regeneration

With age, there is a gradual decline in the regenerative properties of most tissues. The goal of regenerative medicine is to restore cells, tissues, and structures that are lost or damaged after disease, injury, or ageing. The question arises as to whether this is due to the intrinsic ageing of stem cells or, rather, to the impairment of stem-cell function in the aged tissue environment. Since stem cells are necessary to repair lasting wear in tissues, their loss of function is an important contributor to degenerative ageing. Consequently, inefficient replacement of worn-out cells in adult tissues due to the declining function of stem cells over time may be a primary cause of human ageing. Indeed, there is much interest in harnessing the potential of stem cells in the brain (Gage 1998) and heart (Leite et al. 2015) for therapeutic purposes.

Globally, cerebrovascular diseases are the second cause of death being surpassed by ischaemic heart disease by just 1.5%. The consequences of stroke are often devastating and extend well beyond the patients to impact not only families but also employers, caregivers, and social networks. Around 60–83% of survivors will fully recover and achieve independence in self-care by 1 year after a stroke. However, a significant proportion may require temporary or lifelong assistance. Thus, between 10% and 15% of survivors require assistance and primary care in a specialised institution 1 year following the stroke.

In western ageing societies, the incidence of stroke events increases abruptly at the age of about 73 years. Advances in stroke medicine and the adaptation to stroke risk factors were successful in decreasing stroke incidence and increasing the number of stroke survivors in western societies. However, due to the increase in the number of elderly, the incidence of stroke has increased again paralleled by obesity and metabolic syndrome.

Ageing is accompanied by both functional and cognitive decline which are closely related to morphological changes in the brain during ageing. In addition, the increased susceptibility of the aged brain to cerebral ischaemia is associated with cognitive decline and incomplete behavioural recovery (Manwani et al. 2011).

Stroke is one of the most frequent causes of death and permanent disabilities worldwide, for which only limited and unsatisfactory treatments exist. Cell-based neurorestorative therapy has considerable potential for improving stroke recovery. However, the efficacy of all these therapies has been so far discouragingly low. The major causes for the poor efficacy of implanted cells in vivo are directly or indirectly related to (1) host neuroinflammation, (2) poor cell retention, and (3) poor survival and integration.

Initial enthusiasm for stimulating restorative processes in the ischaemic brain with cell-based therapies has meanwhile converted into a more balanced view, recognising impediments related to survival, migration, differentiation, and integration of therapeutic cells in the hostile aged brain environment. Therefore, a current lack of understanding of the fate of transplanted cells means that the safety of cell therapy in stroke patients is still unproven.

Earlier studies on postmortem human brains provided evidence that there might be subventricular zone (SVZ) cell proliferation and neuroblast formation after stroke even in aged patients (Jin et al. 2006; Macas et al. 2006; Minger et al. 2007). The finding that new neurons are continuously added in the adult human striatum (Ernst et al. 2014), along with the presence of an increased number of putative neuroblasts in the human striatum after stroke, lends support to this hypothesis (Macas et al. 2006). However, whether endogenous neurogenesis contributes to spontaneous recovery after stroke has not yet been established. In addition, age, comorbidities, the physical condition of the patient, and the severity of the disease could substantially influence these steps and, therefore, the outcome of the healing process.

In humans, functional reorganisation has been reported in well-recovered patients with subcortical stroke (Zhang et al. 2014). Indeed, studies on postmortem brains provided evidence for enhanced SVZ cell proliferation and neuroblast formation after stroke even in adult and even aged humans (Martí-Fàbregas et al. 2010; Ernst

et al. 2014). One study reported an increased number of new cortical neurons originating from the SVZ in the peri-infarct cortex at 65 days after the insult (Palma-Tortosa et al. 2017).

Most clinical studies conducted so far used neural cells derived from human foetal donors. The techniques to achieve effective survival and growth of neuronal tissues transplanted into the CNS are meanwhile well established (Dunnett 2013). Even though effective, neural grafting has, however, not become a standard treatment for several reasons, including the limited supply of foetal tissue of human origin, and the beneficial effects have been controversial (Morizane et al. 2008). Of the various options, stem-cell therapy presents us with a viable alternative (Stoll 2014). For the example of neural precursor cells (NPCs), the group comprehensively analyzed (a) various routes (intravenous, intraarterial, intraperitoneal, intracerebroventricular, brain parenchymal) and (b) time points of stem/precursor cell delivery, pointing out advantages of systemic (intravenous or intraarterial) over local (intraparenchymal) delivery strategies in the stroke brain. Accordingly, future clinical studies should focus on systemic delivery approaches. Of the small molecule drugs examined, the GABAA α5 antagonist S44819 entered a multicentric randomized phase IIb trial in human stroke patients (RESTORE BRAIN) under the applicant's guidance, which was conducted in 80 institutions in 13 countries based on animal studies by his group (Chabriat et al. 2020; Hermann et al. 2022a).

In order to enable the replacement of lost tissue, cell replacement strategies were used in human stroke patients (Stoll 2014; Strazzullo et al. 2010). However, these early clinical studies lacked appropriate control groups. The RECOVER-Stroke trial conducted by Savitz et al. (2019) may well serve as a role model for future early-stage cell therapy clinical trials in stroke. The study featured an impressive array of safety endpoints and stratified patients according to NIHSS scores ($\leq$15 vs. $\geq$16) and whether the patients suffered from a lacunar versus a cortical stroke. Importantly, this study also represents the first serious attempt to assess the safety of cell delivery by the intra-arterial route in stroke patients. In this study, a special subpopulation of commercially available bone marrow cells that express CD34+ and CD133+ stem and progenitor cell surface markers and high levels of aldehyde dehydrogenase (ALDH) was administered.

Clinical Studies

There are several therapeutic options available during the acute phase of stroke. Thrombolysis using rt-PA and/or mechanical thrombectomy can be used to limit the consequences of acute occlusions of cerebral blood vessels. Clinical trials on patients that received r-tPA treatment within 3 h of the onset still reported functional deficits at 3 and 12 months post-stroke, on a Modified Rankin Scale 2–5 (Langhorne et al. 2009; Jovin et al. 2015). The motor deficit is the most frequent one encountered, however DALYs (Disability-adjusted life years) include cognitive, linguistic, optical, and sensorial deficits (Langhorne et al. 2009). Numerous neuroprotectants have

been tested with promising results in animal models. However, there is a difficult transition to human clinical trials, which may be the cause of slow progress in the field (Paul and Candelario-Jalil 2021) Some of the recently completed clinical trials have tested the therapeutic efficacy of human urinary kallidinogenase (Ni et al. 2017; Dong et al. 2020), 3-methyl-1-phenyl-2-pyrazoline-5-one (Enomoto et al. 2019), nerinetide (Hill et al. 2020), 3K3A-activated protein C (Lyden et al. 2020) with results that are still being evaluated.

The mechanical removal of the clot from the intracranial artery by means of modern stent retriever devices has been recommended since 2015 for acute ischemic stroke (AIS) because of occlusion of the proximal large intracranial artery. According to current guidelines, mechanical thrombectomy (MT) should be proceeded by intravenous Alteplase administration (IVT) unless contraindications exist (Broocks et al. 2023). The classic time window for MT is 6 h; however, in patients fulfilling the criteria of the DAWNtrial (Nogueira et al. 2018) that examined patients presenting between 6 and 24 h after AIS and criteria of the DEFUSE 3 trial that examined patients presenting between 6 and 16 h, the time window for MT can be significantly prolonged (Albers et al. 2018).

It is estimated that up to 10% of AIS patients fulfil the criteria for undergoing MT (Powers et al. 2019; Bhalla et al. 2021). The Neurorehabilitation Training Toolki (NNT) to obtain functional independence was between 3.2 and 7.4 when compared with the best medical treatment (Goyal et al. 2016). Meta-analysis of individual patient data from the key clinical studies includes the following: MR CLEAN (Berkhemer et al. 2015), ESCAPE (Goyal et al. 2015), REVASCAT (Saver et al. 2015), and SWIFT PRIME (Menjot de Champfleur et al. 2017). EXTEND IA (Campbell et al. 2015) indicates that MT caused a significant increase in the chance for complete recovery (mRS score 0–1; 26.9% vs. 12.9%) or independence (mRS score 0–2; 46.0% vs. 26.5%) as compared to standardised treatment, including IVT (Goyal et al. 2016). Despite combined AIS therapy, more than 50% of patients will remain disabled after MT (Goyal et al. 2016).

Plasma Biomarkers Associated with Ischaemic Stroke

The risk of having a stroke event doubles each decade after the age of 55. Therefore, it is of great interest to develop neurorestorative therapies for stroke which occurs mostly in elderly people.

However, to date, patients at risk for these sequels of stroke are not duly diagnosed and treated due to the lack of reliable biomarkers. Extracellular vesicles (EVs) are lipid bilayer-delimited particles that are shed by the brain cells and are able to cross the blood-brain barrier and enter the bloodstream; thus, they may be used to interrogate molecular and cellular events in the brain-damaged area (Driga et al. 2021).

Despite sustained efforts to develop clinically effective drugs, the complex mechanism underlying stroke recovery makes complete functional recovery

unlikely. Therefore, research into the prevention and identification of biomarkers that could potentially improve the response to treatment is currently advisable (Clarkson et al. 2010). By the Biomarkers Definitions (Working Group in 1998) a biological marker or biomarker refers to a vast category of indicators defined as "a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention" (Dagonnier et al. 2021). More and more studies aim to identify useful plasma biomarkers associated with the prognosis of ischaemic stroke. Harmann and colleagues reported a growth in the number of publications that evaluate the role of biomarkers associated with the prognosis of stroke between 2007 and 2018 (Dagonnier et al. 2021), while another recent review made a comprehensive systematic investigation of blood biomarkers associated with physical post-stroke recovery (Norden et al. 2014).

It is well known that the adult brain has a limited capacity to regenerate is aggravated by ageing, and that intensive rehabilitation is able to improve neurological outcomes after stroke; however, providing such treatment for all stroke survivors would place an unbearable organisational and financial burden on European health care systems. To overcome this situation, biomarkers need to be developed that, together with the individual risk factors, e.g., age, comorbidities, and gender, are able to predict long-term outcomes after stroke. Creating such a personalised medicine tool could identify individual patients at risk for bad outcomes after stroke and provide them with preventive neurorehabilitation. While improving patients' lives, such an approach will most likely also reduce healthcare costs significantly.

Neurovascular Unit-Derived Exosomes in Response to Stroke

Recent data strongly suggest that exosomes (EVs) play a critical role in brain homeostasis and plasticity (Holm et al. 2018) by acting as the bidirectional carrier between neurons, glia, vascular and perivascular cells, on the one hand, and between the brain and periphery on the other (Zagrean et al. 2018). Small EVs comprise 70-150 nm sized vesicles released by the late endosomal compartment (Lener et al. 2015). They correspond to the intraluminal vesicles of multivesicular bodies (MVBs) that upon fusion with the plasma membrane are released into the extracellular space (Hermann et al. 2022b). Exosomes are not only involved in the epigenetic regulation of communication between neurons and glial cells within the nervous system but also in brain-body epigenetic interconnection mediated by non-coding RNA and miRNA cargo (Lai et al. 2012).

Of special interest for diagnosis and prediction of post-stroke recovery, brain-EVs cross the blood-brain barrier and reach the plasma, allowing assessment of the events occurring in the post-stroke brain (Rosell and Lo 2008). It has been shown that intravenously delivered MSC-derived small EVs very similarly promoted post-ischemic neurological recovery, endogenous neurogenesis and angiogenesis as intravenously delivered MSCs (Doeppner et al. 2017), suggesting that EVs mediate

restorative MSC responses. Considering their simple handling and the lack of side effects associated with cell therapies, e.g., malignant transformation, MSC-EVs are attractive candidates for stroke treatment. However, given the myriad of post-stroke events, we can only guess what is really happening with regard to cell–cell interactions and how these interactions can be used to improve stroke (Hermann and Chopp 2012); the development of neurorestorative therapies is a true challenge (Hermann et al. 2015). Indeed, it was demonstrated that brain-derived EVs (Brain-EVs) can cross the BBB and can be isolated from plasma samples of patients and harness specific disease-related proteins, such as beta-amyloid and phosphorylated forms of tau in Alzheimer's disease patients (Kanninen et al. 2016; Ngolab et al. 2017; Sardar et al. 2018). Thus, the disease-specific fingerprint profile of Brain-EVs was isolated from the blood and CSF of stroke patients in order to obtain information about the pathophysiological status of the brain after a stroke and to predict the outcome of individual patients. This would enable patients at risk for unfavourable outcomes after stroke to be directed to intensive neurorehabilitation, which will eventually improve patient outcomes.

References

Abbatecola AM, Ferrucci L, Ceda G, Russo CR, Lauretani F, Bandinelli S et al (2005) Insulin resistance and muscle strength in older persons. J Gerontol 60:1278–1282. https://doi.org/10.1093/gerona/60.10.1278

Aguilera G (2011) HPA axis responsiveness to stress: implications for healthy aging. Exp Gerontol 46(2–3):90–95. https://doi.org/10.1016/j.exger.2010.08.023

Albers GW, Marks MP, Kemp S, Christensen S, Tsai JP, Ortega-Gutierrez S, McTaggart RA, Torbey MT, Kim-Tenser M, Leslie-Mazwi T et al (2018) Thrombectomy for stroke at 6 to 16 hours with selection by perfusion imaging. N Engl J Med 378:708–718. https://doi.org/10.1056/NEJMoa1713973

Allsop D, Mayes J, Moore S, Masad A, Tabner BJ (2008) Metal-dependent generation of reactive oxygen species from amyloid proteins implicated in neurodegenerative disease. Biochem Soc Trans 36(6):1293–1298. https://doi.org/10.1042/BST0361293

Amaya-Montoya M, Perez-Londono A, Guatibonza-Garcia V, Vargas-Villanueva A, Mendivil CO (2020) Cellular senescence as a therapeutic target for age-related diseases: a review. Adv Ther 37:1407–1424. https://doi.org/10.1016/j.arr.2020.101251

Baek SJ, Nam GE, Han KD, Choi SW, Jung SW, Bok AR et al (2014) Sarcopenia and sarcopenic obesity and their association with dyslipidemia in Korean elderly men: the 2008–2010 Korea National health and nutrition examination survey. J Endocrinol Investig 37:247–260. https://doi.org/10.1007/s40618-013-0011-3

Barden N (1996) Modulation of glucocorticoid receptor gene expression by antidepressant drugs. Pharmacopsychiatry 29(1):12–22. https://doi.org/10.1055/s-2007-979536

Berkhemer OA, Fransen PS, Beumer D, van den Berg LA, Lingsma HF, Yoo AJ, Schonewille WJ, Vos JA, Nederkoorn PJ, Wermer MJ et al (2015) A randomized trial of intraarterial treatment for acute ischemic stroke. N Engl J Med 372:11–20. https://doi.org/10.1056/nejmoa1411587

Bhalla A, Patel M, Birns J (2021) An update on hyper-acute management of ischaemic stroke. Clin Med 21:215–221. https://doi.org/10.7861/clinmed.2020-0998

Bloom FE (1979) Norepinephrinc mediated synaptic transmission and hypotheses of psychiatric disorders. Research in the psychobiology of human behavior. The Johns Hopkins University Press, Baltimore, p 11 I. https://doi.org/10.3389/fpsyt.2020.00443

Brady LS, Whitfield HJ, Fox RJ, Gold PW, Herkenham M (1991) Long-term antidepressant administration alters corticotropin-releasing hormone, tyrosine hydroxylase and mineralocorticoid receptor gene expression in rat brain: therapeutic implications. J Clin Invest 87:831–837. https://doi.org/10.1172/jci115086

Brennan LA, Kantorow M (2009) Mitochondrial function and redox control in the ageing eye: role of MsrA and other repair systems in cataract and macular degenerations. Exp Eye Res 88:195–203. https://doi.org/10.1016/j.exer.2008.05.018

Brennan LA, McGreal-Estrada R, Logan CM, Cvekl A, Menko AS, Kantorow M (2018) BNIP3L/NIX is required for elimination of mitochondria, endoplasmic reticulum and Golgi apparatus during eye lens organelle-free zone formation. Exp Eye Res 174:173–184. https://doi.org/10.1016/j.exer.2018.06.003

Brewer GJ (2010) Epigenetic oxidative redox shift (EORS) theory of aging unifies the free radical and insulin signaling theories. Exp Gerontol 45:173–179. https://doi.org/10.1016/j.exger.2009.11.007

Broocks G, McDonough R, Bechstein M, Hanning U, Brekenfeld C, Flottmann F, Kniep H, Nawka MT, Deb-Chatterji M, Thomalla G, Sporns P, Yeo LL, Tan BY, Gopinathan A, Kastrup A, Politi M, Papanagiotou P, Kemmling A, Fiehler J, Meyer L, German Stroke Registry – Endovascular Treatment (GSR-ET) (2023) Benefit and risk of intravenous alteplase in patients with acute large vessel occlusion stroke and low ASPECTS. J Neurointerv Surg 15(1):8–13. https://doi.org/10.1136/neurintsurg-2021-017986

Buga AM, Vintilescu R, Pop OT, Popa-Wagner A (2011) Brain aging and regeneration after injuries: an organismal approach. Aging Dis 2(1):64–79

Burkewitz K, Zhang Y, Mair WB (2014) AMPK at the nexus of energetics and aging. Cell Metab 20:10–25. https://doi.org/10.1016/j.cmet.2014.03.002

Campbell BC, Mitchell PJ, Kleinig TJ, Dewey HM, Churilov L, Yassi N, Yan B, Dowling RJ, Parsons MW, Oxley TJ et al (2015) Endovascular therapy for ischemic stroke with perfusion-imaging selection. N Engl J Med 372:1009–1018. https://doi.org/10.1056/nejmoa1414792

Chabriat H, Bassetti CL, Marx U, Audoli-Inthavong ML, Sors A, Lambert E, Wattez M, Hermann DM, RESTORE BRAIN study investigators (2020) Safety and efficacy of $GABA_A$ α5 antagonist S44819 in patients with ischaemic stroke: a multicentre, double-blind, randomised, placebo-controlled trial. Lancet Neurol 19(3):226–233. https://doi.org/10.1016/S1474-4422(20)30004-1

Clarkson AN, Huang BS, Macisaac SE, Mody I, Carmichael ST (2010) Reducing excessive GABA-mediated tonic inhibition promotes functional recovery after stroke. Nature 468:305–309. https://doi.org/10.1038/nature09511

Cullinan WE, Ziegler DR, Herman JP (2008) Functional role of local GABAergic influences on the HPA axis. Brain Struct Funct 213:63–72. https://doi.org/10.1007/s00429-008-0192-2

da Costaa JP, Vitorino R, Silvad GM, Vogeld C, Duartea AC, Rocha-Santos T (2016) A synopsis on aging – theories, mechanisms and future prospects. Ageing Res Rev 29:90–112. https://doi.org/10.1016/j.arr.2016.06.005

Dagonnier M, Donnan GA, Davis SM, Dewey HM, Howells DW (2021) Acute stroke biomarkers: are we there yet? Front Neurol 5(12):619721. https://doi.org/10.3389/fneur.2021.619721

Dickerson SS, Kemeny ME (2004) Acute stressors and cortisol responses: a theoretical integration and synthesis of laboratory research. Psychol Bull 130(3):355–391. https://doi.org/10.1037/0033-2909.130.3.355

Doeppner TR, Bähr M, Hermann DM, Giebel B (2017) Concise review: extracellular vesicles overcoming limitations of cell therapies in ischemic stroke. Stem Cells Transl Med 6(11):2044–2052. https://doi.org/10.1002/sctm.17-0081

Dominguez LJ, Barbagallo M (2007) The cardiometabolic syndrome and sarcopenic obesity in older persons. J Cardiometab Syndr 2:183–189. https://doi.org/10.1111/j.15594564.2007.06673.x

Dong Y, Qu J, Zhang Z, Wang C, Dong Q (2020) Human urinary kallidinogenase in treating acute ischemic stroke patients: analyses of pooled data from a randomized double-blind placebocontrolled phase IIb and phase III clinical trial. Neurol Res 42:286–290. https://doi.org/10.1080/01616412.2020.1711648

Driga MP, Catalin B, Olaru DG, Slowik A, Plesnila N, Hermann DM, Popa-Wagner A (2021) The need for new biomarkers to assist with stroke prevention and prediction of post-stroke therapy based on plasma-derived extracellular vesicles. Biomedicine 9(9):1226. https://doi.org/10.3390/biomedicines9091226

Dunnett SB (2013) Neural tissue transplantation, repair, and rehabilitation. Handb Clin Neurol. https://doi.org/10.1016/B978-0-444-52901-5.00004-6

Dzięgielewska-Gęsiak S, Stołtny D, Brożek A, Muc-Wierzgoń M, Wysocka E (2020) Are insulin-resistance and oxidative stress cause or consequence of aging. Exp Biol Med (Maywood) 245(14):1260–1267. https://doi.org/10.1177/1535370220929621

Edwards MG, Anderson RM, Yuan M, Kendziorski CM, Weindruch R, Prolla TA (2007) Gene expression profiling of aging reveals activation of a p53-mediated transcriptional program. BMC Genomics 8:80. https://doi.org/10.1186/1471-2164-8-80

Enomoto M, Endo A, Yatsushige H, Fushimi K, Otomo Y (2019) Clinical effects of early edaravone use in acute ischemic stroke patients treated by endovascular reperfusion therapy. Stroke 50:652–658. https://doi.org/10.1161/STROKEAHA.118.023815

Ernst A, Alkass K, Bernard S, Salehpour M, Perl S, Tisdale J, Possnert G, Druid H, Frisén J (2014) Neurogenesis in the striatum of the adult human brain. Cell 156:1072–1083. https://doi.org/10.1016/j.cell.2014.01.044

Fang M (2018) Trends in the prevalence of diabetes among US adults: 1999-2016. Am J Prev Med 55:497–505. https://doi.org/10.1016/j.cdtm.2020.11.003

Ferrari E, Cravello L, Muzzoni B, Casarotti D, Paltro M, Solerte SB, Fioravanti M, Cuzzoni G, Pontiggia B, Magri F (2001) Age-related changes of the hypothalamic-pituitary-adrenal axis: pathophysiological correlates. Eur J Endocrinol 144:319–329. https://doi.org/10.1530/eje.0.1440319

Franceschi C, Garagnani P, Parini P, Giuliani C, Santoro A (2018) Inflammaging: a new immune-metabolic viewpoint for age-related diseases. Nat Rev Endocrinol 14:576–590. https://doi.org/10.1038/s41574-018-0059-4

Gage FH (1998) Stem cells of the central nervous system. Curr Opin Neurobiol 8(5):671–676. https://doi.org/10.1016/s0959-4388(98)80098-6

Goyal M, Demchuk AM, Menon BK, Eesa M, Rempel JL, Thornton J, Roy D, Jovin TG, Willinsky RA, Sapkota BL et al (2015) Randomized assessment of rapid endovascular treatment of ischemic stroke. N Engl J Med 372:1019–1030. https://doi.org/10.1056/nejmoa1414905

Goyal M, Menon BK, van Zwam WH, Dippel DW, Mitchell PJ, Demchuk AM, Dávalos A, Majoie CB, van der Lugt A, de Miquel MA et al (2016) Endovascular thrombectomy after large-vessel ischaemic stroke: a meta-analysis of individual patient data from five randomised trials. Lancet 387:1723–1731. https://doi.org/10.1016/S0140-6736(16)00163-X

Gupta D (2014) Hypothalamic-pituitary-adrenal (HPA) axis and aging. Comprehensive Physiol 4. https://doi.org/10.1002/cphy.c130049

Herman JP, Cullinan WE (1997) Neurocircuitry of stress: central control of the hypothalamo-pituitary-adrenocortical axis. Trends Neurosci 20:78–84. https://doi.org/10.1016/S0166-2236(96)10069-2

Hermann DM, Chopp M (2012) Promoting brain remodelling and plasticity for stroke recovery: therapeutic promise and potential pitfalls of clinical translation. Lancet Neurol 11:369–380. https://doi.org/10.1016/S1474-4422(12)70039-X

Hermann DM, Buga AM, Popa-Wagner A (2015) Neurovascular remodeling in the aged ischemic brain. J Neural Transm 122(Suppl 1):S25–S33. https://doi.org/10.1007/s00702-013-1148-0

Hermann DM, Popa-Wagner A, Peruzzotti-Jametti L, Gunzer M (2022a) Editorial: hot topics in cellular neuropathology. Front Cell Neurosci 16:895861. https://doi.org/10.3389/fncel.2022.895861

Hermann DM, Xin W, Bähr M, Giebel B, Doeppner TR (2022b) Emerging roles of extracellular vesicle-associated non-coding RNAs in hypoxia: insights from cancer, myocardial infarction and ischemic stroke. Theranostics 12(13):5776–5802. https://doi.org/10.7150/thno.73931

Heydendael W, Jacobson L (2008) Differential effects of imipramine and phenelzine on corticosteroid receptor gene expression in mouse brain: potential relevance to antidepressant response. Brain Res 1238:93–107. https://doi.org/10.1016/j.brainres.2008.08.018

Hill MD, Goyal M, Menon BK, Nogueira RG, McTaggart RA et al (2020) Efficacy and safety of nerinetide for the treatment of acute ischaemic stroke (ESCAPENA1): a multicentre, double-blind, randomised controlled trial. Lancet 395:878–887. https://doi.org/10.1016/S0140-6736(20)30258-0

Holm MM, Kaiser J, Schwab ME (2018) Extracellular vesicles: multimodal envoys in neural maintenance and repair. Trends Neurosci 41:360–372. https://doi.org/10.1016/j.tins.2018.03.006

Ida H, Boylan SA, Weigel AL, Hjelmeland LM (2003) Age-related changes in the transcriptional profile of mouse RPE/choroid. Physiol Genomics 15(3):258–262. https://doi.org/10.1152/physiolgenomics.00126.2003

Jin K, Wang X, Xie L, Mao XO, Zhu W, Wang Y et al (2006) Evidence for stroke-induced neurogenesis in the human brain. Proc Natl Acad Sci U S A 103(35):13198–13200. https://doi.org/10.1073/pnas.0603512103

Johnson K, Appel LJ, Brands M, Howard BV, Lefevre M, Lustig RH, Steffen L, Wylie-Rosett J (2009) Dietary sugars intake and cardiovascular health. Circulation 120:1011–1120. https://doi.org/10.1161/CIRCULATIONAHA.109.192627

Jovin TG, Chamorro A, Cobo E, de Miquel MA, Molina CA, Rovira A, San Román L, Serena J, Abilleira S, Ribó M et al (2015) Thrombectomy within 8 hours after symptom onset in ischemic stroke. N Engl J Med 372:2296–2306. https://doi.org/10.1056/nejmoa1503780

Kalogeras KT, Calogero AE, Kuribayiashi T, Khan I, Gallucci WT, Kling MA et al (1990) In vitro and in vivo effects of the triazolobenzodiazepine alprazolam on hypothalamic-pituitary-adrenal function: pharmacological and clinical implications. J Clin Endocrinol Metab 70(5):1462–1471. https://doi.org/10.1210/jcem-70-5-1462

Kanfi Y, Naiman S, Amir G, Peshti V, Zinman G, Nahum L, Bar-Joseph Z, Cohen HY (2012) The sirtuin SIRT6 regulates lifespan in male mice. Nature 483:218–221. https://doi.org/10.1038/nature10815

Kanninen KM, Bister N, Koistinaho J, Malm T (2016) Exosomes as new diagnostic tools in CNS diseases. Biochim Biophys Acta 1862:403–410. https://doi.org/10.1016/j.bbadis.2015.09.020

Khadra D, Itani L, Chebaro Y, Obeid M, Jaber M, Ghanem R et al (2019) Association between sarcopenic obesity and higher risk of type 2 diabetes in adults: a systematic review and meta-analysis. World J Diabetes 10:311–323. https://doi.org/10.2174/1573403X16666200214104122

Khan RMM, Chua ZJY, Tan JC, Yang Y, Liao Z, Zhao Y (2019) From pre-diabetes to diabetes: diagnosis, treatments and translational research. Medicina (Kaunas) 55:546. https://doi.org/10.3390/medicina55090546

Kobayashi M, Suhara T, Baba Y, Kawasaki NK, Higa JK, Matsui T (2018) Pathological roles of iron in cardiovascular disease. Curr Drug Targets 19:1068–1076. https://doi.org/10.2174/1389450119666180605112235

Kremastinos DT, Farmakis D (2011) Iron overload cardiomyopathy in clinical practice. Circulation 124:2253–2263. https://doi.org/10.1161/CIRCULATIONAHA.111.050773

Kumar S, Vijayan M, Bhatti JS, Reddy PH (2017) MicroRNAs as peripheral biomarkers in ageing and age-related diseases. Mol Biol Ageing:47–94. https://doi.org/10.1016/bs.pmbts.2016.12.013

Lai M, McCormick JA, Chapman KE, Kelly PA, Seckl JR, Yau JL (2003) Differential regulation of corticosteroid receptors by monoamine neurotransmitters and antidepressant drugs in primary hippocampal culture. Neuroscience 118(4):975–984. https://doi.org/10.1016/S0306-4522(03)00038-1

Lai X, Schmitz U, Gupta SK, Bhattacharya A, Kunz M, Wolkenhauer O, Vera J (2012) Computational analysis of target hub gene repression regulated by multiple and cooperative miRNAs. Nucleic Acids Res 40(18):8818–8834. https://doi.org/10.1093/nar/gks657

Langhorne P, Coupar F, Pollock A (2009) Motor recovery after stroke: a systematic review. Lancet Neurol 8(8):741–754. https://doi.org/10.1016/S1474-4422(09)70150-4

Lee MS, Kim YH, Park WS, Park OK, Kwon SH, Hong KS et al (2016) Temporal variability of glucocorticoid receptor activity is functionally important for the therapeutic action of fluoxetine in the hippocampus. Mol Psychiatry 21(2):252–260. https://doi.org/10.1038/mp.2014.137

Lee J, You JH, Shin D, Roh JL (2020) Inhibition of glutaredoxin 5 predisposes cisplatin-resistant head and neck cancer cells to ferroptosis. Theranostics 10:7775–7786. https://doi.org/10.7150/thno.46903

Leite CF, Almeida TR, Lopes CS, Dias da Silva VJ (2015) Multipotent stem cells of the heart-do they have therapeutic promise? Front Physiol 8(6):123. https://doi.org/10.3389/fphys.2015.00123

Lener T, Gimona M, Aigner L et al (2015) Applying extracellular vesicles based therapeutics in clinical trials - an ISEV position paper. J Extracell Vesicles 4:30087. https://doi.org/10.3402/jev.v4.30087

Lewis JS, Lee JA, Underwood JC, Harris AL, Lewis CE (1999) Macrophage responses to hypoxia: relevance to disease mechanisms. J Leukoc Biol 66(6):889–900. https://doi.org/10.1002/jlb.66.6.889

Luo J, Mills K, le Cessie S, Noordam R, van Heemst D (2020) Ageing, age-related diseases and oxidative stress: what to do next? Ageing Res Rev 57:100982. https://doi.org/10.1016/j.arr.2019.100982

Lyden P, Pryor KE, Coffey CS, Cudkowicz M, Conwit R et al (2020) Final results of the RHAPSODY trial: a multi-center, phase 2 trial using a continual reassessment method to determine the safety and tolerability of 3K3A-APC, a recombinant variant of human activated protein C, in combination with tissue plasminogen activator, mechanical thrombectomy or both in moderate to severe acute ischemic stroke. Ann Neurol 85:125–136. https://doi.org/10.1002/ana.25383

Macas J, Nern C, Plate KH, Momma S (2006) Increased generation of neuronal progenitors after ischemic injury in the aged adult human forebrain. J Neurosci 26(50):13114–13119. https://doi.org/10.1523/JNEUROSCI.4667-06.2006

Manwani B, Liu F, Xu Y, Persky R, Li J, McCullough L (2011) Functional recovery in aging mice after experimental stroke. Brain Behav Immun 25:1689. https://doi.org/10.1016/j.bbi.2011.06.015

Martí-Fàbregas J, Romaguera-Ros M, Gómez-Pinedo U, Martínez-Ramírez S, Jiménez-Xarrié E, Marín R, Martí-Vilalta JL, García-Verdugo JM (2010) Proliferation in the human ipsilateral subventricular zone after ischemic stroke. Neurology 74:357–365. https://doi.org/10.1212/WNL.0b013e3181cbccec

Matulewicz N, Karczewska-Kupczewska M (2016) Insulin resistance and chronic inflammation. Postepy Hig Med Dosw 70:1245–1258. https://doi.org/10.5604/17322693.1226662

McCarthy LH, Bigal ME, Katz M et al (2008) Chronic pain and obesity in elderly people: results from the Einstein aging study. J Am Geriatr Soc 57:115–119. https://doi.org/10.1111/j.1532-5415.2008.02089.x

Mcternan PG, Kusminski CM, Kumar S (2006) Recent advances in the relationship between obesity, inflammation, insulin resistance. Eur Cytokine Netw 17:4–12. https://doi.org/10.1002/cphy.c170040

Menjot de Champfleur N, Saver JL, Goyal M, Jahan R, Diener HC, Bonafe A, Levy EI, Pereira VM, Cognard C, Yavagal DR, Albers GW (2017) Efficacy of stent-retriever thrombectomy in

magnetic resonance imaging versus computed tomographic perfusion-selected patients in SWIFT PRIME trial (solitaire FR with the intention for thrombectomy as primary endovascular treatment for acute ischemic stroke). Stroke 48(6):1560–1566. https://doi.org/10.1161/STROKEAHA.117.016669

Michelson D, Galliven E, Hill L, Demitrack M, Chrousos G, Gold P (1997) Chronic imipramine is associated with diminished hypothalamic-pituitary-adrenal axis responsivity in healthy humans. J Clin Endocrinol Metab 82(8):2601–2606. https://doi.org/10.1210/jcem.82.8.4172

Miller GE, Cohen S, Janicki-Deverts D (2007) Psychological stress and disease. JAMA 298(14): 1685–1687. https://doi.org/10.1001/jama.298.14.1685

Minger SL, Ekonomou A, Carta EM, Chinoy A, Perry RH, Ballard CG (2007) Endogenous neurogenesis in the human brain following cerebral infarction. Regen Med 2(1):69–74. https://doi.org/10.2217/17460751.2.1.69

Morizane A, Li JY, Brundin P (2008) From bench to bed: the potential of stem cells for the treatment of Parkinson's disease. Cell Tissue Res 331(1):323–336. https://doi.org/10.1007/s00441-007-0541-0

Nemeroff CB (1992) New vistas in neuropeptide research: focus on corticotropin-releasing factor. Neuro Psychopharmacol 6:787–794. https://doi.org/10.3389/fpsyt.2020.00443

Ngolab J, Trinh I, Rockenstein E, Mante M, Florio J, Trejo M, Masliah D, Adame A, Masliah E, Rissman RA (2017) Brainderived exosomes from dementia with Lewy bodies propagate α-synuclein pathology. Acta Neuropathol Commun 5:46. https://doi.org/10.1186/s40478-017-0445-5

Ni J, Qu J, Yao M, Zhang Z, Zhong X et al (2017) Re-evaluate the efficacy and safety of human urinary kallidinogenase (RESK): protocol for an open-label, single-arm, multicenter phase IV trial for the treatment of acute ischemic stroke in Chinese patients. Transl Stroke Res 8:341–346. https://doi.org/10.1007/s12975-017-0527-5

Nikisch G (2009) Involvement and role of antidepressant drugs of the hypothalamic-pituitary-adrenal axis and glucocorticoid receptor function. Neuro Endocrinol Lett 30(1):11–16

Nogueira RG, Jadhav AP, Haussen DC, Bonafe A, Budzik RF, Bhuva P, Yavagal DR, Ribo M, Cognard C, Hanel RA et al (2018) Thrombectomy 6 to 24 hours after stroke with a mismatch between deficit and infarct. N Engl J Med 378:11–21. https://doi.org/10.1056/nejmoa170644

Nomura T, Ikeda Y, Nakao S, Ito K, Ishida K, Suehiro T et al (2007) Muscle strength is a marker of insulin resistance in patients with type 2 diabetes: a pilot study. Endocr J 54:791–796. https://doi.org/10.1507/endocrj.K07-055

Norden DM, Fenn AM, Dugan A, Godbout JP (2014) TGFβ produced by IL-10 redirected astrocytes attenuates microglial activation. Glia 62:881–895. https://doi.org/10.1002/glia.22647

Palma-Tortosa S, García-Culebras A, Moraga A, Hurtado O, Perez-Ruiz A, Durán-Laforet V, Parra J, Cuartero MI, Pradillo JM, Moro MA et al (2017) Specific features of SVZ neurogenesis after cortical ischemia: a longitudinal study. Sci Rep 7:16343. https://doi.org/10.1038/s41598-017-16109-7

Paul S, Candelario-Jalil E (2021) Emerging neuroprotective strategies for the treatment of ischemic stroke: an overview of clinical and preclinical studies. Exp Neurol 335:113518. https://doi.org/10.1016/j.expneurol.2020.113518

Pepin MC, Beaulieu S, Barden N (1989) Antidepressants regulate glucocorticoid receptor messenger RNA concentrations in primary neuronal cultures. Brain Res Mol Brain Res 6(1):77–83. https://doi.org/10.1016/0169-328X(89)90031-4

Pescador N, Perez-Barba M, Ibarra JM, Corbaton A, Martinez-Larrad MT, Serrano-Rios M (2013) Serum circulating microRNA profiling for identification of potential type 2 diabetes and obesity biomarkers. PLoS One 8(10):e77251. https://doi.org/10.1371/journal.pone.0077251

Powers WJ, Rabinstein AA, Ackerson T, Adeoye OM, Bambakidis NC, Becker K, Biller J, Brown M, Demaerschalk BM, Hoh B et al (2019) Guidelines for the early management of patients with acute ischemic stroke: 2019 update to the 2018 guidelines for the early management of acute ischemic stroke: a guideline for healthcare professionals from the American Heart

Association/American Stroke Association. Stroke 50:e344–e418. https://doi.org/10.1161/STR.0000000000000211

Ramirez K, Niraula A, Sheridan JF (2016) GABAergic modulation with classical benzodiazepines prevent stress-induced neuro-immune dysregulation and behavioral alterations. Brain Behav Immun 51:154–168. https://doi.org/10.1016/j.bbi.2015.08.011

Rea IM, Gibson DS, McGilligan V, McNerlan SE, Alexander HD, Ross OA (2018) Age and age-related diseases: role of inflammation triggers and cytokines. Front Immunol 9. https://doi.org/10.3389/fimmu.2018.00586

Riera E, Dillin A (2015) Tipping the metabolic scales towards increased longevity in mammals. Nat Cell Biol 17:196–203. https://doi.org/10.1038/ncb3107

Roberts E (1976) Disinhibition as an organizing principle in the nervous system: the role of the GABA system. GABA in nervous system function. Raven, New York, pp 515–539. https://doi.org/10.1016/S1874-6055(99)80014-0

Robins SJ, Lyass A, Zachariah JP, Massaro JM, Vasan RS (2011) Insulin resistance and the relation of a dyslipidemia to coronary heart disease: the Framingham heart study. Arterioscler Thromb Vasc Biol 31:1208–1214. https://doi.org/10.1161/ATVBAHA.110.219055

Rosell A, Lo EH (2008) Multiphasic roles for matrix metalloproteinases after stroke. Curr Opin Pharmacol 8:82–89. https://doi.org/10.1016/j.coph.2007.12.001

Rosnick CB, Snall BJ, McEvoy CL, Borenstein AR, Mortimer JA (2007) Negative life events and cognitive performance in a population of older adults. J Aging Health 19(4):612–629. https://doi.org/10.1177/0898264307300975

Sardar Sinha M, Ansell-Schultz A, Civitelli L, Hildesjö C, Larsson M, Lannfelt L, Ingelsson M, Hallbeck M (2018) Alzheimer's disease pathology propagation by exosomes containing toxic amyloid-beta oligomers. Acta Neuropathol 136:41–56. https://doi.org/10.1007/s00401-018-1868-1

Saver JL, Goyal M, Bonafe A, Diener HC, Levy EI, Pereira VM, Albers GW, Cognard C, Cohen DJ, Hacke W et al (2015) Stent-retriever thrombectomy after intravenous t-PA vs. t-PA alone in stroke. N Engl J Med 372:2285–2295. https://doi.org/10.1056/nejmoa1415061

Savitz SI, Yavagal D, Rappard G, Likosky W, Rutledge N, Graffagnino C, Alderazi Y, Elder JA, Chen PR, Budzik RF Jr, Tarrel R, Huang DY, Hinson JM Jr (2019) A phase 2 randomized, sham-controlled trial of internal carotid artery infusion of autologous bone marrow-derivedALD-401 cells in patients with recent stable ischemic stroke (RECOVER-Stroke). Circulation 139(2):192–205. https://doi.org/10.1161/CIRCULATIONAHA.117.030659

Shoelson SE, Herrero L, Naaz A (2007) Obesity, inflammation, and insulin resistance. Gastroenterology 132:2169–2180. https://doi.org/10.1053/j.gastro.2007.03.059

Simard S, Shail P, MacGregor J, El Sayed M, Duman RS, Vaccarino FM et al (2018) Fibroblast growth factor 2 is necessary for the antidepressant effects of fluoxetine. PLoS One 13(10): e0204980. https://doi.org/10.1371/journal.pone.0204980

Stoll EA (2014) Advances toward regenerative medicine in the central nervous system: challenges in making stem cell therapy a viable clinical strategy. Mol Cell Ther 2:12. https://doi.org/10.1186/2052-8426-2-12

Strazzullo P, D'Elia L, Cairella G, Garbagnati F, Cappuccio F, Scalfi L (2010) Excess body weight and incidence of stroke meta-analysis of prospective studies with 2 million participants. Stroke 41:e418. https://doi.org/10.1161/STROKEAHA.109.576967

Swaab DF, Fliers E, Partiman TS (1985) The suprachiasmatic nucleus of the human brain in relation to sex, age and senile dementia. Brain Res 342:37–44. https://doi.org/10.1016/0006-8993(85)91350-2

Tafet GE, Nemeroff CB (2020) Pharmacological treatment of anxiety disorders: the role of the HPA axis. Front Psych 11:443. https://doi.org/10.3389/fpsyt.2020.00443

Tomimatsu K, Narita M (2015) Translating the effects of mTOR on secretory senescence. Nat Cell Biol 17:1230–1232. https://doi.org/10.1038/ncb3244

Trayhurn P, Wang B, Wood IS (2008) Hypoxia in adipose tissue: a basis for the dysregulation of tissue function in obesity? Brit J Nutr 100(2):227–235. https://doi.org/10.1017/S0007114508971282

Uchino BN, Campo RA, Vaughn A, Maija R, Smith TW (2001) The assessment of positivity and negativity in social networks: the reliability and validity of the social relationships index. J Commun Psychol 37(4):471–486. https://doi.org/10.1002/jcop.20308

Valli A, Harris AL, Kessler BM (2015) Hypoxia metabolism in ageing. Aging 7(7):465–466

Wang M, Tan Y, Shi Y, Wang X, Liao Z, Wei P (2020) Diabetes and sarcopenic obesity: pathogenesis, diagnosis, and treatments. Front Endocrinol (Lausanne) 11:568. https://doi.org/10.3389/fendo.2020.00568

Xu J, Knutson MD, Carter CS, Leeuwenburgh C (2008) Iron accumulation with age, oxidative stress and functional decline. PLoS One 3:e2865. https://doi.org/10.1371/journal.pone.0002865

Yan LL, Daviglus ML, Liu K et al (2006) Midlife body mass index and hospitalization and mortality in older age. JAMA 295:190–198. https://doi.org/10.1001/jamanetworkopen.2022.2318

Zagrean AM, Hermann DM, Opris I, Zagrean L, Popa-Wagner A (2018) Multicellular crosstalk between exosomes and the neurovascular unit after cerebral ischemia. Therapeutic implications. Front Neurosci 12:811. https://doi.org/10.3389/fnins.2018.00811

Zhang J, Meng L, Qin W, Liu N, Shi FD, Yu C (2014) Structural damage and functional reorganization in ipsilesional m1 in well-recovered patients with subcortical stroke. Stroke 45:788–793. https://doi.org/10.1161/STROKEAHA.113.003425

Zhou R-P, Chen Y, Wei X, Yu B, Xiong Z-G, Lu C, Hu W (2020) Novel insights into ferroptosis: implications for age-related diseases. Theranostics 10(26):11976–11997. https://doi.org/10.7150/thno.50663