Advances in Experimental Medicine and Biology 1260
Proteomics, Metabolomics, Interactomics and Systems Biology

Paul C. Guest *Editor*

Reviews on New Drug Targets in Age-Related Disorders

Springer

Advances in Experimental Medicine and Biology

Proteomics, Metabolomics, Interactomics and Systems Biology

Advances in Experimental Medicine and Biology provides a platform for scientific contributions in the main disciplines of the biomedicine and the life sciences. This series publishes thematic volumes on contemporary research in the areas of microbiology, immunology, neurosciences, biochemistry, biomedical engineering, genetics, physiology, and cancer research. Covering emerging topics and techniques in basic and clinical science, it brings together clinicians and researchers from various fields. *Advances in Experimental Medicine and Biology* has been publishing exceptional works in the field for over 40 years, and is indexed in SCOPUS, Medline (PubMed), Journal Citation Reports/Science Edition, Science Citation Index Expanded (SciSearch, Web of Science), EMBASE, BIOSIS, Reaxys, EMBiology, the Chemical Abstracts Service (CAS), and Pathway Studio. 2018 Impact Factor: 2.126.

Content of this series is reviewed.

This series of volumes focuses on concepts, techniques and recent advances in the field of proteomics, interactomics, metabolomics and systems biology. Recent advances in various 'omics' technologies enable quantitative monitoring of myriad various biological molecules in a high-throughput manner, and allow determination of their variation between different biological states on a genomic scale. Now that the sequencing of various genomes, from prokaryotes to humans, has provided the list and linear sequence of proteins and RNA that build living organisms, defining the complete set of interactions that sustain life constitutes one of the key challenges of the postgenomic era. This series is intended to cover experimental approaches for defining protein-protein, protein-RNA, protein-DNA and protein-lipid interactions; as well as theoretical approaches dealing with data analysis, integration and modeling and ethical issues.

More information about this series at http://www.springer.com/series/15040

Paul C. Guest
Editor

Reviews on New Drug Targets in Age-Related Disorders

Springer

Editor
Paul C. Guest
Laboratory of Neuroproteomics
Department of Biochemistry and Tissue Biology
Institute of Biology
University of Campinas (UNICAMP)
Campinas, Brazil

ISSN 0065-2598 ISSN 2214-8019 (electronic)
Advances in Experimental Medicine and Biology
ISBN 978-3-030-42666-8 ISBN 978-3-030-42667-5 (eBook)
https://doi.org/10.1007/978-3-030-42667-5

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

Preface

Aging is an inevitable part of life and is becoming a worldwide social, economic, and health problem. This is mainly due to the fact that the increasing proportion of individuals in the advanced age category has a higher probability of developing age-related disorders, such as type II diabetes mellitus, cardiovascular disorders, sarcopenia, and neurodegenerative conditions. New therapeutic approaches are still in need to decrease or slow the effects of such diseases in this advanced age category. Advances in "omic" technologies such as genomics, transcriptomics, proteomics, and metabolomics have significantly enhanced our understanding of disease in multiple medical areas along with the analysis of multiple molecular networks to provide a more integrated view of healthy and disease pathways. It is hoped that emerging hits from these analyses might be prioritized for further screening as potential novel drug targets for increasing the human healthspan in line with the lifespan. In turn, this will lead to new therapeutic strategies as well as drug development projects by the pharmaceutical industry.

This book presents a series of reviews describing studies, which have resulted in identification of potential new drug targets for age-related disorders. Much of this information has come from "omic" comparisons of healthy and disease states or from testing the effects of potential new therapeutic approaches. The authors in this series come from five of the six habitable continents from countries such as Australia, Brazil, Canada, Chile, China, Germany, India, Iran, Italy, Russia, Ukraine, the United Kingdom, and the United States. This underscores the keen interest in this topic throughout the world.

Chapter 1 discusses the closely linked relationship between reactive oxygen species, autophagy, and apoptosis in cancer therapy. Chapter 2 looks at the effects of micronutrient supplementation on immune function during aging. Chapter 3 describes how methods designed to restore bioactive lipids to normal levels can prevent age-related disorders and enhance longevity and health. Chapter 4 shows how modifications in the intestinal levels of short-chain fatty acids are linked with age-related pathologies including metabolic diseases and type 2 diabetes, hypertension, cardiovascular and neurodegenerative diseases, and cancer. Chapter 5 looks at the evidence of the rejuvenating effects of youthful systemic milieu on the aging

processes in the nervous system, skeletal muscle, heart, liver, and other organs. Chapter 6 focuses on physical exercise as a strategy to reduce skeletal muscle loss during aging. Chapter 7 describes how a combined intervention of polyphenols and regular physical exercise provides cognitive benefits for the aging brain. Chapter 8 presents a strategy involving identification and implementation of biomarker tests for diagnosis during the prodromal or early stages of Alzheimer's disease for better clinical outcomes. Chapter 9 looks at how understanding age-related changes in the circadian clock and minimizing circadian dysfunction may be crucial components to promote healthy aging. Chapter 10 highlights the etiology of depression in patients affected by Alzheimer's disease and speculates on more appropriate and alternative therapeutics. Chapter 11 describes how essential oils or formulations that contain terpenoids as major components may serve as important aromatherapeutics for relief of anxiety and depression. Chapter 12 summarizes the current knowledge of nutrition-based therapies for counteracting the effects of sarcopenia. Finally, Chap. 13 discusses the latest research on the use of the antidiabetic drug metformin as a potential intervention to reduce the risk of age-related disorders.

The book will be of high interest to researchers in the areas of aging and chronic disease, as well as to clinical scientists, physicians, and major drug companies since it gives insights into the latest ideas and technologies enabling progress in the area of healthy aging. It will provide important information on disease mechanisms related to the aging process, as each chapter will be presented in the context of specific chronic diseases or different therapeutic strategies.

Campinas, Brazil Paul C. Guest

Contents

Chapter 1
Targeting ROS-Mediated Crosstalk Between Autophagy and Apoptosis in Cancer

Lixia Gao, Jenni Loveless, Chloe Shay, and Yong Teng

1 Introduction

Reactive oxygen species (ROS) are generated as a by product in cellular oxidative metabolism processes (Fig. 1.1) [1–3]. The superoxide (O_2-), hydroxyl (OH•), peroxy1 (RO_2•) and hydroperoxy1 (HO_2•) radicals belong to oxygen radicals, while the non-radical oxidizing agents are hydrogen peroxide (H_2O_2), hypochlorous acid (HOCl), and ozone (O_3). Under specific conditions, the non-oxygen radicals can easily convert to radicals [4, 5]. This redox-balanced environment is maintained by

L. Gao
International Academy of Targeted Therapeutics and Innovation, Chongqing University of Arts and Sciences, Chongqing, P. R. China

J. Loveless
Department of Oral Biology and Diagnostic Sciences, Dental College of Georgia, Augusta University, Augusta, GA, USA

C. Shay
Department of Pediatrics, Emory Children's Center, Emory University, Atlanta, GA, USA
e-mail: chloe.shay@emory.edu

Y. Teng (✉)
Department of Oral Biology and Diagnostic Sciences, Dental College of Georgia, Augusta University, Augusta, GA, USA

Georgia Cancer Center, Department of Biochemistry and Molecular Biology, Medical College of Georgia, Augusta University, Augusta, GA, USA

Department of Medical Laboratory, Imaging and Radiologic Sciences, College of Allied Health, Augusta University, Augusta, GA, USA
e-mail: yteng@augusta.edu

P. C. Guest (ed.), *Reviews on New Drug Targets in Age-Related Disorders*,
Advances in Experimental Medicine and Biology 1260,
https://doi.org/10.1007/978-3-030-42667-5_1

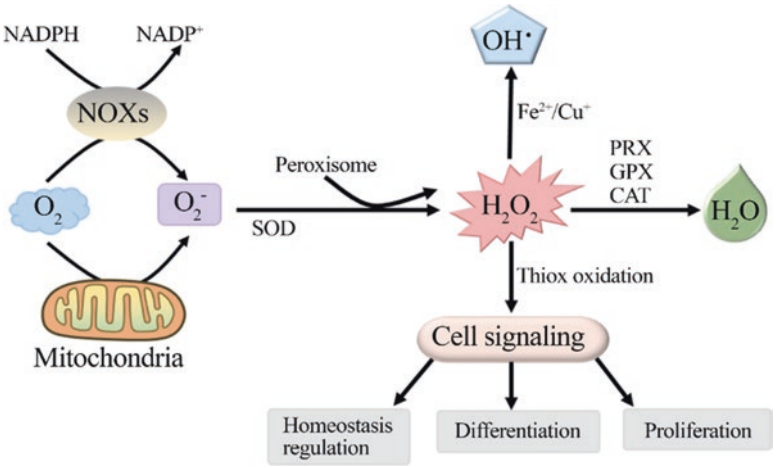


Fig. 1.1 ROS generation and regulation. The mitochondria and membrane bound NADPH oxidases (NOXs) are the two main sources to produce ROS. SOD is rapidly converted O2− into H_2O_2. In the cytosol, H_2O_2 promotes the production of OH• by reacting with metal ions ($Fe2^+$ or Cu^+). This process often induces the oxide stress to damage DNA, lipids, and proteins. H_2O_2 can be converted into H_2O and O_2 by cellular antioxidant proteins, such as peroxiredoxins (PRx), glutathione peroxidase (GPx), and catalase (CAT), as well as controlling cell signaling through protein thiol oxidation to regulate the homeostasis, differentiation and proliferation

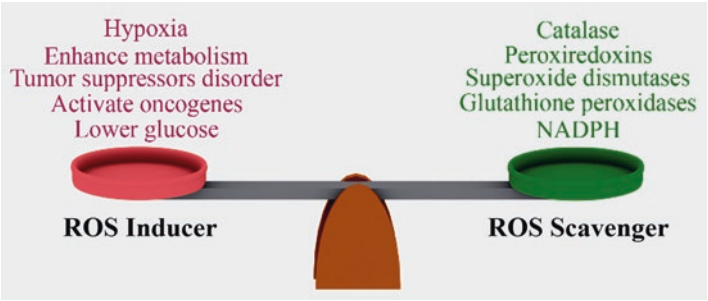


Fig. 1.2 A double-edged sword of ROS in cancer cells. Normally, under conditions of hypoxia, oncogene activation or tumor suppressor loss, metabolic activity is enhanced and low glucose will produce ROS in cancer cells. To maintain optimal ROS levels, cancer cells will use catalase, peroxiredoxins, superoxide dismutases, glutathione peroxidases and NADPH to remove excess ROS. The balance between ROS production and clearance has important effects in signal transduction

various enzymes such as superoxide dismutase (SOD), catalase and glutathione peroxidase, as well as substances such as ascorbate and α-tocopherol [6]. When a cell is in a state of dynamic equilibrium, it increases the release of ROS to exert important influence on body metabolism [7]. ROS not only participate in normal metabolism for enzyme reaction, mitochondrial electron transfer, signal transduction, and gene expression, but also contribute to some diseases, such as the occur-

rence and development of tumors (Fig. 1.2). One of the main reasons is because an excess of ROS in the mitochondria might induce oxidative stress and metabolic disorders, leading to damaged organelles, cell death and inflammation [8–10].

Typically, ROS participate in the interplay between autophagy and apoptosis by its ability to mediate the redox signaling pathways [11–13]. This triggers an autophagy response, which further induces oxidative stress when the autophagy function is disrupted. The elevated ROS created by oxidative stress eventually leads to apoptosis. Interestingly, autophagy and/or apoptosis in cancer cells can be triggered by ROS under various stressors [14, 15]. In this review, we focused on the importance and regulatory role of ROS in the interplay of autophagy and apoptosis in cancer therapy.

2 The Function of ROS in Cancer Development and Treatment

ROS as signaling molecules have an important role in physiological and pathology processes [16]. When compared to normal cells, recent studies have found elevated levels of ROS in cancer cells [17, 18]. This finding suggests that the levels of ROS are associated with the development of cancer. The physiological or optimal levels of ROS promote proliferation and differentiation in cancer cells [19], while the pathology or high levels of ROS cause oxidative damage of lipids, proteins and DNA in tandem with inducing cancer cell senescence and cell death [2, 10]. Oxidative stress is an important factor in both tumor development and cancer therapy [20]. Many signaling pathways are linked to tumorigenesis by regulating the metabolism of ROS through direct or indirect mechanisms. Weinberg and his colleagues reported the role of mitochondrial metabolism and ROS generation in Kras-mediated tumorigenicity [21]. Their study indicated that ROS generated from the mitochondrial metabolism regulated the extracellular signal-regulated kinase/mitogen-activated protein kinase (ERK/MAPK) signaling pathway in the process of Kras-induced anchorage-independent growth in the HCT116 human colon cancer cell line.

Interestingly, increased ROS can also promote cancer cells oncogenic mutations, leading to loss of tumor suppressor genes and accelerate cellular metabolism [22, 23]. At this point, the production of ROS is beneficial for cancer proliferation and differentiation. Takahashi et al. explored how ROS induced Transient receptor potential cation channel, subfamily A, member 1 (TRPA1) activation leading to Ca^{2+} influx is a defensive mechanism in cancer cells that promotes anti-apoptotic signalling [24]. They established that TRPA1 functioned to resist oxidative stress in cancer cells and revealed that TRPA1 oxidization differs from the ROS scavenging antioxidant defense program, as it can induce Ca^{2+} flux into cells to promote cancer cell survival under high levels of ROS. TRPA1 promotes cancer cell survival through

the upregulation of Ca^{2+}-dependent anti-apoptotic pathways, which have no association with increased antioxidant capacity of cancer cells.

In addition, ROS stimulate numerous genetic mutations, epigenetic changes and alter cell sensitivity to anticancer drugs [25, 26], leading to intrinsic and acquired resistance in cancer cells. Ye et al. showed that mitochondrial serine hydroxymethyltransferase (SHMT2) catabolism can be favorable for tumor growth by regulating the mitochondrial reduction-oxidation (redox) balance [27]. They found that knockdown of SHMT2 affected the cellular reduced/oxidized ratio of nicotinamide adenine dinucleotide phosphate ($NADPH/NADP^+$) in Myc-dependent cells along with ROS levels, resulting in hypoxia-mediated cell death. This finding indicates that mitochondrial serine catabolism contributes to the maintenance of mitochondrial redox balance and cell survival. Our group recently showed that extremely low ROS levels in hepatocellular carcinoma (HCC) cells are one compelling mechanism that underpins how HCC cells escape from sorafenib-induced apoptosis [28]. Thus far, many studies have confirmed the positive correlation between excessive ROS and cancer cell death [29, 30]. Lee's group found that a dual stimuli-responsive hybrid anticancer drug, QCA, can preferentially kill cancer cells by amplifying oxidative stress in vitro and in vivo [31]. QCA activates H_2O_2 and acidifies pH to generate antioxidant reduced glutathione (GSH)-depleting quinone methide (QM) and ROS generating cinnamaldehyde, respectively. This novel drug inhibits antioxidant systems and increases ROS stressors to induce cancer cell death. Ge et al. reported that a natural chemical, deoxypodophyllotoxin (DPT), triggers glioma cell death by generating excessive ROS [32]. They found that DPT promoted up-regulation of poly (ADP-ribose) polymerase 1 (PARP-1), cytoplasmic accumulation of PAR polymer, and nuclear translocation of apoptosis-inducing factor (AIF). Moreover, DPT not only induced glioma cell death in vitro, but also inhibited the growth of xenograft glioma in vivo. This phenomenon disappeared though when using the PARP-1 inhibitor, 3AB, to treat glioma cells. Along with this finding, the antioxidant NAC inhibited the excessive ROS production by DPT, suggesting that DPT triggers cell death in glioma cells due to the generation of excessive ROS.

3 ROS-Mediated Cancer Cell Autophagy

Accumulation of ROS induces autophagy and, in turn, autophagy serves to reduce ROS levels. ROS and autophagy have a similar function in cancer cell survival and cell death. This is why some of the current treatment strategies have been developed based on one or both of these factors. In general, ROS are targeted in two contradicting manners in cancer therapy: (i) inhibition of ROS formation to promote the cellular survival signaling pathway and (ii) induction of ROS formation to trigger the death signaling pathway. Similarly, autophagy has two targeted pathways: (i) blockade of autophagy inhibiting its cytoprotection and (ii) induction of high levels of cellular autophagy increasing cellular death. Thus, the interaction and the balance between ROS and autophagy can be a key part of regulating cellular homeo-

stasis. It is well established that both ROS and autophagy are strongly associated with cancer development and progression, but clarifying the functional relationship of the two mechanisms seems to be difficult because of their dual role in cancer processes [33–35].

Autophagy is a lysosomal degradation pathway that is essential for survival, differentiation, development, and homeostasis. The various stages of autophagy are regulated by the autophagy signaling molecule transcription factor EB (TFEB), the mechanistic target of rapamycin complex 1 (mTOR1) and lysosomes. Zhang's group reported that lysosomes regulate the response of autophagy to oxidative stress by the TFEB and mTOR1 pathways [36]. They provided evidence that the mitochondrial ROS levels can induce Ca^{2+} release through activation of the transient receptor potential cation channel, mucolipin subfamily, member 1 (MCOLN1), leading to activation of the TFEB nuclear translocation to induce autophagy. This is a good example that ROS have the ability to regulate autophagy by the MCOLN1-lysosome Ca^{2+}-TFEB pathway. Recently, related studies have shown that the autophagy-related protein 4 (ATG4) is regulated by changes in redox potential under different conditions and in specific subcellular microenvironments [37]. Elazar et al. mainly described the role of ROS as signaling molecules in starvation induced autophagy [38]. In a state of starvation, the cancer cells prefer to produce ROS, especially H_2O_2. This oxidation process is necessary because it can affect the formation of autophagosomes. They further showed that hydrogen peroxide can induce the cysteine protease HsAtg4 and activate a cysteine residue located near the HsAtg4 catalytic site. This demonstrated that regulating the expression of HsAtg4 may be a molecular mechanism for redox regulation of autophagy processes.

Mitochondria are a significant source of pathological ROS production as well as the sites in which ROS-activated signaling pathways converge [33]. However, how mitochondria mediate autophagy under stress through controlling ROS levels and status remains to be elucidated. Perez et al. described mitochondrial DNA regulated cell autophagy by the ROS-AMPK-ULK1 signaling pathway [35]. When chemical hypoxia was induced in Human SK-Hep-1 wild-type and mtDNA depleted (Rho) cells, they found that chemical hypoxia can cause down-regulation of hypoxia inducible factor-1α-dependent autophagy and suppression of Bcl-2 and mTOR signaling, whereas the AMPK/ULK1-mediated pro-autophagy pathway was activated in wild-type cells. They also found that chemical hypoxia could induce the lower levels of ROS in Rho cells in comparison to wild-type cells. Mitochondrial dysfunction can reduce the ROS in cancer cells and affect the autophagy process.

ROS accumulation in cancer cells can induce oxidative stress, whereas the autophagy pathway responds to oxidative stress in cells. If the autophagy is unable to recover from the oxidative stress, they will have to face the fate of death. Expression of ATG5 expression as an autophagy target gene can influence the level of autophagy and cell death by the amount of ROS involved. Agostinis's group studied the role of ROS and autophagy in regulating immunogenic cell death (ICD) by hypericin-mediated photodynamic (Hyp-PDT) [39], which induced ROS-based

endoplasmic reticulum stress and triggered ICD. Based on this work, ATG5 knockdown cancer cells were developed for exploration of the autophagy signaling pathway. This work indicated that autophagy-attenuated cancer cells can express enhanced ecto-CALR induction and immunogenic cell proliferation under Hyp-PDT. This study suggested that ROS-induced autophagy has an important function between dying cancer cells and the immune system.

4 ROS-Mediated Cancer Cell Apoptosis

ROS are mainly produced endogenously by mitochondrial damage or exogenously in cells exposed to oxidative stressors [40, 41]. Regulation of apoptosis is complicated as there are two apoptotic pathways, the death receptor pathway and the mitochondrial pathway. Although these regulatory pathways are defined separately, their pathways are interactive [42]. As far as we know, the mitochondria can release cytochrome c, which activates caspases. Therefore, the mitochondria play a regulatory role in metabolic-redox in the ROS-mediated cancer cell apoptosis [12]. ROS are generated mainly from the electrons leaking out of mitochondrial electron transport chain, and reverse ROS could damage mitochondrial function [43]. This has been described as "a double-edged sword" in cancer therapy [44]. On the one hand, high levels of ROS can induce cancer apoptosis. On the other hand, ROS can negatively influence the genetic stability resulting in cancer drug resistance [45]. The occurrence of chemotherapy drug resistance is the main reason for the prognosis of cancer treatment, and it is also a problem that has been plaguing researchers.

Some anticancer drugs were reported to induce apoptosis by ROS-mediated processes [46, 47]. Ouyang's group investigated the anticancer ability of Baicalin and its mechanism in human osteosarcoma cells [48], showing that Baicalin induced apoptosis through a ROS-mediated mitochondrial pathway. Zinc oxide (ZnO) nanoparticles have been widely used in cosmetics and sunscreen products. However, whether or not it is toxic to the human body has yet to be explored. To solve this puzzle, Dhawan et al. studied the effect of ZnO nanoparticles in human HCC HepG2 cells [49]. When ZnO nanoparticles (14–20 μg/mL) were treated for 12 h, they found that these nanoparticles induced apoptosis in the HepG2 cells through upregulation of cellular oxidative damage. In the study, ROS decreased the mitochondrial membrane potential and increased the ratio of the apoptotic markers Bax/Bcl-2, leading to cell apoptosis.

When cancer cell apoptosis fails, ROS activate drug resistance [50, 51]. In our previous work, we investigated the regulatory function of ROS involved in the fibroblast growth factor 19/fibroblast growth factor receptor 4 (FGF19/FGFR4) signaling pathway in order to sensitize resistance of HCC cells to sorafenib [28]. FGFR4 has the ability to regulate ROS levels upon FGF19 signaling in HCC cells. We found that over-expression of FGF19 led to a defensive mechanism against oxidative stress from ROS and apoptosis in the HCC cells. Either loss of FGF19 or FGFR4

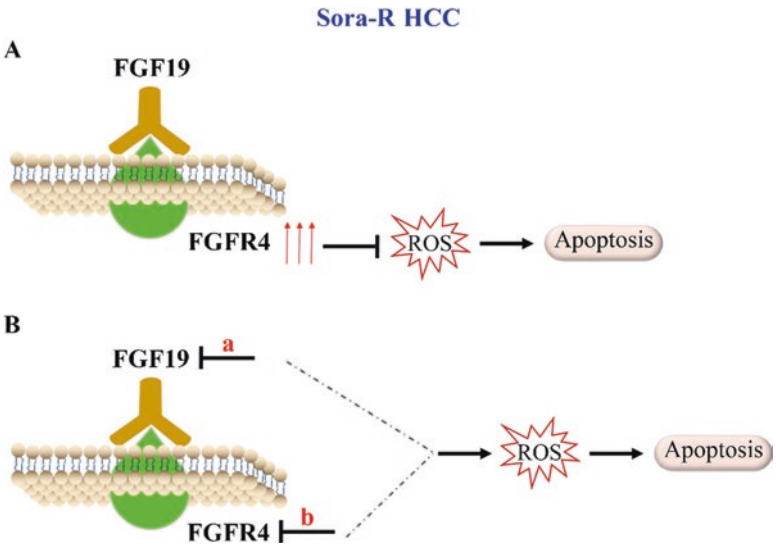


Fig. 1.3 FGFR4-mediated ROS is involved the sorafenib-induced HCC cell apoptosis. (**a**) In sorafenib-resistant (Sora-R) HCC cells, overactivity of FGFR4 inhibits ROS, which in turn blocks its mediated apoptosis. (**b**) Inactivation of the FGF19-FGFR4 axis by inhibition of (a) FGF19 expression or (b) suppression of FGFR4 phosphorylation can overcome the resistance of HCC cells to sorafenib by enhanced ROS-dependent apoptosis

expression counteracted these phenotypes. We further demonstrated that inhibition of FGFR4 by the pan-FGFR inhibitor ponatinib or the FGFR4 selective inhibitor BLU9931 can overcome sorafenib-induced resistance through enhancing ROS-induced apoptosis in HCC cells (Fig. 1.3) [28, 52]. The results from our study may lead to a novel anticancer strategy to improve the therapeutic efficacy of sorafenib in patients with HCC. We also showed that the proto-oncogene B-Raf $(BRAF)^{V600E}$ inhibitor, PLX4032, can induce generation of $O2^{-}$ and nitric oxide (NO) in $BRAF^{V600E}$ mutant A375 cells [33]. Coinciding with this finding, we also found that PLX4032 reduced mitochondrial membrane potential in human malignant melanoma-derived A375 cells. These observations suggest that PLX4032 induces apoptosis in part by accumulation of ROS production in melanoma cells.

5 ROS-Mediated Complex Interplay Between Autophagy and Apoptosis in Cancer Cells

The relationship between apoptosis and autophagy is extremely complex in cancer cells. Under certain environmental conditions, autophagy and apoptosis can exert synergistic effects, whereas in other conditions, autophagy and apoptosis can inhibit each other [53–55]. The molecular mechanisms between autophagy and apoptosis

in cancer are always the focus of researchers [55, 56]. These include the discussion of the different effects of autophagy on apoptosis [57], and the finding that Licarin A induces autophagy and apoptosis by ROS in non-small cell lung cancer cells [58]. In addition, chemotherapeutic agents, such as cisplatin or 5FU, have been reported to induce autophagy in HCC cells [59]. When autophagy was blocked by treatment with 3-methyladenine (3-MA) or depletion of Beclin 1, chemotherapy-induced apoptosis was enhanced in the HCC cells. Further studies revealed that the combination of an autophagy inhibitor (chloroquine, CQ) and chemotherapy significantly increased cell apoptosis and inhibited tumor growth in a mouse xenograft tumor model [59]. The tumor suppressor gene of p53 plays an important role in the process of tumor development. According to some reports, p53 involves ROS-dependent autophagy and apoptosis in cancer cells. Moreover, some drugs have the potential to induce cell apoptosis and autophagy by the ROS/c-Jun N-terminal kinase (JNK) signaling pathway [60, 61]. In addition, we explored CYT997, a novel microtubule-disrupting agent. When CYT997 is in combination with the autophagy inhibitor, hydroxychloroquine (HCQ), it can enhance the anticancer capacity by modulating the levels of ROS in head and neck squamous cell carcinoma (HNSCC) (Fig. 1.4) [62]. CYT997 triggered autophagy in HNSCC cells by accumulating ROS levels as evidenced by appearance of numerous autophagic vacuoles and increased levels of

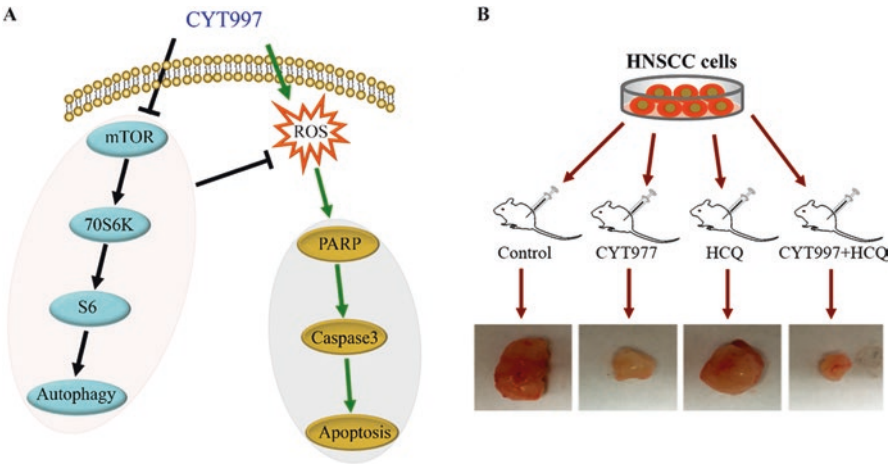


Fig. 1.4 The importance of ROS in crosstalk between autophagy and apoptosis in CYT997 treatment. (**a**) Upregulation of autophagy by mTOR-dependent pathways appears to have a cytoprotective role in preventing apoptosis by inhibiting CYT997-induced excessively high levels of ROS. (**b**) Blockade of autophagy using HCQ sensitizes HNSCC cell to CYT997 through enhancing apoptosis

autophagy-related protein 7 (ATG7). Our investigation also uncovered that upregulation of autophagy was mTOR-dependent, which played a cytoprotective role in preventing apoptosis in CYT997 treatment (Fig. 1.4). Blockade of autophagy using HCQ increased sensitivity of HNSCC cells to CYT997 through inhibition of CYT997-induced excessively high levels of ROS. Similar results were obtained in the xenograft mouse model of HNSCC. Therefore, we suggest that the addition of autophagy blockade may have potential in enhancing the therapeutic outcomes of microtubule-targeting drugs in the treatment of HNSCC.

6 Conclusions

Autophagy and apoptosis are two distinct self-destructive processes that influence the normal clearance of dying cells. The disruption of the relationship between autophagy and apoptosis has important pathophysiological consequences, including affecting oncogenesis at multiple stages ranging from transformation to metastasis. Control of the crosstalk between autophagy and apoptosis in tumor cells may remove a critical barrier to comprehensive and efficacious cancer treatment. It is clear that ROS play a pivotal role in regulating the balance between autophagy and apoptosis. Therefore, how to tightly control ROS levels in cancer cells can have a significant influence on the response of cancer cells to cancer therapy. In the future, antioxidants, autophagy inhibitors and apoptosis inducers will be a potential therapeutic strategy during the course of treatment of cancer patients.

Competing Interests The authors declare no competing financial interests.

References

1. Apel K, Hirt H (2004) Reactive oxygen species: metabolism, oxidative stress, and signal transduction. Annu Rev Plant Biol 55:373–399
2. Reczek CR, Chandel NS (2015) ROS-dependent signal transduction. Curr Opin Cell Biol 33:8–13
3. Rabinovitch RC, Samborska B, Faubert B, Ma EH, Gravel SP, Andrzejewski S et al (2017) AMPK maintains cellular metabolic homeostasis through regulation of mitochondrial reactive oxygen species. Cell Rep 21(1):1–9
4. Bayir H (2005) Reactive oxygen species. Crit Care Med 33(12 Suppl):S498–S501
5. Di Meo S, Reed TT, Venditti P, Victor VM (2016) Role of ROS and RNS sources in physiological and pathological conditions. Oxidative Med Cell Longev 2016:1245049. https://doi.org/10.1155/2016/1245049
6. Fruehauf JP, Meyskens FL Jr (2007) Reactive oxygen species: a breath of life or death? Clin Cancer Res 13(3):789–794
7. Lobo V, Patil A, Phatak A, Chandra N (2010) Free radicals, antioxidants and functional foods: impact on human health. Pharmacogn Rev 4(8):118–126

8. Busciglio J, Yankner BA (1995) Apoptosis and increased generation of reactive oxygen species in Down's syndrome neurons in vitro. Nature 378(6559):776–779
9. Irani K (2000) Oxidant signaling in vascular cell growth, death, and survival: a review of the roles of reactive oxygen species in smooth muscle and endothelial cell mitogenic and apoptotic signaling. Circ Res 87(3):179–183
10. Schumacker PT (2006) Reactive oxygen species in cancer cells: live by the sword, die by the sword. Cancer Cell 10(3):175–176
11. Scherz-Shouval R, Elazar Z (2007) ROS, mitochondria and the regulation of autophagy. Trends Cell Biol 17(9):422–427
12. Circu ML, Aw TY (2010) Reactive oxygen species, cellular redox systems, and apoptosis. Free Radic Biol Med 48(6):749–762
13. Simon HU, Haj-Yehia A, Levi-Schaffer F (2000) Role of reactive oxygen species (ROS) in apoptosis induction. Apoptosis 5(5):415–418
14. Chen Y, McMillan-Ward E, Kong J, Israels SJ, Gibson SB (2008) Oxidative stress induces autophagic cell death independent of apoptosis in transformed and cancer cells. Cell Death Differ 15(1):171–182
15. Li HY, Zhang J, Sun LL, Li BH, Gao HL, Xie T et al (2015) Celastrol induces apoptosis and autophagy via the ROS/JNK signaling pathway in human osteosarcoma cells: an in vitro and in vivo study. Cell Death Dis 6:e1604. https://doi.org/10.1038/cddis.2014.543
16. Finkel T (2011) Signal transduction by reactive oxygen species. J Cell Biol 194(1):7–15
17. Tong L, Chuang CC, Wu S, Zuo L (2015) Reactive oxygen species in redox cancer therapy. Cancer Lett 367(1):18–25
18. Schumacker PT (2015) Reactive oxygen species in cancer: a dance with the devil. Cancer Cell 27(2):156–157
19. Mittler R (2017) ROS are good. Trends Plant Sci 22(1):11–19
20. Gorrini C, Harris IS, Mak TW (2013) Modulation of oxidative stress as an anticancer strategy. Nat Rev Drug Discov 12(12):931–947
21. Weinberg F, Hamanaka R, Wheaton WW, Weinberg S, Joseph J, Lopez M et al (2010) Mitochondrial metabolism and ROS generation are essential for Kras-mediated tumorigenicity. Proc Natl Acad Sci U S A 107(19):8788–8793
22. Dixon SJ, Stockwell BR (2014) The role of iron and reactive oxygen species in cell death. Nat Chem Biol 10(1):9–17
23. Panieri E, Santoro MM (2016) ROS homeostasis and metabolism: a dangerous liason in cancer cells. Cell Death Dis 7(6):e2253. https://doi.org/10.1038/cddis.2016.105
24. Takahashi N, Chen HY, Harris IS, Stover DG, Selfors LM, Bronson RT et al (2018) Cancer cells co-opt the neuronal redox-sensing channel TRPA1 to promote oxidative-stress tolerance. Cancer Cell 33(6):985–1003.e7. https://doi.org/10.1016/j.ccell.2018.05.001
25. Pelicano H, Carney D, Huang P (2004) ROS stress in cancer cells and therapeutic implications. Drug Resist Updat 7(2):97–110
26. Priya LB, Baskaran R, Huang CY, Padma VV (2017) Neferine ameliorates cardiomyoblast apoptosis induced by doxorubicin: possible role in modulating NADPH oxidase/ROS-mediated NFκB redox signaling cascade. Sci Rep 7(1):12283. https://doi.org/10.1038/s41598-017-12060-9
27. Ye J, Fan J, Venneti S, Wan YW, Pawel BR, Zhang J et al (2014) Serine catabolism regulates mitochondrial redox control during hypoxia. Cancer Discov 4(12):1406–1417
28. Gao L, Wang X, Tang Y, Huang S, Hu CA, Teng Y (2017) FGF19/FGFR4 signaling contributes to the resistance of hepatocellular carcinoma to sorafenib. J Exp Clin Cancer Res 36(1):8. https://doi.org/10.1186/s13046-016-0478-9
29. Reczek CR, Chandel NS (2017) The two faces of reactive oxygen species in cancer. Annu Rev Cancer Biol 1(1):79–98
30. Nogueira V, Hay N (2013) Molecular pathways: reactive oxygen species homeostasis in cancer cells and implications for cancer therapy. Clin Cancer Res 19(16):4309–4314

31. Noh J, Kwon B, Han E, Park M, Yang W, Cho W et al (2015) Amplification of oxidative stress by a dual stimuli-responsive hybrid drug enhances cancer cell death. Nat Commun 6:6907. https://doi.org/10.1038/ncomms7907
32. Ma D, Lu B, Feng C, Wang C, Wang Y, Luo T et al (2016) Deoxypodophyllotoxin triggers parthanatos in glioma cells via induction of excessive ROS. Cancer Lett 371(2):194–204
33. Gao L, Jauregui CE, Teng Y (2017) Targeting autophagy as a strategy for drug discovery and therapeutic modulation. Future Med Chem 9(3):335–345
34. Sena LA, Chandel NS (2012) Physiological roles of mitochondrial reactive oxygen species. Mol Cell 48(2):158–167
35. Marin JJG, Lozano E, Perez MJ (2016) Lack of mitochondrial DNA impairs chemical hypoxia-induced autophagy in liver tumor cells through ROS-AMPK-ULK1 signaling dysregulation independently of HIF-1alpha. Free Radic Biol Med 101:71–84
36. Zhang X, Yu L, Xu H (2016) Lysosome calcium in ROS regulation of autophagy. Autophagy 12(10):1954–1955
37. Scherz-Shouval R, Shvets E, Fass E, Shorer H, Gil L, Elazar Z (2007) Reactive oxygen species are essential for autophagy and specifically regulate the activity of Atg4. EMBO J 26(7):1749–1760
38. Scherz-Shouval R, Sagiv Y, Shorer H, Elazar Z (2003) The COOH terminus of GATE-16, an intra-Golgi transport modulator, is cleaved by the human cysteine protease HsApg4A. J Biol Chem 278(16):14053–14058
39. Garg AD, Dudek AM, Ferreira GB, Verfaillie T, Vandenabeele P, Krysko DV et al (2013) ROS-induced autophagy in cancer cells assists in evasion from determinants of immunogenic cell death. Autophagy 9(9):1292–1307
40. Shadel GS, Horvath TL (2015) Mitochondrial ROS signaling in organismal homeostasis. Cell 163(3):560–569
41. Zorov DB, Juhaszova M, Sollott SJ (2014) Mitochondrial reactive oxygen species (ROS) and ROS-induced ROS release. Physiol Rev 94(3):909–950
42. Igney FH, Krammer PH (2002) Death and anti-death: tumour resistance to apoptosis. Nat Rev Cancer 2(4):277–288
43. Guzy RD, Hoyos B, Robin E, Chen H, Liu L, Mansfield KD et al (2005) Mitochondrial complex III is required for hypoxia-induced ROS production and cellular oxygen sensing. Cell Metab 1(6):401–408
44. Liou GY, Storz P (2010) Reactive oxygen species in cancer. Free Radic Res 44(5):479–496
45. Okon IS, Zou MH (2015) Mitochondrial ROS and cancer drug resistance: implications for therapy. Pharmacol Res 100:170–174
46. Chen B, Cao X, Lu H, Wen P, Qi X, Chen S et al (2018) N-(3-oxo-acyl) homoserine lactone induced germ cell apoptosis and suppressed the over-activated RAS/MAPK tumorigenesis via mitochondrial-dependent ROS in C. Elegans. Apoptosis 23(11–12):626–640
47. Moon DO, Kim MO, Choi YH, Hyun JW, Chang WY, Kim GY (2010) Butein induces G(2)/M phase arrest and apoptosis in human hepatoma cancer cells through ROS generation. Cancer Lett 288(2):204–213
48. Wan D, Ouyang H (2018) Baicalin induces apoptosis in human osteosarcoma cell through ROS-mediated mitochondrial pathway. Nat Prod Res 32(16):1996–2000
49. Sharma V, Anderson D, Dhawan A (2012) Zinc oxide nanoparticles induce oxidative DNA damage and ROS-triggered mitochondria mediated apoptosis in human liver cells (HepG2). Apoptosis 17(8):852–870
50. Shen Y, Yang J, Zhao J, Xiao C, Xu C, Xiang Y (2015) The switch from ER stress-induced apoptosis to autophagy via ROS-mediated JNK/p62 signals: a survival mechanism in methotrexate-resistant choriocarcinoma cells. Exp Cell Res 334(2):207–218
51. Chen Z, Teo AE, McCarty N (2016) ROS-induced CXCR4 signaling regulates mantle cell lymphoma (MCL) cell survival and drug resistance in the bone marrow microenvironment via autophagy. Clin Cancer Res 22(1):187–199

52. Gao L, Shay C, Lv F, Wang X, Teng Y (2018) Implications of FGF19 on sorafenib-mediated nitric oxide production in hepatocellular carcinoma cells – a short report. Cell Oncol (Dordr) 41(1):85–91
53. Cheng Y, Zhang Y, Zhang L, Ren X, Huber-Keener KJ, Liu X et al (2012) MK-2206, a novel allosteric inhibitor of Akt, synergizes with gefitinib against malignant glioma via modulating both autophagy and apoptosis. Mol Cancer Ther 11(1):154–164
54. Kang R, Zeh HJ, Lotze MT, Tang D (2011) The Beclin 1 network regulates autophagy and apoptosis. Cell Death Differ 18(4):571–580
55. Mariño G, Niso-Santano M, Baehrecke EH, Kroemer G (2014) Self-consumption: the interplay of autophagy and apoptosis. Nat Rev Mol Cell Biol 15(2):81–94
56. Moscat J, Diaz-Meco MT (2009) p62 at the crossroads of autophagy, apoptosis, and cancer. Cell 137(6):1001–1004
57. Jain MV, Paczulla AM, Klonisch T, Dimgba FN, Rao SB, Roberg K et al (2013) Interconnections between apoptotic, autophagic and necrotic pathways: implications for cancer therapy development. 1. J Cell Mol Med 17(1):12–29
58. Maheswari U, Ghosh K, Sadras SR (2018) Licarin A induces cell death by activation of autophagy and apoptosis in non-small cell lung cancer cells. Apoptosis 23(3–4):210–225
59. Guo XL, Li D, Hu F, Song JR, Zhang SS, Deng WJ et al (2012) Targeting autophagy potentiates chemotherapy-induced apoptosis and proliferation inhibition in hepatocarcinoma cells. Cancer Lett 320(2):171–179
60. Wang G, Zhang T, Sun W, Wang H, Yin F, Wang Z et al (2017) Arsenic sulfide induces apoptosis and autophagy through the activation of ROS/JNK and suppression of Akt/mTOR signaling pathways in osteosarcoma. Free Radic Biol Med 106:24–37
61. Wang H, Zhang T, Sun W, Wang Z, Zuo D, Zhou Z et al (2016) Erianin induces G2/M-phase arrest, apoptosis, and autophagy via the ROS/JNK signaling pathway in human osteosarcoma cells in vitro and in vivo. Cell Death Dis 7(6):e2247. https://doi.org/10.1038/cddis.2016.138
62. Gao L, Zhao X, Lang L, Shay C, Andrew Yeudall W, Teng Y (2018) Autophagy blockade sensitizes human head and neck squamous cell carcinoma towards CYT997 through enhancing excessively high reactive oxygen species-induced apoptosis. J Mol Med (Berl) 96(9):929–938

Chapter 2
Micronutrients that Affect Immunosenescence

Behnaz Abiri and Mohammadreza Vafa

1 Introduction

Over the past few decades, longevity has significantly elevated and, as a result, the health system is currently facing the growing emergence of age-related diseases [1]. Food itself is a cause of age-related disease since the production of reactive oxygen species (ROS), advanced glycation end products, advanced lipoxidation end products and inflammatory mediators lead to multiple tissue damage (Fig. 2.1) [2]. In addition, it has been repeatedly reported that a normal nutritional status is essential for optimal immune function. However, the prevalence rate of malnutrition is generally higher among the elderly, especially in community-dwelling and nursing home residents [3]. It is believed that malnutrition or insufficient intake of certain nutrients found in the elderly constitutes another adverse factor further contributing to the dysregulation of immune function developed with aging. On this basis, in free-living elderly individuals, aging is characterized by low-grade inflammation, the so-called "inflammaging", which may evolve toward a chronic inflammatory condition when the accumulation of metabolic products becomes excessive, thus aggravating tissue damage (Fig. 2.2) [4]. Immunosenescence reflects the decrease with age of the immune response in humans and abnormal immunity contributes to the complications of age-related diseases. Immune abnormalities in the elderly have

B. Abiri
Department of Nutrition, Faculty of Paramedicine, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran

M. Vafa (✉)
Department of Nutrition, School of Public Health, Iran University of Medical Sciences, Tehran, Iran

Pediatric Growth and Development Research Center, Institute of Endocrinology and Metabolism, Iran University of Medical Sciences, Tehran, Iran
e-mail: vafa.m@iums.ac.ir

P. C. Guest (ed.), *Reviews on New Drug Targets in Age-Related Disorders*,
Advances in Experimental Medicine and Biology 1260,
https://doi.org/10.1007/978-3-030-42667-5_2

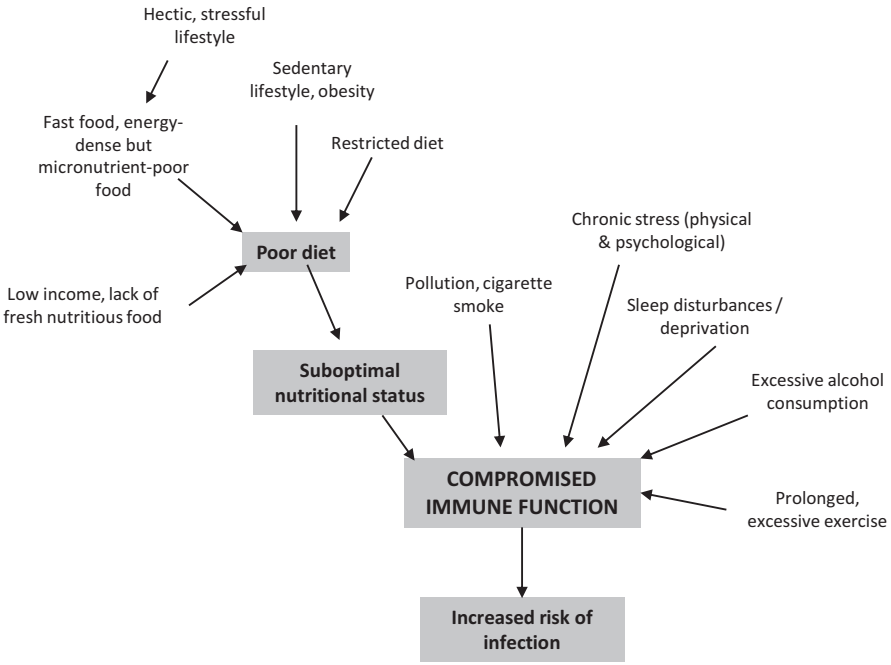


Fig. 2.1 Lifestyle-associated factors affecting immune function

been found in both the innate and the adaptive immune system. In particular, dysfunction of granulocytes, monocytes/macrophages (Mθ) and natural killer (NK) cells has been reported. On the other hand, changes in both T and B lymphocytes have been indicated in senescence. Further details on immune cells will be discussed in this chapter. Easier access of pathogens into the aged host plays a pathogenic role in the aggravation of inflammatory and dysmetabolic states (obesity and diabetes), atherosclerosis and neurodegeneration [5]. Moreover, in the elderly, imbalanced immune networks can account for the elevated frequency of autoimmune disease and cancer, thus justifying therapeutic efforts to correct impaired immunity in the elderly [6]. In this chapter, an overview of the major changes of the immune system in the elderly will be provided. In addition, treatment of age-related disease by micronutrients and their mechanisms will be discussed. Thus, novel therapeutic attempts to correct the aged immune responsiveness with micronutrients will be described.

2 The Immune System in Aging

As the body ages, so does the immune system [7] and most people over the age of 60 years experience some immune dysregulation that makes them less able to respond to immune challenges [8, 9]. Immune cells are continually renewed from

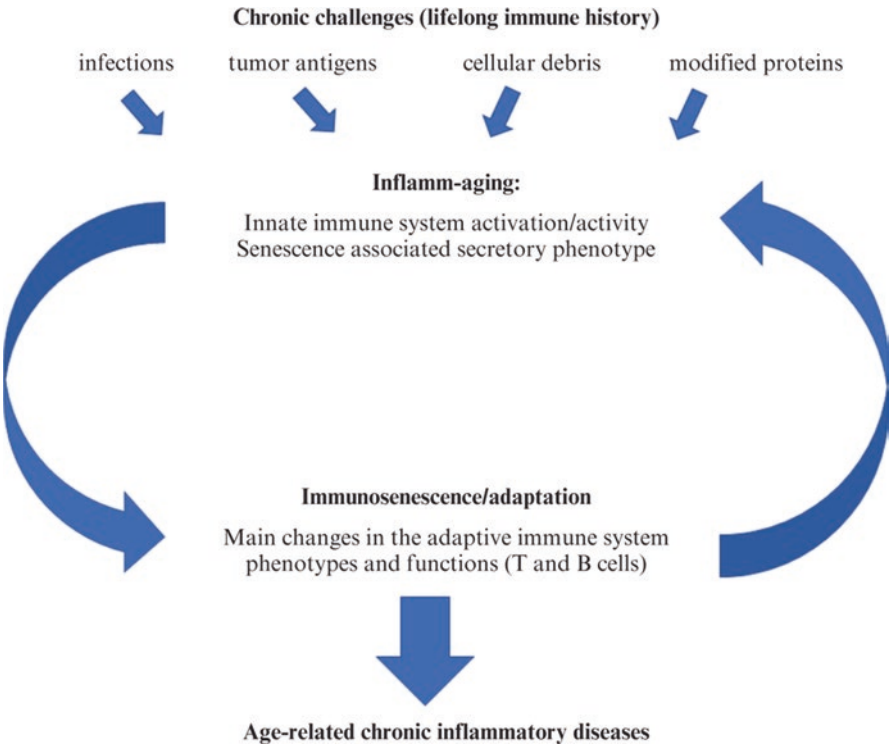


Fig. 2.2 The association between inflammaging and immunosenescence/adaptation

hematopoietic stem cells but these mature with age and become less able to generate lymphocytes. Furthermore, the total amount of hematopoietic tissue declines [9, 10]. A loss of immune cells and a reduction in the number of circulating lymphocytes are characteristic in the immune systems of older people [11], consistent with decreased production of T cells in the involuted thymus, as well as diminished function of mature lymphocytes in secondary lymphoid tissues [9, 12]. Alterations in the innate immune system also occur with advancing age. Skin and mucous membranes—the first line of defense against invading pathogens—become less effective as skin cell replacement reduces and dermal and subcutaneous atrophy occurs [13]. After 60 years of age, there is a reduction in secretory IgA, which forms part of the first line of defense against pathogens that manage to invade the mucosal surfaces [14]. In elderly people, functional activity of immune cells including phagocytes and the intracellular respiratory burst necessary to kill pathogens are decreased [13]. Indeed, a longer inflammatory process is induced in elderly people [15]. Increased levels of circulating pro-inflammatory cytokines such as tumor-necrosis factor alpha (TNF-α), interleukin (IL)-1, and IL-6 characterize low-grade chronic inflammation in elderly people, a process known as inflammaging [13]. Inflammaging is a physiological response to lifelong antigenic stress and, if kept under control by anti-inflammatory cytokines such as IL-10 [13], represents an efficient defense

mechanism in older people. Elevated production of anti-inflammatory molecules is an important counter-regulatory process in aging, as inflammaging would otherwise be damaging [16]. Many of the most common chronic diseases related to aging, such as atherosclerosis, Alzheimer's disease, osteoporosis and diabetes [13], are associated with low-grade inflammation [7]. Oxidative stress also has a role in inflammaging, emphasizing the role of oxidative stress in the complex mechanisms of aging [16]. Immune cells, which contain a high percentage of polyunsaturated fatty acids in their plasma membrane and so are inclined to lipid peroxidation, are particularly sensitive to alterations in the oxidant–antioxidant balance [17]. Hence, oxidative damage can compromise the integrity of immune cell membranes and change transmission of signals both within and between various immune cells, resulting in an impaired immune response [17]. It has been proposed that, in elderly people, many immune markers of immunosenescence may actually be more associated with prolonged exposure to antigen stimulation and to oxidative stress involving the production of reactive oxygen species, rather than to "aging" of the immune system [10–12].

An extensive set of receptors is involved in the phagocytic process, such as complement receptors, toll-like receptors (TLRs), fragment crystallizable receptors and scavenger receptors in response to pathogens and autologous apoptotic cells [18]. In older people, both polymorphonuclear cells (PMN) and Mθ demonstrate impaired phagocytosis and oxidative burst [19]. In elderly humans, dendritic cells also exhibited a decreased capacity to phagocytose apoptotic cells in vitro [20]. The abovementioned abnormalities may justify the high frequency of infectious events (such as winter infections) in aging. On the other hand, decreased phagocytic clearance of apoptotic cells may result in accumulation of debris which, in turn, triggers inflammation or autoimmune reactions in the aged host [21]. With regards to NK cells, evidence has been reported that these cells undergo age-associated alterations, which may explain the increased incidence of viral infections in the elderly [22]. Also, in the case of T regulatory (Treg) cells, some changes have been shown in aging [23]. All of these modifications may account for an elevated incidence of autoimmune diseases, cancer and infections in the elderly, even if their mechanisms of action require more detailed evaluations. Indeed, the tuning of Treg cell-mediated immune suppression in aging is very delicate and its imbalance may shift from a protective impact (anti-inflammatory activity) to a detrimental status of disease outcome. Th17 cells polarize the immune response toward an inflammatory profile and have an important effect in the development of autoimmune and chronic inflammatory disease, hence overcoming the anti-inflammatory impacts exerted by Treg cells. Th17 cells are elevated in aging with a decrease of Treg cells [24]. However, after activation the Th17/Treg cell ratio tends to reduce with an elevation in forkhead box (Fox)P3 and IL-10. Thus, this ratio may represent an important target for controlling autoimmune and inflammatory disease in the elderly. The decrease in murine B cell lymphopoiesis with increasing age appears to depend on the inability of hemopoietic stem cells to generate B cells, thus leading to a lack of early B cell lineage precursors [25]. In addition, IL-7 production by stromal cells of the bone marrow is decreased with advancing age, thus retarding B cell development in the early phase with a significant decrease of pre-B cell numbers. In humans aged

CD19+ B cell number reduces [26], while the aging-related B cell (ABC) subset can be detected in peripheral blood [27]. In in vitro studies, ABCs have been indicated to respond to innate stimuli with polarization toward Th17 cells and secretion of autoantibodies.

Genetic and environmental factors (such as nutritional status) may play a role in immune function throughout aging, but these have yet to be described.

3 Intake of Micronutrients in the Elderly

The daily intake of micronutrients is often inadequate in the elderly, owing to many causes, such as poor socio-economic states, loss of appetite, lack of teeth, changed intestinal absorption of food and low requirement of energy [28]. The important micronutrients with immunomodulating properties will be discussed later.

3.1 Micronutrient Requirements and Reported Deficiencies

Although the recommended dietary allowances (RDAs) for elderly people demonstrate that their energy requirements are lower than their younger counterparts, micronutrient needs are mostly the same [29]. Many older people have chronic health states needing hospitalization live in care homes, or tend to eat less and make different food choices [30, 31]. An inadequate intake of micronutrients in elderly people has been reported both in the community and at a higher prevalence rate in long-term care facilities [32], while lower food intake has been related to lower intakes of calcium, iron, zinc, B vitamins and vitamin E in elderly people [31]. In addition, menopause influences utilization of micronutrients. For example, vitamin C gradually reduces as menopause advances, associated negatively with body mass index [33]. As in younger adults, a sufficient supply of antioxidants such as, vitamin C, selenium, and zinc is required to counteract the oxidative stress that is an important factor in immune dysregulation in elderly people. However, older people lose their ability to generate endogenous antioxidants compared with younger adults [34]. The skin of older adults is less able to synthesize vitamin D, and synthesis is approximately 75% slower in people aged over 65 years than in younger adults [35].

3.2 Clinical Effects of Micronutrient Deficiencies and Supplementation

An insufficient intake of micronutrients at any stage of life impacts various functions within the immune system, manifesting as reduced resistance to infections and an increase in the severity of symptoms. For example, zinc deficiency can elevate

thymic atrophy, reduce lymphocyte number and activity, and elevate oxidative stress and inflammation by changing cytokine production [36, 37]. As a result, the risk of all types of infection (bacterial, viral, and fungal but particularly diarrhea and pneumonia) is elevated [38]. A low vitamin C condition also elevates susceptibility to infections such as pneumonia [39], probably due to low levels of antioxidants such as vitamin C being unable to combat the observed oxidative stress [40]. Elevated production of ROS during the immune response to pathogens may reduce vitamin C levels further [41]. Vitamin D deficiency elevates the risk of infection and autoimmune diseases such as multiple sclerosis and diabetes, possibly associated with the activity of vitamin D receptors, which are present throughout the immune system [42, 43].

Given the importance of micronutrients in immunity, and the fact that many people of all ages have single or multiple micronutrient deficiencies that can have adverse immunological impacts, there is a rationale for micronutrient supplementation to restore concentrations to recommended levels, particularly after an infection, and to support immune function and maintenance. To avoid any unwanted side effects, it is of course necessary to ensure that supplementation does not exceed recommended tolerable upper intake levels, the highest level of daily nutrient intake that is likely to pose no risk of detrimental health impacts in most people [29]. As no single biomarker exists that accurately reflects the impacts of supplementation on the immune response, clinical outcomes are instead used to estimate the effectiveness of supplementation [38, 44]. Damaged immunity in older people, often resulting from multiple micronutrient deficiencies, is evident in the elevated incidence and severity of common infections that impact the upper and lower respiratory tracts, as well as the urinary and genital tracts [8, 45]. Supplementation with modest amounts of a combination of micronutrients can have advantageous effects [8]. Higher levels of CD4+ and CD8+ T cells and an increased lymphocyte proliferative response to mitogens have been reported with vitamin A, C and E supplementation [46], while micronutrient supplementation with higher levels of vitamins C, E and beta-carotene elevated the number of various subsets of T-cells, increased lymphocyte response to mitogens, enhanced IL-2 production and NK-cell activity, elevated the response to the influenza virus vaccine, and led to fewer days of infection [47]. Supplementation with a complex micronutrient combination in older people enhanced the number of different types of immune cells, such as total lymphocytes [48]. Multiple micronutrient supplementations in elderly people may also decrease antibiotic usage and result in higher post-vaccination immune responses [8]. Marginal zinc deficiency is prevalent in older people, as their dietary intakes are generally lower and plasma zinc levels decrease with age, possibly due to impaired absorption, changes in cellular uptake, and epigenetic dysregulation of DNA methylation or the methionine/transsulfuration pathway [36].

Adequate vitamin C is also important in elderly people, who are at risk of vitamin C deficiency, particularly females [49]. Sufficient vitamin C intakes can optimize cell and tissue concentrations and help to protect against respiratory and systemic infections, while higher levels are needed during infection to compensate

for the enhanced inflammatory response and metabolic requirement induced by the pathogen, and hence help to decrease the duration and severity of symptoms [50].

Vitamin E supplementation in older people has been shown to significantly ameliorate NK cytotoxic activity, neutrophil chemotaxis and the phagocytic response, and increase mitogen-related lymphocyte proliferation and IL-2 production [51]. Vitamin E can also ameliorate T-cell-mediated immunity and enhance the production of antibodies in response to the hepatitis B and tetanus vaccines [52]. As a lipid-soluble antioxidant, vitamin E plays an important role in protecting the integrity of cell membranes from oxidative damage. Since vitamin E is especially enriched in the membranes of immune cells, it is not surprising that vitamin E deficiency harms both humoral and cell-mediated immune functions [53, 54]. Vitamin E supplementation has an advantageous impact on the immune system, particularly in aged individuals who have compromised immune function. It has been found that vitamin E supplementation enhanced lymphocyte proliferation [55, 56], IL-2 production [55], and delayed type hypersensitivity (DTH) response in old mice [55]. In addition, old mice had a damaged response to infection such as decreased NK cell activity and neutrophil recruitment [57], as well as the related higher viral titers [58], which are all restored by vitamin E supplementation. Given the observed effectiveness of vitamin E in restoring cell-mediated immunity as well as ameliorating innate immunity in aged animals, several clinical trials have been carried out to investigate these impacts in the elderly. In one study, short-term (1 month) supplementation of vitamin E with 800 mg/day significantly ameliorated the DTH response, T cell proliferation and IL-2 production, while reducing plasma lipid peroxide and prostaglandin E2 (PGE2) production in healthy subjects (≥60 years) [59]. Another study which administered lower doses for a longer period (4.5 months) showed that supplementation with vitamin E at 200 mg/day compared to 0, 60 or 800 mg/day was the most effective in enhancing DTH response and antibody titers to hepatitis B and tetanus vaccine in free living, healthy elderly individuals (≥65 years) [52].

The risk of upper respiratory tract infections, particularly common cold, was significantly lower after vitamin E supplementation in nursing home residents, although there was no evident impact on lower respiratory tract infections [58]. However, not all studies have reported beneficial impacts on respiratory tract infections with vitamin E supplementation in older people [36].

The working mechanisms underlying the immuno-modulating impact of vitamin E have been investigated mainly with experiments in cell cultures and animal models. As a whole, vitamin E can increase T cell-mediated function by directly impacting membrane integrity and signal transduction in T cells or indirectly, by decreasing production of suppressive factors such as PGE2 [60, 61].

Results from murine infection models have extended our understanding of how aging influences immunosenescence with clinical implications and how vitamin E functions during the process. Upon infection with influenza A/Port Chalmers/1/73 (H3N2), old mice exhibited higher lung viral titers [58, 62] and damaged IL-2 response [62], all of which were ameliorated by vitamin E supplementation (500 mg/kg diet) for 8 weeks. It seems that the protective impact of vitamin E against

influenza virus may be associated with an increase in Th1 cytokine production. Bou Ghanem et al. showed that old mice were more vulnerable to Streptococcus pneumonia than young mice, along with higher pulmonary bacterial burden, lethal septicemia, and lung inflammation, which were decreased by vitamin E supplementation (500 mg/kg diet) for 4 weeks [57]. These findings suggest that vitamin E can increase resistance of aged mice to bacterial pneumonia by improving the innate immune response.

However, the results from clinical studies of vitamin E supplementation thus far have been discrepant or even contradictory. This may in part be due to the different conditions in vitamin E administration regimen and subject characteristics. On the subject side, both baseline vitamin E condition and the individual's specific genetic background should be considered. It has been shown that polymorphisms of genes including apolipoprotein E, SR-BI scavenger receptor, α-tocopherol transfer protein, CD36 scavenger receptor, and lipoprotein lipase, may affect the bioavailability and cellular activity of vitamin E [63]. Hence, it is probable that the polymorphisms of the genes associated with vitamin E bioactivity may influence the impacts of vitamin E supplementation. As such, the interaction of vitamin E with genes associated with its bioactivity and immune response should be further investigated in the elderly population, which may help to provide a better understanding of vitamin E's effectiveness in restoring age-related immune response.

Like vitamin E, Vitamin D is a lipid-soluble vitamin. However, unlike vitamin E, vitamin D is primarily produced in the skin during sun exposure rather than absorbed from the food. 1,25-(OH)2D circulates to different target tissues to exert its endocrine effects that are mediated by the vitamin D receptor (VDR). A long-recognized role of 1,25-(OH)2D involves calcium homeostasis and bone health. However, extraskeletal effects of vitamin D have been shown along with the discovery of the VDR in tissue and cells that are not involved in maintaining mineral and bone homeostasis. One of the most prominent impacts is in immune cells. Thus, higher circulating 25-(OH)D concentrations are likely needed for optimal intracrine effects of 1,25-(OH)2D, whereas inadequate vitamin D concentrations may be related to dysregulated immune function and probably infectious diseases. Vitamin D inadequacy is prevalent in community-dwelling elderly and more so among the institutionalized elderly [64].

Several in vitro studies have suggested a major role of vitamin D as an important modulator in both innate and adaptive immunity. For example, 1,25-(OH)2D stimulates differentiation of precursor monocytes to mature phagocytic MΦ [65, 66]. In particular, 1,25-(OH)2D supplementation can elevate production of the antimicrobial peptides cathelicidin by MΦ [67] and β-defensin by endothelial cells [68]. However, 1,25-(OH)2D suppresses T cell proliferation [69], in particular T helper 1 (Th1) cells, which are a subset of CD4+ effector T cells capable of producing IL-2 and IFN-γ and activating MΦ [70]. Thus, vitamin D may help to limit the potential tissue impairment related to Th1 cellular immune response. Investigations found that 1,25-(OH) 2D can inhibit pro-inflammatory Th17 cells [71] while enhancing Treg [72], which may be a pivotal mechanism for the potential of vitamin D in mitigating autoimmune disorders.

The immunosuppressive mechanism of vitamin D on T cells may be in part described by its impacts on dendritic cells (DCs). 1,25-(OH)2D was found to suppress the maturation of monocyte-derived DCs, thereby inhibiting their capacity to present antigens to T cells [72]. According to these cellular studies, it seems that vitamin D can stimulate innate immune responses, which can help remove invading bacteria and viruses, whereas the regulatory impact of vitamin D on T cells can be advantageous under a number of states such as T cell-mediated autoimmune inflammatory diseases. Since elderly people are at higher risk for poor vitamin D status, mainly because of limited sunlight exposure, decreased ability of the skin to produce vitamin D, and decreased vitamin D intake [73–75], it is plausible that low vitamin D status in older people may also contribute to their compromised immune response and their enhanced prevalence of infection, which can be relieved by sufficient intake of vitamin D.

Epidemiological studies have indicated a relationship between low 25-(OH)D levels and chronic diseases such as infections [76, 77]. One study in 34 healthy females over 60 years-old found that those with high 25-(OH)D levels (>75 nM) had a higher percentage of effector CD8+ T cells, suggesting that enhanced vitamin D may hasten CD8+ T cell senescence. However, 25-(OH)D status impacted neither the T cell proliferative response nor the serum concentrations of the inflammatory cytokines IL-1, IL-6, and IL-17, as well as the Th2 cytokine IL-4 [78]. Another study found no relationship between 25-(OH)D levels and humoral immune responses against seasonal influenza, vaccines, or alterations in subpopulations of immune cells, and cytokine/chemokine response in adults ≥50 years-old [79].

As mentioned above, vitamin D has an impact on multiple aspects of immune system, enabled by expression of the VDR and the enzyme 1α-hydroxylase present in most immune cells [67, 80, 81]. Vitamin D has been demonstrated to increase numerous innate immune functions necessary for combating against microbial infections. Local synthesis of 1,25(OH)2D plays an important role in respiratory infection. Upon infection, pathogen-associated molecular patterns (PAMPs) on pathogens trigger pathogen recognition TLRs in the host [82]. TLRs when triggered lead to induction of the gene for 1α-hydroxylase (CYP27B1), which in turn induces local generation of 1,25-(OH)2D. Importantly, 1,25-(OH)2D is a direct inducer of antimicrobial peptide gene expression [67, 68]. Moreover, it has been proposed that vitamin D's useful impact in infection may be associated with its anti-inflammatory properties. Vitamin D has been shown to suppress the production of pro-inflammatory cytokines (IL-6, IL-8, IL-12, IFN-γ, TNF-α) in the innate immune response, and in the adaptive immune response, it inhibits T-cell activation and modulates CD4+ T-cell differentiation by favoring polarization toward Th2 and Treg phenotypes while inhibiting Th1 and Th17 differentiation [71, 83–86]. Another probable mechanism suggested involves direct suppression of pathogen replication by vitamin D in the host, but there is no solid evidence supporting this hypothesis. Also, an in vitro study showed no direct impact of vitamin D on rhinovirus replication in epithelial cells [87].

Since vitamin D regulates immune function via the VDR, genetic variants in this receptor could influence its function, which would alter the biological impacts of

vitamin D. The current recommendations for vitamin D intake are primarily based on bone health and mortality, but not for infection prophylaxis. More specifically, clinical trials are required to definitively characterize the impact of vitamin D supplementation on immune function and risk of infection in the elderly with different basal serum levels of 25- (OH)D and to investigate optimal doses for achieving sustained sufficiency in vitamin D status.

Before such information is available and due to the high prevalence of vitamin D deficiency/insufficiency and well-established relationship between vitamin D deficiency and infection incidence, vitamin D intake should be raised to 1000 IU/day for the majority of people in order to achieve circulating levels >50 nmol/L as proposed [88, 89]. Regardless of the baseline levels, the dose of 1000 IU/day should be considered safe given the large distance from the current tolerable upper intake level of 4000 IU/day.

Zinc is a trace element essential for DNA synthesis, membrane integrity, and cell proliferation, as well as being an important micronutrient for cell performance in the immune system [90, 91]. Since there is no specialized zinc storage system in the body, zinc deficiency can rapidly deplete the zinc supply to immune cells leading to compromised immune function [92]. It has been demonstrated that zinc deficiency can profoundly change immuno-homeostasis which involves both innate and adaptive immunity, causing damaged phagocytosis and intracellular killing activity of phagocytes, reduced NK cell activity, thymus involution and reduced thymic output, and reduced lymphocyte proliferation, IL-2 production, DTH response, and antibody response to vaccines [93–96]. All of these manifestations are similar to those observed in the aged immune system. Consistent with these findings, reduced zinc intake and low zinc conditions have been reported in older people [97–100], and a low zinc status in the elderly has been shown to contribute to age-related dysregulation of the immune response [101].

In a study, institutionalized healthy elderly (>70 years) individuals who received 1 month of daily supplementation with 440 mg zinc sulfate had a significant elevation in the proportion of circulating T cells, DTH response, and anti-tetanus toxin IgG titers, all of which depend on T cell response [102]. Similarly, 60 mg zinc acetate supplementation for 4.5 months significantly increased plasma zinc levels and ameliorated the DTH response in zinc deficient elderly persons who showed an anergic response to skin antigen tests [103]. In contrast, Bogden et al. did not find any difference in DTH response nor lymphocyte proliferation between the placebo and zinc-supplemented groups [104]. A number of studies that have evaluated the effect of zinc on immune cell phenotype have been inconclusive. For example, free-living elderly persons receiving zinc at 10 mg/day for 7 weeks showed a decrease in activated (CD25+) CD4+ T cells [105], whereas institutionalized healthy elderly individuals who consumed 25 mg/day zinc for 3 months showed an enhancement in the number of activated (HLA-DR+) CD4+ T cells and cytotoxic T cells [106]. In another study, the number of circulating T cells were enhanced in a group of zinc deficient nursing home elderly persons (serum zinc <70 μg/dL) who had been supplemented with 30 mg zinc for 3 months, and this was accompanied by elevated lymphocyte proliferation with T cell stimulants [107]. Since participants with serum zinc ≤60 μg/dL failed to obtain sufficient concentrations of serum zinc (≥70 μg/dL)

despite supplementation [107], those with low a zinc status may require a larger dose or longer duration of supplementation to achieve higher zinc levels sufficient to reverse T-cell immunosenescence.

Supplementation with low to moderate doses of zinc in healthy elderly people can help to restore thymulin activity, enhance the number of cytotoxic T cells, decrease the number of activated Th cells and elevate the cytotoxicity of NK cells [36]. Such changes are immunologically advantageous that help to decrease the incidence of infections such as common cold, cold sores and influenza [108], as well as the incidence rate and morbidity of pneumonia [109]. There are some reports that a sufficient zinc supply could prevent degenerative age-associated diseases including infection and cancer [110].

Copper (Cu) actively contributes to a series of reactions enhancing growth and development and an important source of this metal in food is meat [111]. The basal storage of Cu is in the liver, where it is contained in a membrane-bound form as metallothioneins. Ceruloplasmin, a blood protein synthesized by the liver, contains multiple molecules of Cu, hence this protein represents a biomarker of Cu status in the body [112].

Reduced Cu in older adults appears to be associated with a decreased intake of food and beverages, particularly milk, which increases Cu absorption at the intestinal level along with glucose [113]. Cu obtained via dietary sources is not only distributed in the body but also accelerates iron (Fe) metabolism [114]. Dietary Cu deficiency results in a variety of immune abnormalities. In this regard, neutropenia, damage of Mθ and NK cell functions and decreased IL-2 production have been indicated in Cu deficiency [115]. A full recovery of immune functions was obtained following Cu supplementation in Cu-deficient subjects [116]. Since pro-inflammatory cytokines manage synthesis and secretion of Cu-containing molecules by the liver, enhanced plasma Cu levels have been found in some age-related diseases including atherosclerosis and Alzheimer's disease [117]. On the other hand, in the case of severe Cu deficiency, a damaged immune response as well as disturbed anti-oxidant activity and altered metabolism have been found in elderly subjects, and Cu supplementation appears to be essential for recovery of cellular functions [118].

Fe is another essential micronutrient for the proliferation and differentiation of cells. In addition to oxygen transport, Fe is mostly involved in the catalyzation of hydroxyl radical formation, which are impacted by the activity of transcription agents such as hypoxia inducible factor-1 or nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) [119]. Fe deficiency leads to a number of immune dysfunctions in the elderly and this state is related to anemia [120]. Thymic atrophy, depletion of T cells and NK cells with polarization toward Th2 cells have been reported in Fe-deficient subjects [121]. Thus, Fe supplementation in anemic elderly subjects appears to be essential for maintenance of immune homeostasis and prevention of degenerative diseases. Clinical trials in healthy elderly participants have shown that Fe supplementation is useful in Fe-deficiency anemia and the associated chronic inflammation and oxidative impairment in age-related diseases [122].

Selenium (Se) has anti-oxidant activities via its incorporation into selenoproteins which, in turn, control ROS and redox status as well as inflammation and immune responses [123]. In view of the enhancement in oxidative damage in senescence, Se

has been administered as a supplement in the elderly. In aged people, Se supplementation alone or in combination with beta-carotene, caused an increase of CD4+ T cells that persisted for 2 months following discontinuation of the administration regimen [124]. In other studies of aged people, the Se concentration was positively associated with an enhancement in the number of NK cells. In addition, a relationship was found between low levels of Se and severity of IL-6-mediated inflammation [125]. Moreover, in aged individuals, several nutritional markers were investigated for their relationship with proliferation of peripheral blood lymphocytes. Se was one of the four nutrients that was positively associated with lymphocyte proliferation [126].

The effects of micronutrient deficiency and supplementation are summarized in Table 2.1.

Table 2.1 Effects of micronutrient deficiency and supplementation on immune function

Micronutrient	Effects of deficiency	Effects of supplementation
Vitamin C	Increased oxidative damage, increased incidence and severity of infections, reduced resistance to infections, reduced DTH response [38–40]	Protected against oxidative stress, reduced duration and incidence of pneumonia and common cold symptoms [36, 39, 41]
Vitamin A	Influences immune functions, and vulnerability to infections [36]	Reduced risk of morbidity and mortality from infectious disease. Not advantageous in pneumonia [36, 39]
Vitamin E	Impairs humoral, B and T cell function [36]	Improved DTH response and T cell proliferation [59]
Vitamin D	Increased vulnerability to infections, increased morbidity and mortality, increased risk of autoimmune diseases [36, 38]	Enhanced immune function in infectious disease [82]
Zinc	Reduced lymphocyte number and function, enhanced thymic atrophy, changed cytokine production toward oxidative stress and inflammation, increased infectious diseases [36, 39]	Enhanced number of T cells, reduced incidence of infections, enhanced natural killer cell cytotoxicity [36, 39]
Copper	Abnormal low levels of neutrophil, increased vulnerability to infections [36]	Decreased antibody production in response to influenza vaccine [14]
Iron	Decline in immune response, reduced lymphocyte bactericidal activity [38]	Theoretically increase immunity to infections, but untargeted supplementation may increase availability of iron for pathogen growth [39]
Selenium	Suppression of immune function, damaged humoral and cell-mediated immunity [36, 38]	Ameliorates cell-mediated immunity and increases immune response in individuals with deficiency, but may deteriorate allergic asthma and damage the immune response to parasites [36]

DTH delayed type hypersensitivity

4 Conclusions

Aging-associated damage in the immune system is well established and is a main factor contributing to the elevated morbidity and mortality related to infection in the elderly. Optimal immune function depends on a normal, well-balanced nutritional state, although the prevalence of malnutrition is generally greater among the elderly, which further damages the aged immune system. Hence, nutritional intervention may have a promising potential in mitigating the negative influence of aging on immune function, hence ameliorating resistance to infection in the elderly population. Although epidemiological studies have proposed a relationship between certain nutrients and dietary components with risk of mortality and morbidity associated with infections, the results of interventional studies have been discrepant and limited success has been achieved in this regard. This demonstrates that effective nutrients and dietary components may work together generating additive and synergistic impacts, which cannot be obtained by taking single nutritional supplements. Thus, it is advised that intake of nutrients should focus first on consumption of a sufficient and balanced daily diet. However, it is worth emphasizing that higher amounts of certain nutrients above the currently recommended intake levels may be required for elderly people, particularly for those who are ill, on medication, or in a lower socioeconomic status. The available clinical data proposes that micronutrient supplementation can decrease the risk and severity of infection and support a faster recovery. However, more research is needed into the impact of micronutrient supplementation on immune functions and on clinical outcomes. Nevertheless, current knowledge regarding the importance of micronutrients on immune function, the impact of micronutrient deficiencies on the risk and severity of infections, and the worldwide prevalence rate of an insufficient micronutrient state form a basis for the use of a targeted multiple micronutrient supplement program to support immune function throughout a person's lifetime.

References

1. Magrone T, Jirillo E (2013) The interaction between gut microbiota and age-related changes in immune function and inflammation. Immun Aging 10:31
2. Magrone T, Perez de Heredia F, Jirillo E, Morabito G, Marcos A, Serafini M (2013) Functional foods and nutraceuticals as therapeutic tools for the treatment of diet-related diseases. Can J Physiol Pharmacol 91(6):387–396
3. Ahmed T, Haboubi N (2010) Assessment and management of nutrition in older people and its importance to health. Clin Interv Aging 5:207–216
4. Franceschi C, Capri M, Monti D, Giunta S, Olivieri F, Sevini F et al (2007) Inflammaging and anti-inflammaging: a systemic perspective on aging and longevity emerged from studies in humans. Mech Aging Dev 128(1):92–105
5. Li W (2013) Phagocyte dysfunction, tissue aging and degeneration. Aging Res Rev 12(4):1005–1012

6. Candore G, Caruso C, Jirillo E, Magrone T, Vasto S (2010) Low grade inflammation as a common pathogenetic denominator in age related diseases: novel drug targets for anti-aging strategies and successful aging achievement. Curr Pharm Des 16(6):584–596
7. Fulop T, Witkowski JM, Pawelec G, Alan C, Larbi A (2014) On the immunological theory of aging. Interdiscip Top Gerontol 39:163–176
8. Chandra R (2002) Nutrition and the immune system from birth to old age. Eur J Clin Nutr 56(Suppl 3):S73–S76
9. Montecino-Rodriguez E, Berent-Maoz B, Dorshkind K (2013) Causes, consequences, and reversal of immune system aging. J Clin Investig 123(3):958–965
10. Ventura MT, Casciaro M, Gangemi S, Buquicchio R (2017) Immunosenescence in aging: between immune cells depletion and cytokines up-regulation. Clin Mol Allergy 15:21. https://doi.org/10.1186/s12948-017-0077-0
11. Brodin P, Davis MM (2017) Human immune system variation. Nat Rev Immunol 17(1):21–29
12. Pawelec G (2017) Does the human immune system ever really become "senescent"? F1000Rese 6:1323. pii: F1000 Faculty Rev-1323. https://doi.org/10.12688/f1000research.11297.1
13. Castelo-Branco C, Soveral I (2014) The immune system and aging: a review. Gynecol Endocrinol 30(1):16–22
14. Jafarzadeh A, Sadeghi M, Karam GA, Vazirinejad R (2010) Salivary IgA and IgE levels in healthy subjects: relation to age and gender. Braz Oral Res 24(1):21–27
15. Maggini S, Maldonado P, Cardim P, Fernandez Newball C, Sota Latino E (2017) Vitamins C., D and zinc: synergistic roles in immune function and infections. Vitam Miner 6:167. https://doi.org/10.4172/2376-1318.1000167
16. Fulop T, Larbi A, Dupuis G, Le Page A, Frost E, Cohen A et al (2017) Immunosenescence and inflamm-aging as two sides of the same coin: friends or foes? Front Immunol 8:1960. https://doi.org/10.3389/fimmu.2017.01960
17. Haryanto B, Suksmasari T, Wintergerst E, Maggini S (2015) Multivitamin supplementation supports immune function and ameliorates conditions triggered by reduced air quality. Vitam Miner 4:1–15. https://doi.org/10.4172/2376-1318.1000128
18. Napoli I, Neumann H (2010) Protective effects of microglia in multiple sclerosis. Exp Neurol 225(1):24–28
19. Tortorella C, Piazzolla G, Spaccavento F, Jirillo E, Antonaci S (1999) Age-related effects of oxidative metabolism and cyclic AMP signaling on neutrophil apoptosis. Mech Aging Dev 110(3):195–205
20. Agrawal A, Agrawal S, Cao JN, Su H, Osann K, Gupta S (2007) Altered innate immune functioning of dendritic cells in elderly humans: a role of phosphoinositide 3-kinase-signaling pathway. J Immunol 178(11):6912–6922
21. Aprahamian T, Takemura Y, Goukassian D, Walsh K (2008) Aging is associated with diminished apoptotic cell clearance in vivo. Clin Exp Immunol 152(3):448–455
22. Hazeldine J, Lord JM (2013) The impact of aging on natural killer cell function and potential consequences for health in older adults. Aging Res Rev 12(4):1069–1078
23. Fessler J, Ficjan A, Duftner C, Dejaco C (2013) The impact of aging on regulatory T-cells. Front Immunol 60(2):130–137
24. Schmitt V, Rink L, Uciechowski P (2013) The Th17/Treg balance is disturbed during aging. Exp Gerontol 48(12):1379–1386
25. Kogut I, Scholz JL, Cancro MP, Cambier JC (2012) B cell maintenance and function in aging. Semin Immunol 24(5):342–349
26. Ademokun A, Wu YC, Dunn-Walters D (2010) The aging B cell population: composition and function. Biogerontology 11(2):125–137
27. Rubtsov AV, Rubtsova K, Fischer A, Meehan RT, Gillis JZ, Kappler JW et al (2011) Toll-like receptor 7 (TLR7)-driven accumulation of a novel CD11c+ B-cell population is important for the development of autoimmunity. Blood 118(5):1305–1315
28. Kant AK (2000) Consumption of energy-dense, nutrient-poor foods by adult Americans: nutritional and health implications. The third National Health and Nutrition Examination Survey, 1988–1994. Am J Clin Nutr 72(4):929–936

29. Institute of Medicine (2011) Dietary reference intakes for calcium and vitamin D; Institute of Medicine (US) committee to review dietary reference intakes for vitamin D and calcium; Ross AC, Taylor CL, Yaktine AL, Del Valle HB (eds). The National Academies Press, Washington, DC. ISBN-10: 0309163943
30. Montgomery SC, Streit SM, Beebe ML, Maxwell PJ 4th (2014) Micronutrient needs of the elderly. Nutr Clin Pract 29(4):435–444
31. Drenowski A, Shultz J (2001) Impact of aging on eating behaviors, food choices, nutrition, and health status. J Nutr Health Aging 5(2):75–79
32. High K (2001) Nutritional strategies to boost immunity and prevent infection in elderly individuals. Clin Infect Dis 33(11):1892–1900
33. Wiacek M, Zubrzycki IZ, Bojke O, Kim HJ (2013) Menopause and age-driven changes in blood level of fat- and water-soluble vitamins. Climacteric 16(6):689–699
34. Karaouzene N, Merzouk H, Aribi M, Merzouk SA, Berrouiguet AY, Tessier C et al (2011) Effects of the association of aging and obesity on lipids, lipoproteins and oxidative stress biomarkers: a comparison of older with young men. Nutr Metab Cardiovasc Dis 21(10):792–799
35. World Health Organization, Food and Agricultural Organization of the United Nations. (2006) Part 2. Evaluating the public health significance of micronutrient malnutrition. In Guidelines on food fortification with micronutrients. World Health Organization, Geneva. ISBN-10: 9241594012
36. Micronutrient Information Center. Immunity in depth. http://lpi.oregonstate.edu/mic/health-disease/immunity. Accessed 17 Apr 2018
37. Savino W, Dardenne M (2010) Nutritional imbalances and infections affect the thymus: consequences on T-cell-mediated immune responses. Proc Nutr Soc 69(4):636–643
38. Calder P, Prescott S, Caplan M (2007) Scientific review: the role of nutrients in immune function of infants and young children; emerging evidence for long-chain polyunsaturated fatty acids. Mead Johnson & Company, Glenview. https://eprints.soton.ac.uk/152657/
39. Prentice S (2017) They are what you eat: can nutritional factors during gestation and early infancy modulate the neonatal immune response? Front Immunol 8:1641. https://doi.org/10.3389/fimmu.2017.01641
40. Hemilä H (2017) Vitamin C and infections. Nutrients 9(4). pii: E339. https://doi.org/10.3390/nu9040339
41. Hemilä H, Chalker E (2013) Vitamin C for preventing and treating the common cold. Cochrane Database Syst. Rev (1):CD000980. https://doi.org/10.1002/14651858.CD000980.pub4
42. Aranow C (2011) Vitamin D and the immune system. J Investig Med 59(6):881–886
43. Mangin M, Sinha R, Fincher K (2014) Inflammation and vitamin D: the infection connection. Inflamm Res 63(10):803–819
44. Albers R, Bourdet-Sicard R, Braun D, Calder PC, Herz U, Lambert C et al (2013) Monitoring immune modulation by nutrition in the general population: identifying and substantiating effects on human health. Br J Nutr 110(Suppl 2):S1–S30
45. Hamer DH, Sempértegui F, Estrella B, Tucker KL, Rodríguez A, Egas J (2009) Micronutrient deficiencies are associated with impaired immune response and higher burden of respiratory infections in elderly Ecuadorians. J Nutr 139(1):113–119
46. Penn ND, Purkins L, Kelleher J, Heatley RV, Mascie-Taylor BH, Belfield PW (1991) The effect of dietary supplementation with vitamins A, C, and E on cell-mediated immune function in elderly long-stay patients: a randomized controlled trial. Age Aging 20(3):169–174
47. Chandra R (1992) Effect of vitamin and trace-element supplementation on immune responses and infection in elderly subjects. Lancet 340(8828):1124–1127
48. Schmoranzer F, Fuchs N, Markolin G, Carlin E, Sakr L, Sommeregger U (2009) Influence of a complex micronutrient supplement on the immune status of elderly individuals. Int J Vitam Nutr Res 79(5–6):308–318
49. Elmadfa I, Meyer A, Nowak V, Hasenegger V, Putz P, Verstraeten R et al (2009) European nutrition and health report. Ann Nutr Metab 55(Suppl 2):1–40
50. Carr A, Maggini S (2017) Vitamin C and immune function. Nutrients 9(11). pii: E1211. https://doi.org/10.3390/nu9111211

51. De la Fuente M, Hernanz A, Guayerbas N, Victor VM, Arnalich F (2008) Vitamin E ingestion improves several immune functions in elderly men and women. Free Radic Res 42(3):272–280
52. Meydani SN, Meydani M, Blumberg JB, Leka LS, Siber G, Loszewski R et al (1997) Vitamin E supplementation and in vivo immune response in healthy elderly subjects. A randomized controlled trial. JAMA 277(17):1380–1386
53. Gebremichael A, Levy EM, Corwin LM (1984) Adherent cell requirement for the effect of vitamin E on in vitro antibody synthesis. J Nutr 114(7):1297–1305
54. Kowdley KV, Mason JB, Meydani SN, Cornwall S, Grand RJ (1992) Vitamin E deficiency and impaired cellular immunity related to intestinal fat malabsorption. Gastroenterology 102(6):2139–2142
55. Meydani SN, Meydani M, Verdon CP, Shapiro AA, Blumberg JB, Hayes KC (1986) Vitamin E supplementation suppresses prostaglandin E1(2) synthesis and enhances the immune response of aged mice. Mech Aging Dev 34(2):191–201
56. Sakai S, Moriguchi S (1997) Long-term feeding of high vitamin E diet improves the decreased mitogen response of rat splenic lymphocytes with aging. J Nutr Sci Vitaminol (Tokyo) 43(1):113–122
57. Bou Ghanem EN, Clark S, Du X, Wu D, Camilli A, Leong JM et al (2015) The alpha-tocopherol form of vitamin E reverses age-associated susceptibility to streptococcus pneumoniae lung infection by modulating pulmonary neutrophil recruitment. J Immunol 194(3):1090–1099
58. Hayek MG, Taylor SF, Bender BS, Han SN, Meydani M, Smith DE et al (1997) Vitamin E supplementation decreases lung virus titers in mice infected with influenza. J Infect Dis 176(1):273–276
59. Meydani SN, Barklund MP, Liu S, Meydani M, Miller RA, Cannon JG et al (1990) Vitamin E supplementation enhances cell-mediated immunity in healthy elderly subjects. Am J Clin Nutr 52(3):557–563
60. Meydani SN, Han SN, Wu D (2005) Vitamin E and immune response in the aged: molecular mechanisms and clinical implications. Immunol Rev 205:269–284
61. Wu D, Meydani SN (2008) Age-associated changes in immune and inflammatory responses: impact of vitamin E intervention. J Leukoc Biol 84(4):900–914
62. Han SN, Wu D, Ha WK, Beharka A, Smith DE, Bender BS et al (2000) Vitamin E supplementation increases T helper 1 cytokine production in old mice infected with influenza virus. Immunology 100(4):487–493
63. Mocchegiani E, Costarelli L, Giacconi R, Malavolta M, Basso A, Piacenza F et al (2014) Vitamin E-gene interactions in aging and inflammatory age-related diseases: implications for treatment. A systematic review. Aging Res Rev 14:81–10
64. Mosekilde L (2005) Vitamin D and the elderly. Clin Endocrinol (Oxf) 62(3):265–281
65. Xu H, Soruri A, Gieseler RK, Peters JH (1993) 1,25-Dihydroxyvitamin D3 exerts opposing effects to IL-4 on MHC class-II antigen expression, accessory activity, and phagocytosis of human monocytes. Scand J Immunol 38(6):535–540
66. Abe E, Miyaura C, Tanaka H, Shiina Y, Kuribayashi T, Suda S et al (1983) 1 alpha,25-dihydroxyvitamin D3 promotes fusion of mouse alveolar macrophages both by a direct mechanism and by a spleen cell-mediated indirect mechanism. Proc Natl Acad Sci U S A 80(18):5583–5587
67. Liu PT, Stenger S, Li H, Wenzel L, Tan BH, Krutzik SR et al (2006) Toll-like receptor triggering of a vitamin D-mediated human antimicrobial response. Science 311(5768):1770–1773
68. Wang TT, Nestel FP, Bourdeau V, Nagai Y, Wang Q, Liao J et al (2004) Cutting edge: 1,25-dihydroxyvitamin D3 is a direct inducer of antimicrobial peptide gene expression. J Immunol 173(5):2909–2912
69. Rigby WF, Stacy T, Fanger MW (1984) Inhibition of T lymphocyte mitogenesis by 1,25-dihydroxyvitamin D3 (calcitriol). J Clin Invest 74(4):1451–1455
70. Lemire JM, Archer DC, Beck L, Spiegelberg HL (1995) Immunosuppressive actions of 1,25- dihydroxyvitamin D3: preferential inhibition of Th1 functions. J Nutr 125(6 Suppl):1704S–1708S

71. Daniel C, Sartory NA, Zahn N, Radeke HH, Stein JM (2008) Immune modulatory treatment of trinitrobenzene sulfonic acid colitis with calcitriol is associated with a change of a T helper (Th) 1/Th17 to a Th2 and regulatory T cell profile. J Pharmacol Exp Ther 324(1):23–33
72. Penna G, Adorini L (2000) 1 Alpha,25-dihydroxyvitamin D3 inhibits differentiation, maturation, activation, and survival of dendritic cells leading to impaired alloreactive T cell activation. J Immunol 164(5):2405–2411
73. van der Wielen RP, Lowik MR, van den Berg H, de Groot LC, Haller J, Moreiras O et al (1995) Serum vitamin D concentrations among elderly people in Europe. Lancet 346(8969):207–210
74. Oliveri B, Plantalech L, Bagur A, Wittich AC, Rovai G, Pusiol E et al (2004) High prevalence of vitamin D insufficiency in healthy elderly people living at home in Argentina. Eur J Clin Nutr 58(2):337–342
75. Portela ML, Monico A, Barahona A, Dupraz H, Sol Gonzales-Chaves MM, Zeni SN (2010) Comparative 25-OH-vitamin D level in institutionalized women older than 65 years from two cities in Spain and Argentina having a similar solar radiation index. Nutrition 26(3):283–289
76. Holick MF (2007) Vitamin D deficiency. N Engl J Med 357(3):266–281
77. Bikle DD (2008) Vitamin D and the immune system: role in protection against bacterial infection. Curr Opin Nephrol Hypertens 17(4):348–352
78. Hwang YG, Hsu HC, Lim FC, Wu Q, Yang P, Fisher G et al (2013) Increased vitamin D is associated with decline of naive, but accumulation of effector, CD8 T cells during early aging. Adv Aging Res 2(2):72–80
79. Sadarangani SP, Ovsyannikova IG, Goergen K, Grill DE, Poland GA (2016) Vitamin D, leptin and impact on immune response to seasonal influenza A/H1N1 vaccine in older persons. Hum Vaccin Immunother 12(3):691–698
80. Hewison M (2010) Vitamin D and the immune system: new perspectives on an old theme. Endocrinol Metab Clin North Am 39:365–379, table of contents
81. Greiller CL, Martineau AR (2015) Modulation of the immune response to respiratory viruses by vitamin D. Nutrients 7:4240–4270
82. Medzhitov R (2001) Toll-like receptors and innate immunity. Nat Rev Immunol 1(2):135–145
83. Boonstra A, Barrat FJ, Crain C, Heath VL, Savelkoul HF, O'Garra A (2001) 1alpha,25-Dihydroxyvitamin d3 has a direct effect on naive CD4(+) T cells to enhance the development of Th2 cells. J Immunol 167(9):4974–4980
84. Gorman S, Kuritzky LA, Judge MA, Dixon KM, McGlade JP, Mason RS et al (2007) Topically applied 1,25-dihydroxyvitamin D3 enhances the suppressive activity of CD4+CD25+ cells in the draining lymph nodes. J Immunol 179(9):6273–6283
85. Hamzaoui A, Berraies A, Hamdi B, Kaabachi W, Ammar J, Hamzaoui K (2014) Vitamin D reduces the differentiation and expansion of Th17 cells in young asthmatic children. Immunobiology 219(11):873–879
86. Penna G, Roncari A, Amuchastegui S, Daniel KC, Berti E, Colonna M et al (2005) Expression of the inhibitory receptor ILT3 on dendritic cells is dispensable for induction of CD4+Foxp3+ regulatory T cells by 1,25-dihydroxyvitamin D3. Blood 106(10):3490–3497
87. Brockman-Schneider RA, Pickles RJ, Gern JE (2014) Effects of vitamin D on airway epithelial cell morphology and rhinovirus replication. PLoS One 9:e86755. https://doi.org/10.1371/journal.pone.0086755
88. Zittermann A, Pilz S, Hoffmann H, Marz W (2016) Vitamin D and airway infections: a European perspective. Eur J Med Res 21:14. https://doi.org/10.1186/s40001-016-0208-y
89. Zittermann A, Ernst JB, Gummert JF, Borgermann J (2014) Vitamin D supplementation, body weight and human serum 25-hydroxyvitamin D response: a systematic review. Eur J Nutr 53(2):367–374
90. Vallee BL, Falchuk KH (1993) The biochemical basis of zinc physiology. Physiol Rev 73(1):79–118
91. Ibs KH, Rink L (2003) Zinc-altered immune function. J Nutr 133(5 Suppl 1):1452S–1456S
92. Rink L, Gabriel P (2001) Extracellular and immunological actions of zinc. Biometals 14(3–4):367–383

93. Fraker PJ, King LE (2004) Reprogramming of the immune system during zinc deficiency. Annu Rev Nutr 24:277–298
94. Shankar AH, Prasad AS (1998) Zinc and immune function: the biological basis of altered resistance to infection. Am J Clin Nutr 68(2 Suppl):447S–4463S
95. Mitchell WA, Meng I, Nicholson SA, Aspinall R (2006) Thymic output, aging and zinc. Biogerontology 7(5–6):461–470
96. Mocchegiani E, Giacconi R, Cipriano C, Malavolta M (2009) NK and NKT cells in aging and longevity: role of zinc and metallothioneins. J Clin Immunol 29:416–425
97. Sandstead HH, Henriksen LK, Greger JL, Prasad AS, Good RA (1982) Zinc nutriture in the elderly in relation to taste acuity, immune response, and wound healing. Am J Clin Nutr 36(5 Suppl):1046–1059
98. Prasad AS, Fitzgerald JT, Hess JW, Kaplan J, Pelen F, Dardenne M (1993) Zinc deficiency in elderly patients. Nutrition 9(3):218–224
99. Briefel RR, Bialostosky K, Kennedy-Stephenson J, McDowell MA, Ervin RB, Wright JD (2000) Zinc intake of the U.S. population: findings from the third National Health and Nutrition Examination Survey, 1988–1994. J Nutr 130(5S Suppl):1367S–1373S
100. Lindeman RD, Clark ML, Colmore JP (1971) Influence of age and sex on plasma and red-cell zinc concentrations. J Gerontol 26(3):358–363
101. Mocchegiani E, Giacconi R, Muzzioli M, Cipriano C (2000) Zinc, infections and immunosenescence. Mech Aging Dev 121(1–3):21–35
102. Duchateau J, Delepesse G, Vrijens R, Collet H (1981) Beneficial effects of oral zinc supplementation on the immune response of old people. Am J Med 70(5):1001–1004
103. Cossack ZT (1989) T-lymphocyte dysfunction in the elderly associated with zinc deficiency and subnormal nucleoside phosphorylase activity: effect of zinc supplementation. Eur J Cancer Clin Oncol 25(6):973–976
104. Bogden JD, Oleske JM, Lavenhar MA, Munves EM, Kemp FW, Bruening KS et al (1990) Effects of one year of supplementation with zinc and other micronutrients on cellular immunity in the elderly. J Am Coll Nutr 9(3):214–225
105. Kahmann L, Uciechowski P, Warmuth S, Malavolta M, Mocchegiani E, Rink L (2006) Effect of improved zinc status on T helper cell activation and TH1/TH2 ratio in healthy elderly individuals. Biogerontology 7(5–6):429–435
106. Fortes C, Forastiere F, Agabiti N, Fano V, Pacifici R, Virgili F et al (1998) The effect of zinc and vitamin A supplementation on immune response in an older population. J Am Geriatr Soc 46(1):19–26
107. Barnett JB, Dao MC, Hamer DH, Kandel R, Brandeis G, Wu D et al (2016) Effect of zinc supplementation on serum zinc concentration and T cell proliferation in nursing home elderly: a randomized, double-blind, placebo-controlled trial. Am J Clin Nutr 103(3):942–951
108. Prasad A (2007) Zinc: mechanisms of host defense. J Nutr 137(5):1345–1349
109. Meydani SN, Barnett JB, Dallal GE, Fine BC, Jacques PF, Leka LS et al (2007) Serum zinc and pneumonia in nursing home elderly. Am J Clin Nutr 86(4):1167–1173
110. Mocchegiani E, Romeo J, Malavolta M, Costarelli L, Giacconi R, Diaz LE et al (2013) Zinc: dietary intake and impact of supplementation on immune function in elderly. Age (Dordr) 35(3):839–860
111. Hartmann HJ, Felix K, Nagel W, Weser U (1993) Intestinal administration of copper and its transient release into venous rat blood serum concomitantly with metallothionein. Biometals 6(2):115–118
112. Harvey LJ, Ashton K, Hooper L, Casgrain A, Fairweather-Tait SJ (2009) Methods of assessment of copper status in humans: a systematic review. Am J Clin Nutr 89(6):2009S–2024S
113. Wapnir RA (1998) Copper absorption and bioavailability. Am J Clin Nutr 67(5 Suppl):1054S–1060S
114. Osaki S, Johnson DA (1969) Mobilization of liver iron by ferroxidase (ceruloplasmin). J Biol Chem 244:5757–5758

115. Koller LD, Mulhern SA, Frankel NC, Steven MG, Williams JR (1987) Immune dysfunction in rats fed a diet deficient in copper. Am J Clin Nutr 45(5):997–1006
116. Bonham M, O'Connor JM, Hannigan BM, Strain JJ (2002) The immune system as a physiological indicator of marginal copper status? Br J Nutr 87(5):393–403
117. Brewer GJ (2009) The risks of copper toxicity contributing to cognitive decline in the aging population and to Alzheimer's disease. J Am Coll Nutr 28(3):238–242
118. Mocchegiani E, Costarelli L, Giacconi R, Piacenza F, Basso A, Malavolta M (2012) Micronutrient (Zn, Cu, Fe)-gene interactions in aging and inflammatory age-related diseases: implications for treatments. Aging Res Rev 11(2):297–319
119. Wang J, Pantopoulos K (2011) Regulation of cellular iron metabolism. Biochem J 434(3):365–381
120. Guyatt GH, Patterson C, Ali M, Singer J, Levine M, Turpie I et al (1990) Diagnosis of irondeficiency anemia in the elderly. Am J Med 88(3):205–209
121. Ekiz C, Agaoglu L, Karakas Z, Gurel N, Yalcin I (2005) The effect of iron deficiency anemia on the function of the immune system. Hematol J 5(7):579–583
122. Welch KD, Reilly CA, Aust SD (2002) The role of cysteine residues in the oxidation of ferritin. Free Radic Biol Med 33(3):399–408
123. Huang Z, Rose AH, Hoffmann PR (2012) The role of selenium in inflammation and immunity: from molecular mechanisms to therapeutic opportunities. Antioxid Redox Signal 16(7):705–743
124. Wood SM, Beckham C, Yosioka A, Darban H, Watson RR (2000) beta-Carotene and selenium supplementation enhances immune response in aged humans. Integr Med 2(2):85–92
125. Ershler WB, Keller ET (2000) Age-associated increased interleukin-6 gene expression, late-life diseases, and frailty. Annu Rev Med 51:245–270
126. Wardwell L, Chapman-Novakofski K, Herrel S, Woods J (2008) Nutrient intake and immune function of elderly subjects. J Am Diet Assoc 108(12):2005–2012

Chapter 3
Bioactive Lipids in Age-Related Disorders

Undurti N. Das

1 Introduction

Aging is inevitable but can be slowed. As we age, cells, tissues, organs and systems are bound to become senile and develop several health-related issues that may ultimately affect the health and lead to the onset of various diseases. But aging can be healthy so that disease occurrence and progression can be prevented, postponed or altogether avoided. Despite the fact that aging is believed to be a genetically programmed event, understanding the biochemical changes that predispose to the development of various disorders associated with aging may lead to development of strategies that keep the human organism healthy. Several studies in the field of aging have been performed with specific reference to changes in gene expression and their proteins, alterations in the activity of various enzymes and consequently alterations in the cellular functions that results in the aging process.

There are two dominant theories of aging: damage-based and programmed. The damage-based theory suggests that aging results from a continuous process of damage, presumably to DNA, which leads to alterations in several metabolic pathways. This theory implies that DNA damage occurs throughout the entire lifespan induced by byproducts released during the normal cellular process or a consequence of inefficient repair systems. In contrast to this, the programmed theory of aging argues that aging is a genetically-regulated process but not as a result of random or stochastic events. Although both the theories appear different, the underlying mechanism is in both is damage to DNA that results in altered gene(s) expression or alterations in gene expression as a programmed process. Thus, in theory, it is possible to stop, or

U. N. Das (✉)
UND Life Sciences, Battle Ground, WA, USA

BioScience Research Centre and Department of Medicine, GVP Medical College and Hospital, Visakhapatnam, India
e-mail: undurti@lipidworld.com

P. C. Guest (ed.), *Reviews on New Drug Targets in Age-Related Disorders*,
Advances in Experimental Medicine and Biology 1260,
https://doi.org/10.1007/978-3-030-42667-5_3

even reverse aging provided that the altered gene(s) expressions can be identified and restored to normal. In essence both theories have a strong genetic component. It can be said that the damage-based theory of aging suggests that genetic factors such as defensive or protective genes, have a role in aging while the programmed theory does recognize that some forms of damage to DNA contribute to aging and that environmental factors have a significant role in the aging process. So the difference between these two theories lies in the underlying mechanism. Damage-based theories of aging argue that aging is predominantly a result of interactions with the environment and/or damage from chemical reactions, while the programmed theories argue that aging is predetermined and occurs on a fixed schedule triggered by genetic programs. Other theories of aging include: (i) extrinsic or intrinsic factors that cause an accumulation of damage; and (ii) changes in gene expression that are either programmed or derived from DNA structural changes. It is apparent that there is a certain amount of overlap between all of these theories of aging.

In essence, aging is unavoidable and influenced by various endogenous and exogenous factors that result in gradual cellular deterioration. Studies have revealed that certain interventions can increase life expectancy and inhibit the aging process [1]. For instance, aging can be postponed or delayed (i) in mice, worms and fruit flies by inhibiting the insulin/IGF-1 axis that results in a transcription factor (DAF-16 in C. elegans, FoXo in mice) entering the nucleus to stimulate the expression of genes encoding survival-promoting proteins such as the Klotho protein; (ii) scavenging the highly toxic reactive oxygen species (ROS) produced mainly in the mitochondria, whose accumulation leads to DNA, lipid and protein changes (that results in cell dysfunction and aging); (iii) preventing shortening of the telomeres by enhancing telomerase activity; and (iv) correcting defective autophagy that uses lysosomes to destroy altered proteins to retain cell homeostasis. Studies of genetically mediated aging disorders such as progeria have revealed the importance of laminins (intermediate nuclear filaments) which fail to mature causing accelerated aging and premature death. It is agreed that there is no single biological marker of aging but measuring a combination of markers of specific diseases associated with aging may be of help in preventing or at least prevent or postpone some disorders of aging. For example, measurement of Nt-proBNP, troponin I, C-reactive protein and cystatin that are increased in atheroma and cardiovascular diseases may help in preventing these diseases by employing suitable remedial measures. Different organs age in different ways. Vessel walls become rigid due to protein glycation and develop atheroma, the heart is invaded by fibrosis, the brain suffers from neurofibrillar degeneration and senile plaques (responsible for Alzheimer's disease), the retina undergoes macular degeneration, renal function declines due to a gradual decrease in the nephron pool, and immune defenses become less effective due to the functional degradation of B and T lymphocytes and thymus involution, resulting in the development of cancer or autoimmune disorders. Despite all of the advances made in molecular and biochemical understanding of cell function(s), the only two measures that are known to slow the aging process are physical exercise and dietary restriction in the form of reduced calorie intake.

Recent studies by us and others showed that there are some distinct changes in the metabolism of bioactive lipids (BALs) that may predispose to the development of age-related disorders such as obesity, type 2 diabetes mellitus, hypertension, atherosclerosis, coronary heart disease (CHD), immune dysfunction and cancer. This implies that abnormalities in BAL metabolism can lead to the occurrence of these disorders at an early age and rectifying BAL metabolism could form a novel therapeutic approach for these disorders. Since most of these disorders are common with advancing age, restoring BALs to normal may have implications in preventing aging itself or may aid in healthy aging. It is possible that occurrence of some of these disorders is an indication of premature aging. This suggests that plasma levels or specific tissue levels of various BALs could be used as a measure of aging. It is interesting to note that both physical exercise and diet restriction enhance BAL metabolism such that inappropriate inflammation and imbalance in the immune system are restored to normal.

2 Essential Fatty Acid Metabolism

Our dietary essential fatty acids (EFAs): cis-linoleic acid (LA, 18:2 n-6) and alpha-linolenic acid (ALA, 18:3 n-3) are converted to their long-chain metabolites by the action of delta-6- desaturase and delta-5-desaturase and elongases. Thus, LA is converted to gamma-linolenic acid (GLA, 18:3 n-6), dihomo-gamma-linolenic acid (DGLA, 20:3 n-6) and arachidonic acid (AA, 20:4 n-6) whereas ALA is converted to eicosapentaenoic acid (EPA, 20:5 n-3) and docosahexaenoic acid (DHA, 22:6 n-3) by the same set of enzymes (Figs. 3.1 and 3.2; for EFA metabolism). It is noteworthy that several vitamins, minerals and trace elements influence EFA metabolism (Fig. 3.2) and deficiencies in these can result in the formation of higher amounts of pro-inflammatory and a decrease in the synthesis of anti-inflammatory metabolites. It is noteworthy that vitamins B1, B6, B12 and C are needed for adequate synthesis of GLA, DGLA, AA, EPA and DHA, precursors that in turn are needed for the formation of prostaglandin E1 (PGE1), prostacyclin (PGI2), lipoxins, resolvins protectins and maresins, which have potent anti-inflammatory, vasodilator, anti-platelet anti-aggregator and cytoprotective actions [2–11]. AA is the precursor of 2 series PGs, thromboxanes (TXs) and 4 series leukotrienes (LTs) whereas EPA is the precursor of 3 series PGs, TXs and 5 series LTs. PGs, TXs and LTs have pro-inflammatory actions. It is noteworthy that 3 series PGs, TXs and 5 series LTs are also pro-inflammatory in nature but are less potent compared to 2 series PGs TXs and 4 series LTs. Hence the suggestion that PGs, TXs and LTs formed from EPA have anti-inflammatory actions is not correct. AA, EPA and DHA are also metabolized by cytochrome P450 enzyme system to form various products that have been outlined in Figs. 3.3, 3.4, 3.5 and 3.6 in addition to the action of COX and LOX enzymes. In general, more detailed studies have been performed on the metabolic products formed by the action of COX and LOX enzymes compared to the cytochrome P450 enzymes system. One needs to consider P450 products formed

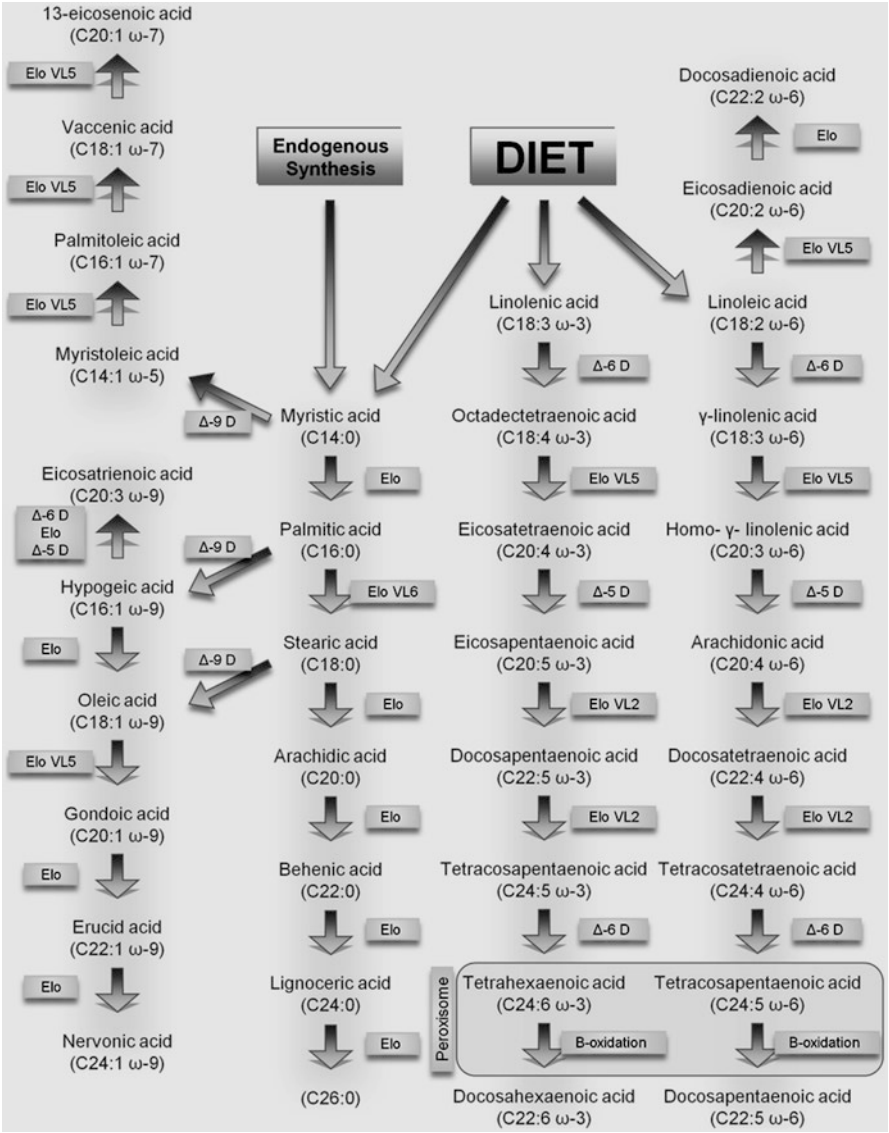


Fig. 3.1 Scheme showing metabolism of EFAs (LA and ALA) and also the metabolism of n-7 and n-9 fatty acids. Although all fatty acids are important for normal health, n-3 and n-6 seems to be more critical. It is to be noted that "n" is same as "ω". A simplified version and other products formed from EFAs are given in Fig. 3.2

from AA, EPA and DHA while studying the actions of various metabolites of EFAs and PUFAs.

In contrast to the pro-inflammatory actions of PGs, LTs and TXs, certain specific anti-inflammatory products can also be formed from AA, EPA and DHA. These

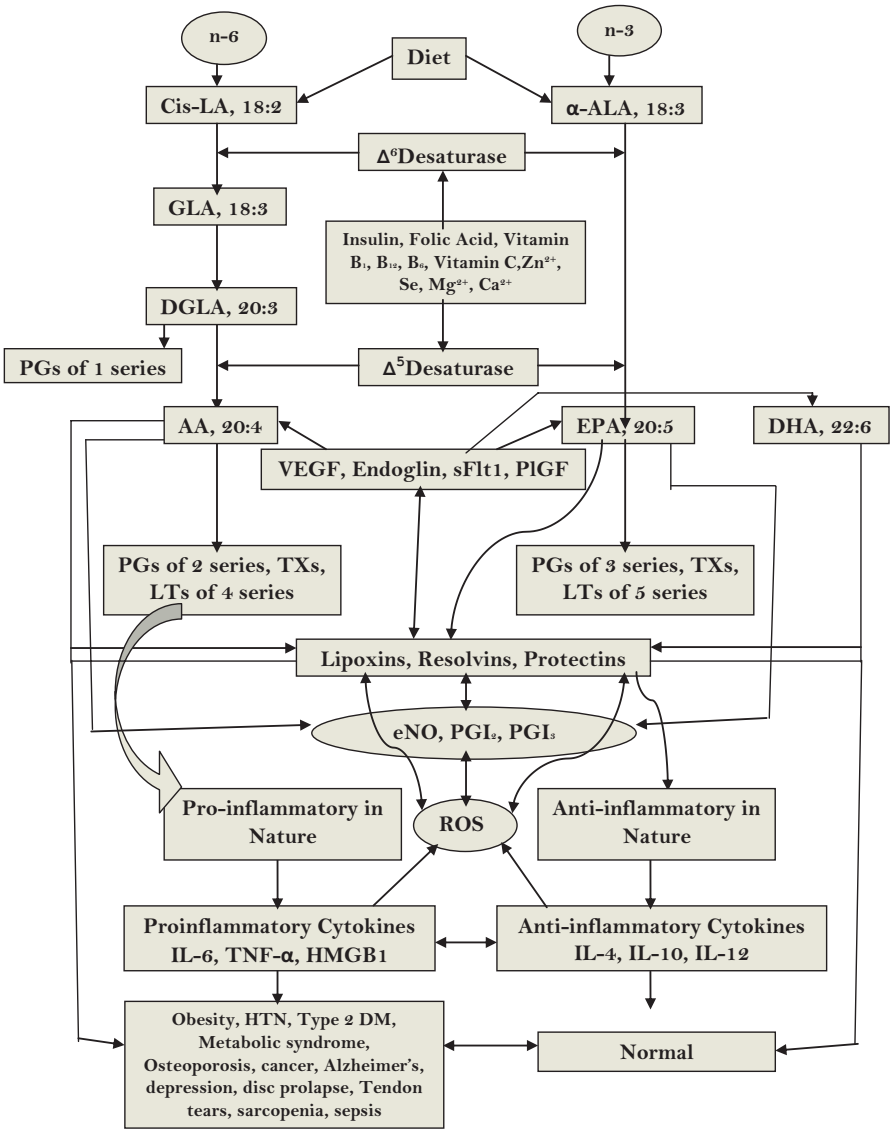


Fig. 3.2 Scheme showing effect of vitamins on metabolism of essential fatty acids and their role in various diseases

include lipoxins from AA, resolvins of E series from EPA and resolvins of D series and protectins and maresins from DHA (Fig. 3.3). Hence, it is likely that under physiological conditions a balance is maintained between pro- and anti-inflammatory compounds formed from GLA, DGLA, AA, EPA, DPA and DHA. The production of various PGs, TXs, LTs, lipoxins, resolvin, protectins and maresins from their respective precursors depends on the concentrations of GLA, DGLA, AA, EPA,

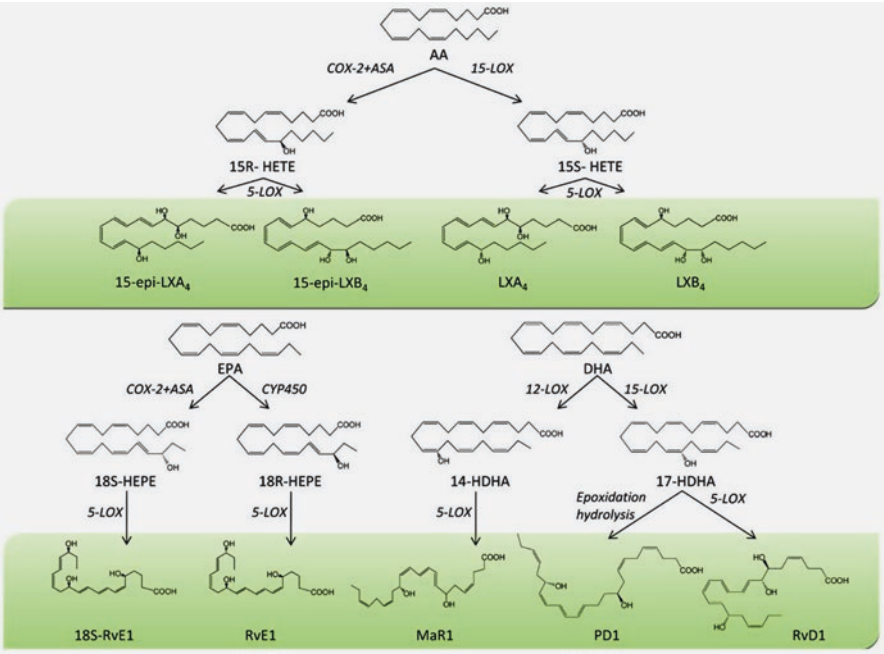


Fig. 3.3 Metabolism of AA, EPA and DHA by COX and LOX enzymes in the presence of aspirin that leads to the formation of lipoxins, resolvins, protectins and maresins

DPA and DHA in the cell membrane lipid pool of these fatty acids released by the action of the enzyme phospholipase A2 (PLA2). It is known that PLA2 is activated by various stimuli including injury, infection, LPS (lipopolysaccharide), IL-6, TNF-α, IL-1, IL-2, IL-4, HMGB1 and other inciting agents that are capable of perturbing the cell membrane. It is predicted that PLA2 is able to activate in a specific and coordinated fashion such that the release of GLA, DGLA, AA, EPA, DPA and DHA from the cell membrane is determined based on the context and necessity of the local events either to initiate and perpetuate inflammation and/or suppress inflammation and initiate resolution of inflammation and restore homeostasis. Thus, the release of PGs, TXs, LTs, lipoxins, resolvins, protectins and maresins appears to occur in a deliberately coordinated and smooth manner to shift the local events from pro-inflammatory status to resolution of the inflammation phase. How this occurs is not completely clear. However, it is known that local factors play a significant role in this sequence of events. Some of these local factors that may have the ability to regulate the production of BALs include pH, lactate, potassium, sodium, magnesium, and glucose and its metabolite concentrations. It is noteworthy that EFAs and PUFAs and other BAL molecules are capable of altering the function of mechanosensitive channels such as PIEZO-1 and PIEZO-2 and TRPV and thus, regulate the structure and functions of various receptors located on the cell membrane. In addition, local infiltrating leukocytes, T cells, NK cells and macrophages including

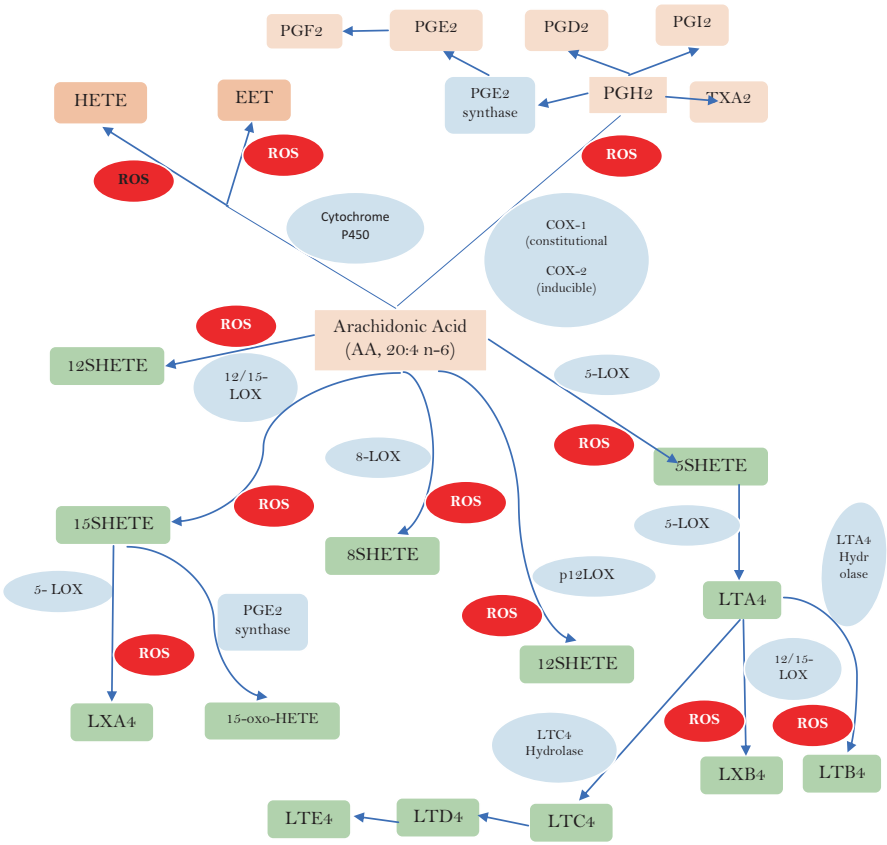


Fig. 3.4 A detailed metabolism of AA showing the formation of various products and generation of ROS during its metabolism

endothelial cells, fibroblasts and cellular milieu are also capable of influencing the activity of PLA2, desaturases, elongases, COXs, LOXs, PG synthase, 15-PGDH (15-prostaglandin dehydrogenase) and other eicosanoid catabolic enzymes that can alter local concentrations of EFAs, PUFAs, PGs, LTs, TXs, lipoxins, resolvins, protectins and maresins. Thus, the formation of actions of BALs are complex. At the same time, in view of their large number of actions, EFAs and BALs are capable of influencing a number of cellular events and thus participate in a number of disorders that include inflammatory, immunological and degenerative disorders as discussed below. Understanding the various actions of EFAs and BALs and their role in various diseases opens a new window of opportunity to exploit these as potential drug targets for various disorders.

Note that the term EFAs is used for LA and ALA, PUFAs refer to GLA, DGLA, AA, EPA, DPA and DHA and BAL refers to EFAs, PUFAs and lipoxins, resolvins, protectins and maresins in the present discussion.

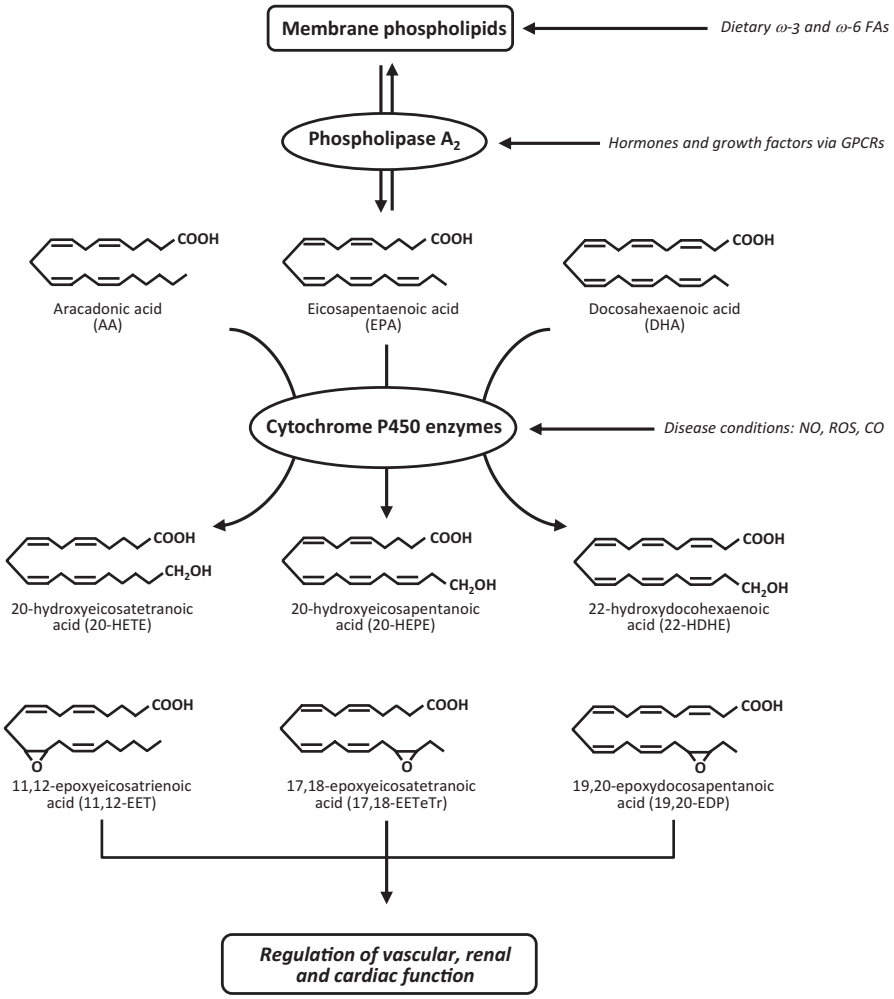


Fig. 3.5 Metabolism of AA, EPA and DHA by cytochrome P450 enzymes and various products formed from this pathway. These products have regulatory action on vascular, renal and cardiac tissues

3 Actions of Bioactive Lipids

BALs have several important actions that may explain their involvement in many cellular functions and biological processes and disorders. Some of these significant actions of BALs include: (i) participation in inflammatory processes; (ii) modulation of the immune response in cancer other immunological disorders; (iii) influencing the actions of ion channels including Piezo1 and Piezo 2 and voltage gated ion channels such as the transient receptor potential cation channel subfamily V member 1 (TrpV1) in the cell mitotic process, cell signaling, cell cycle progression, and

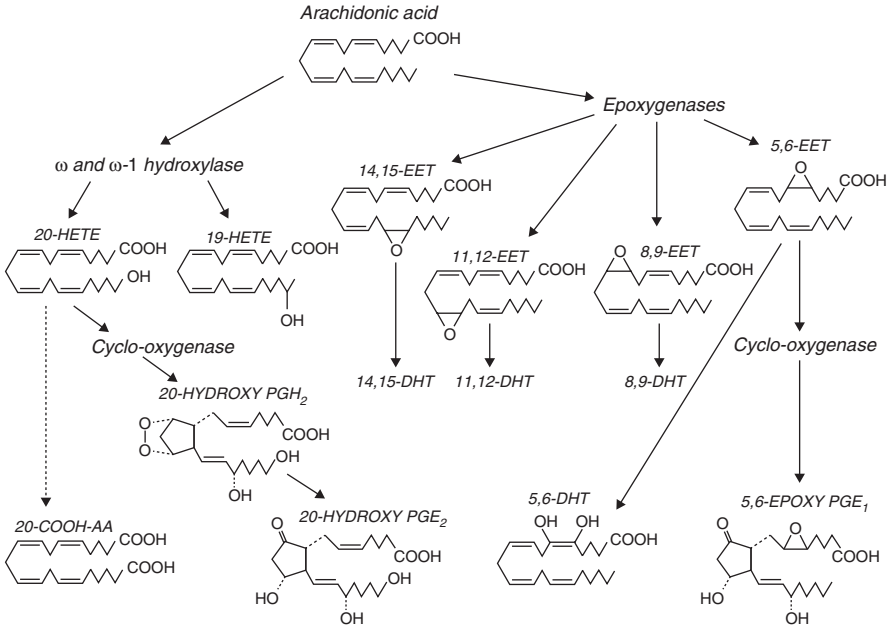


Fig. 3.6 Scheme showing metabolism of AA by the cytochrome P450 enzyme system. Both EPA and DHA also undergo similar metabolism by the cytochrome P450s. AA is metabolized by cytochrome P450 mono-oxygenases to ω- and ω-1-hydroxyeicosatetraenoic acids (HETEs), epoxyeicosatrienoic acids (epoxides, EETs), and dihydroxyeicosatrienoic acids (diols, DHTs). 20-HETE and 5,6-EET can be converted by COX to analogues of PGs

cell volume regulation; (iv) altering cell membrane fluidity, influencing the structure and composition of intermediate filaments and their multiple binding partners involved in cellular mechanics and gene regulation; (v) and regulating mitochondrial processes, telomerase activity; and G-protein–mediated signal transduction.

4 Inflammation

It is interesting to note that GLA, DGLA, AA, EPA, DPA and DHA have potent anti-inflammatory actions by themselves without the necessity of formation of their respective metabolites: PGs, TXs, LTs, lipoxins, resolvins, protectins and maresins. GLA, DGLA, AA, EPA and DHA inhibit the formation of pro-inflammatory cytokines interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α), IL-1, and HMGB1 (high mobility group box-1) [12–22]. Since both AA and EPA form precursors to pro-inflammatory PGs, LTs, TXs and anti-inflammatory lipoxins and resolvins, it is likely that their concentrations in the cell membrane, the amount(s) of each of these fatty acids that are released in response to PLA2 activation, their conversion to the respective metabolites (PGs, LTs, TXs *vs* lipoxins and resolvins), and their

degradation determines the final outcome of the inflammatory process. Thus, it is anticipated that there is a delicate balance between the pro- and anti-inflammatory products of AA and EPA. Hence, it is likely that inflammation is triggered and perpetuated if the pro-inflammatory PGs, LTs and TXs are produced in excess whereas inflammation is suppressed, and resolution ensues if the production of lipoxins and resolvins are formed in adequate amounts. It is noteworthy that lipoxins and resolvins are capable of suppressing the production and antagonizing the actions of PGs, LTs and TXs and thus, inhibiting inflammation and initiating inflammation resolution processes [2, 23–29]. DHA, the precursor of resolvins of D series, protectins and maresins have anti-inflammatory, cytoprotective and wound healing properties [1, 23, 30, 31]. It is also evident that lipoxins and maresins are capable of reducing inflammatory edema, neuropathic pain, and enhancing tissue regeneration partly by acting on the TRPV1 channels [31]. Our studies revealed that both AA and LXA4 (lipoxin A4) not only inhibit inflammation by decreasing the production of IL-6 and TNF-α but also suppress NF-kB and COX-2 expression and enhance the proliferation of pancreatic β cells [32, 33]. The beneficial actions of AA appear to be due to formation of LXA4. Surprisingly, we observed that experimental animals treated with EPA, DHA and other fatty acids also showed enhanced levels of LXA4 despite the fact that LXA4 is derived from AA. This suggests that fatty acids other than AA when administered can displace AA from the cell membrane lipid pool and this displaced AA could be converted to LXA4. The other possibility is that there is a crosstalk among lipoxins, resolvins, protectins and maresins such that they are able to augment the production of each other as the situation demands. If this is true, it is not clear why this crosstalk needs to occur. The fact that lipoxins, resolvins, protectins and maresins that have similar anti-inflammatory, inflammation resolution and wound healing properties but are produced from different precursors suggests that there are more well-designed but separate actions that are critical for wound healing and other beneficial actions. In this context, it is noteworthy that lipoxins, resolvins, protectins and maresins also show cytoprotective and cell proliferation regulatory actions in addition to their anti-inflammatory role. This suggests that lipoxins, resolvins, protectins and maresins may have more selective, specific and beneficial actions in addition to their action on inflammation. Such an assumption is supported by the observation that LXA4 has more potent anti-diabetic action compared to resolvins (unpublished data). Similarly, LXA4 is more potent that the resolvins in suppressing the production of IL-6 and TNF-α in alloxan and streptozotocin-induced type 1 and type 2 diabetes mellitus animal models. Thus, although lipoxins, resolvins, protectins and maresins show anti-inflammatory actions, their potency is variable. No studies have been performed to assess such variations in their potency but some preliminary predictions are possible as shown in Fig. 3.7. In this context, the role of PLA2 in inflammation and formation of PGs, LTs, TXs and lipoxins, resolvins, protectins and maresins needs attention.

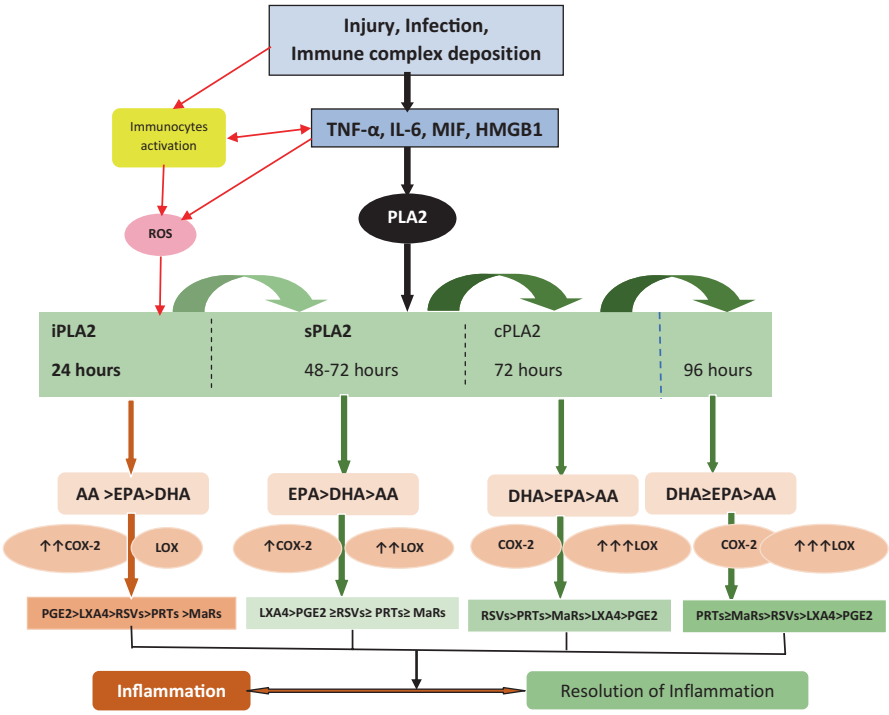


Fig. 3.7 Scheme showing the relationship among pro- and anti-inflammatory cytokines, PGs, LTs, lipoxins, resolvins, protectins and maresins and steroids. Metabolism and actions of AA are shown as a representative of various PUFAs (DGLA, EPA and DHA). For further details see the text. RSVs resolvins, PRTs protectins; MaRs maresins. During the inflammatory process it is expected that there will be activation of desaturases, COX-2 and LOX enzymes depending on the stage and duration of inflammation. The proposed levels of anti-inflammatory lipoxins (LXA4), RSVs, PRTs and MaRs, and pro-inflammatory PGE2 can be as follows (it needs to be noted that PGE2 is depicted as a representative of all pro-inflammatory lipids and the relationship among cytokines and the bioactive lipids is given in Fig. 3.8): 24 h: PGE2↑↑↑↑; LXA4↑; RSVs↔; PRTs↔; MaRs↔. 48 h: PGE2↑↑↑; LXA4↑↑; RSVs↑; PRTs↑; MaRs↑. 72 h: PGE2↑↑; LXA4↑↑↑; RSVs↑↑; PRTs↑↑↑; MaRs↑↑↑. 96 h: PGE2↑; LXA4↑↑; RSVs↑↑↑; PRTs↑↑↑; MaRs↑↑↑↑. >96 h: PGE2↑; LXA4↑; RSVs↑↑; PRTs↑↑; MaRs↑↑↑. The actions of these compounds in the inflammation and wound healing process can be as follows: LXA4 → anti-inflammatory >resolution >protection >proliferation; RSVs → resolution > anti-inflammatory >protection >proliferation; PRTs → protection > resolution > anti-inflammatory > proliferation; MaRs → proliferation > protection > resolution > anti-inflammatory. Resolution refers to resolution of inflammation. Protection refers to protection of normal cells/tissues from injurious agents. Proliferation refers to proliferation of stem cells and other cells in order to replace damaged cells/tissues. Despite the fact that all compounds have similar and overlapping actions and possess anti-inflammatory properties, each lipid may show one particular action more compared to the other actions

5 Bioactive Lipids and the Immune Response

It is likely that under normal physiological conditions, a balance is maintained between pro-inflammatory PGS, TXs, LTs and IL-6, TNF-α, IL-1β, HMGB1 and other pro-inflammatory cytokines and anti-inflammatory lipoxins, resolvins, protectins and maresins and anti-inflammatory cytokines IL-4, IL-10, TGF-β. It is noteworthy that once the inflammatory process reaches its peak, the anti-inflammatory pathway is stimulated and the formation of anti-inflammatory lipoxins, resolvins, protectins and maresins and the needed anti-inflammatory cytokines occurs. These events are likely to be accompanied by suppression of ROS (reactive oxygen species) generation and increase in the much-needed antioxidant defences. These events trigger the initiation of the inflammation resolution process and healing of the wound to restore homeostasis. Lipoxins, resolvins, protectins and maresins inhibit PMNLs (polymorphonuclear leukocytes) trans-endothelial migration, reduce leucocyte infiltration, and suppress dendritic cell (DC) migration and IL-12 production in order to suppress inflammation and enhance the anti-inflammatory process. Lipoxins, resolvins, protectins and maresins have the ability to augment the expression of antiinflammatory genes and attenuate LTB4-stimulated proinflammatory signals [2, 23].

It is known that an interaction exists among pro- and anti-inflammatory cytokines and PUFA metabolism. Proinflammatory cytokines IL-1, IL-6, TNF-α and IFN-γ are known to activate phospholipases, augment ROS generation [34–38], and enhance the activities of COX-2 and LOX enzymes that are needed for the production of PGE2, TXA2 and LTs to initiate and perpetuate inflammation and subsequently to suppress inflammatory process as and when the purpose of inflammation is achieved. The precursors that are common (especially AA and EPA) for the formation of both pro-inflammatory (PGs, LTs TXs from AA and EPA) and anti-inflammatory (lipoxins from AA and resolvins from EPA and DHA and protectins and maresins from DHA) lipids are derived from the cell membrane pool by the activation of phospholipase A2 (PLA2). This implies that there could be two waves of release of PUFAs especially AA, EPA and DHA from the cell membrane lipid pool. The first one to enhance the formation of pro-inflammatory PGs, LTs and TXs and the second to trigger the formation of lipoxins, resolvins, protectins and maresins by their respective and specific phospholipases (Fig. 3.8).

The three classes of phospholipases that regulate the release of PUFAs are calcium independent PLA2 (iPLA2), secretory PLA2 (sPLA2), and cytosolic PLA2 (cPLA2). Each class of PLA2 is further divided into isoenzymes for which there are 10 for mammalian sPLA2, at least 3 for cPLA2, and 2 for iPLA2. The first wave of release of PUFAs from the cell membrane is due to the action of iPL2 that results in the formation of pro-inflammatory PGE2, TXA2 and LTB4. The second wave of release of PUFAs is due to the action of sPLA2 and cPLA2 that occurs at the time of initiation of resolution of inflammation. This results in the formation of lipoxins, resolvins, protectins and maresins that suppress inflammation, initiate resolution of

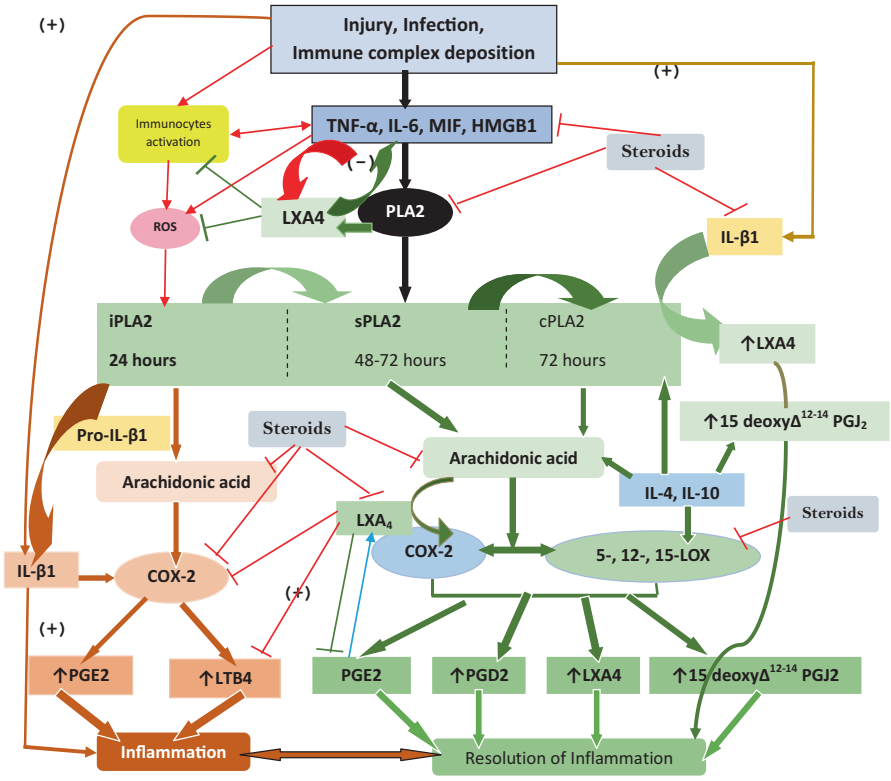


Fig. 3.8 Scheme showing the relationship among pro- and anti-inflammatory cytokines, PGs, LTs, lipoxins, resolvins, protectins and maresins and steroids. Metabolism and actions of arachidonic acid is shown as a representative of various PUFAs (GLA, DGLA, EPA, DPA and DHA). For further details see the text. (+) Indicates increase in the synthesis/action or positive effect. (−) Indicates decrease in the synthesis/action or negative effect

the inflammatory process, cytoprotection of surrounding normal cells/tissues and regeneration of normal cells to replace the dead and damaged cells and tissue to restore homeostasis. It is noteworthy that adequate amounts of PGE2 are needed to induce optimal inflammation and also trigger initiation of resolution of inflammation. Thus, the inflammatory stimuli that induces the release of PUFAs by activating iPLA2 are utilized for the synthesis of pro-inflammatory PGs, TXs and LTs. In contrast, PUFAs released from the same cell membrane stores by the action of sPLA2 and cPLA2 at the time of initiation of resolution of inflammation are directed to form lipoxins, resolvins, protectins and maresins. This delicate yet and imperceptible and orderly switch over from pro-inflammatory to anti-inflammatory molecules seems to be determined by the type of PLAs that are activated and the activities of COX-2 and 5-, 12- and 15-LOX enzymes. Thus, a close co-operation, association and interaction(s) among PLAs, COX-2, LOX enzymes and various cytokines is

needed for the appropriate inflammation to occur and to induce a gradual, smooth and orderly onset of anti-inflammatory events, resolution of inflammation and restoration of homeostasis [2]. Any defects in this process (dysfunction of cytokines, PLAs, COX, LOX enzymes, cell membrane stores of PUFAs, etc.) can lead to persistance of inflammation and damage to the target tissues as seen in autoimmune diseases, chronic infections such as tuberculosis and in aging (Figs. 3.2, 3.7 and 3.8). With advancing age, there is a decrease in the activities of desaturases, an increase in COX-2 activity and a change in the expression of 5-, 12-, and 15-LOX enzymes that can result in a decrease in the concentrations of GLA, DGLA, AA, EPA and DHA in the cell membrane pool, an increase in the formation of PGE2 and decreased synthesis and release of lipoxins, resolvins, protectins and maresins (Figs. 3.7, 3.8 and 3.9) [39–54]. A similar relationship exists between pro- and anti-inflammatory cytokines and any imbalance in their concentrations can lead to inappropriate inflammation (Figs. 3.7 and 3.8).

A significant inverse correlation was noted between age and the LXA4/LTs ratio suggesting that aging is associated with a dramatic change in AA (and possibly also of GLA, DGLA, EPA and DHA) metabolism such that LXA4 (and other anti-inflammatory lipid molecules) levels are decreased whereas those of LTs (a pro-inflammatory molecule) is increased and may contribute to the development of diseases that are common in the elderly such as type 2 diabetes mellitus, hypertension, coronary heart disease (CHD), atherosclerosis, cancer, Alzheimer's disease, depression and immune dysfunction. This may also include other inflammatory and immunological disorders such as disc prolapse, lupus and arthritis, osteoporosis and tendon tears. It is noteworthy that all these are inflammatory conditions and have an immunological component in the form of an increase in the local and/or systemic concentrations of pro-inflammatory cytokines IL-6, TNF-α, IL-1β and HMGB1 and a concomitant change in bioactive lipids seen as low plasma or tissue levels of GLA, DGLA, AA, EPA and DHA (one or more of these fatty acids or all) and an increase in pro-inflammatory molecules PGE2, LTs, TXs and a deficiency of lipoxins, resolvins, protectins and maresins [2, 6–13, 23–27, 30–33, 46–85]. Thus, bioactive

Fig. 3.9 (continued) It should be noted that suppression of inflammation is not equal to resolution of inflammation. To suppress inflammation, LXA4 inhibits leukocyte infiltration. While resolvins are needed for resolution of inflammation (such as removing the debris of a wound, phagocytosis of dead leukocytes, etc.); protectins may perform the important function of protecting normal cells/tissues from further damage and thus, maintain tissue integrity. Maresins may act on stem cells (to induce their differentiation) for the repair process to occur and restore tissue homeostasis. The figure also shows how this sequence of orderly activation and deactivation of PLA2, COX-2 and formation of PGE2 and LXA4 are likely to get deranged in the face of failure of resolution of inflammation processes. Patients with hypertension, diabetes mellitus and advanced age have low-grade systemic inflammation as a result of sustained activation of COX-2 and formation of PGE2 and failure of formation of adequate amounts of LXA4 and other anti-inflammatory compounds. Failure of the inflammation resolution process may lead to the onset of age-associated disorders such as hypertension, type 2 diabetes mellitus, atherosclerosis, CHD, cancer, osteoporosis and sarcopenia and when this inflammatory process is severe it can lead to the onset of sepsis and septic shock, which are common in the elderly

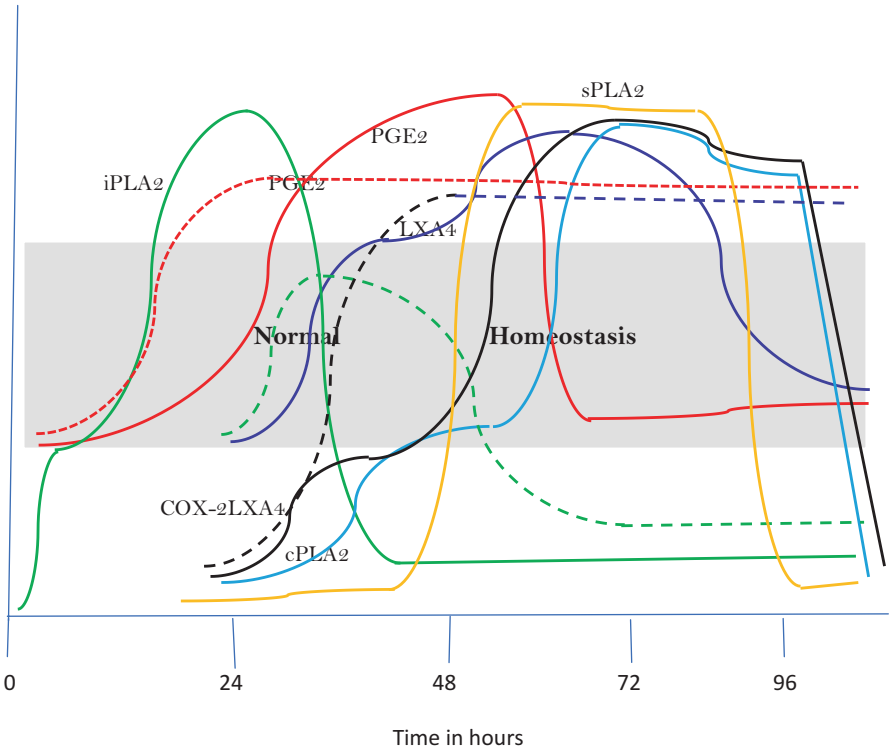


Fig. 3.9 Scheme showing possible relationship among PGE2, LXA4 and various PLA2 enzymes, as seen in inflammation and inflammation resolution processes. PGE2; LXA4; iPLA2; sPLA2; cPLA2; COX-2. All of these concentrations and activities of enzymes are presented as expected to behave during normal inflammatory process (which finally resolve spontaneously). PGE2 when inflammation persists; COX-2 when inflammation persists; LXA4 when resolution of inflammation is defective. Possible changes that may occur in the activities of various PLA2s are not shown in the figure, they are likely to behave in tune with the concentrations of PGE2 and LXA4. Despite the fact that LXA4, resolvins, protectins and maresins have anti-inflammatory actions, there could be subtle differences in their major and minor actions with some amount of overlap in their anti-inflammatory actions (Fig. 3.7). Although the role of nitrolipids is not shown, it is expected to behave similarly to LXA4. As already discussed in the text and shown in Figs. 3.7 and 3.8, there are two waves of release of AA (and other PUFAs). The first one occurs in the early period of inflammation (within the first 24 h due to activation of iPLA2) which predominantly leads to the formation of PGE2 and other pro-inflammatory molecules. Once the concentrations of PGE2 reach the optimum level (say by the end of 24–48 h), a second wave of AA release occurs (due to activation of sPLA2 and cPLA2) that results in the formation of LXA4 (resolvins, protectins and maresins from EPA and DHA), capable of inducing resolution of inflammation. The activation of cPLA2 occurs around 48–72 h to initiate and accelerate the resolution of inflammation. The activation of iPLA2 and formation of PGE2 are closely associated with the activation of COX-2. In this process of inflammation and resolution of inflammation, there is a critical role for the PGDH enzyme needed for catabolism of PGE2. It is noteworthy that LXA4, resolvins, protectins and maresins are anti-inflammatory molecules but may have slight but critically important differences in their actions to resolve the inflammation and enhance wound healing. For instance, LXA4 is needed to induce anti-inflammatory events.

lipids seem to have a significant role in many inflammatory and immune-mediated disorders that are common in the elderly. This implies that occurrence of these inflammatory and immune-mediated diseases such as obesity, type 2 diabetes mellitus, hypertension, atherosclerosis, coronary heart disease, lupus, cancer and osteoporosis is a sign of aging. In essence, all these evidences suggest that an increase in pro-inflammatory PGs, LTs and TXs and cytokines and a decrease or deficiency of LXA4, resolvins protectins and maresins and anti-inflammatory cytokines occur in many diseases associated with aging. This implies that restoring the balance between pro- and anti-inflammatory cytokines and BALs may form a novel approach in the prevention and management of several inflammatory and immunological disorders as summarized previously (Fig. 3.10) [47].

6 IL-6, TNF-α and Corticosteroids Induce a Bioactive Lipid Deficiency State

It is interesting to note that IL-6, TNF-α, HMGB1 and other pro-inflammatory cytokines and corticosteroids suppress desaturase activity that leads to decreased formation of metabolites of EFAs (LA and ALA) such as GLA, DGLA, AA, EPA and DHA. Due the presence of decreased concentrations of GLA, DGLA, AA, EPA and DHA, their metabolites such as PGE1 (from DGLA), PGI2 and LXA4 (from AA), resolvins (from EPA and DHA), protectins and maresins (from DHA) form in low amounts due to precursor deficiency but ironically excess formation of PGs, LTs and TXs occurs [2, 23, 55]. In contrast, IL-6 and TNF-α and other pro-inflammatory cytokines activate PLA2, COX-2 and LOX enzymes whereas corticosteroids suppress them. Thus, corticosteroids are potent anti-inflammatory molecules since they (i) suppress desaturases, (ii) inhibit PLA2 activity, and (iii) block COX and LOX enzymes. As a result of these actions, (i) decreased conversion of LA and ALA to their long-chain metabolites such as GLA, DGLA and AA from LA and EPA and DHA from ALA (due to suppression of desaturases) and hence, a deficiency of GLA, DGLA, AA, EPA and DHA, occurs in the cells; (ii) decreased formation and

Fig. 3.10 (continued) PUFAs can give rise to FAHFAs (fatty acid hydroxy fatty acids) that have anti-inflammatory properties and enhance the formation of NO, CO and H_2S, and mediate exercise-induced anti-inflammatory actions. PUFAs and lipoxins, resolvins, protectins and maresins suppress IL-6, TNF-α and PG, LT and TX production. It is not yet known but possible that FAHFAs suppress tumor cell growth and inhibit inflammatory events in hypothalamus. Although the role of p53 in aging and diseases is not discussed in detail here, it may be noted that p53 is the guardian of the genome. PUFAs and their metabolites, cytokines, NO, CO, H2S, ROS, GDF-11, GnRH and NAE may modulate the action of p53. For instance, exercise reduces the incidence of cancer, possibly by augmenting the production of IL-6 and TNF-α that are cytotoxic to tumor cells either by their direct action and/or by their ability to enhance the production of ROS that are tumoricidal. Exercise enhances the expression and action of p53 that leads to apoptosis of cancer cells. PUFAs have tumoricidal action by enhancing the production of free radicals and accumulation of toxic lipid peroxides in tumor cells

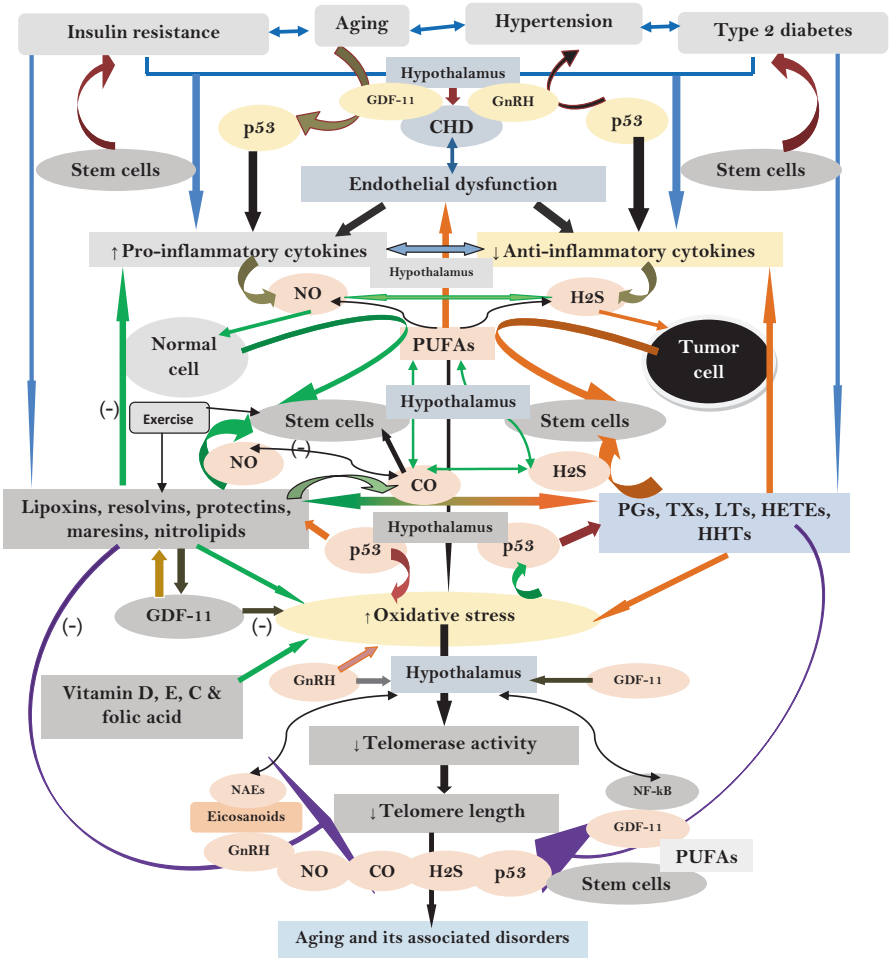


Fig. 3.10 Scheme showing aging and its associated disorders and their relationship to hypothalamus, oxidative stress, PUFA metabolism, CO (carbon monoxide), NO (nitric oxide), H_2S (hydrogen sulfide) and telomere length. High calorie diet stimulates ROS generation that may overwhelm the antioxidant system in adipose and other tissues, enhance the synthesis of pro-inflammatory cytokines, and decrease the formation of anti-inflammatory cytokines, leading to the onset of low-grade systemic inflammation, induction of DNA damage and aging. These events cause aging of endothelial cells, shorten telomere length, and inhibit p53 expression. They also induce endothelial dysfunction and insulin resistance that leads to the development of hypertension, type 2 diabetes mellitus, atherosclerosis, CHD and aging. A high calorie diet, insulin resistance and lack of exercise suppress D6 and D5 desaturases leading to reduced formation of GLA, DGLA, AA, EPA and DHA, the precursors of lipoxins, resolvins, protectins and maresins and other anti-inflammatory products. Deficiency of these molecules impairs resolution of inflammation, DNA damage persists, telomere shortening occurs, p53 dysfunction sets in, and stem cell function becomes inappropriate, leading to the onset and progression of aging and age-associated disorders. These events will result in decreased CO, NO and H_2S production. PUFAs and their metabolites influence stem cell biology and thus, affect the aging process and age-associated disorders including Alzheimer's disease.

release of PGs, LTs and TXs is seen due to substrate deficiency; (iii) as a consequence of inhibition of COX and LOX enzymes reduced formation of PGs, LTs and TXs occurs; and (iv) due to the inhibitory action of corticosteroids on PLA2 activity there is a decrease in the release of GLA, DGLA, AA, EPA and DHA from the cell membrane lipid pool and so, the availability of precursors of PGs, LTs and TXs is significantly low. Thus, in the short-term corticosteroids are potent anti-inflammatory compounds. However, in the long-run they induce an EFA and PUFA deficiency state leading to continuation of the inflammatory state and failure of resolution of the injury/inflammation as a result of decreased formation of lipoxins, resolvins, protectins and maresins that are needed for wound healing and restoration of homeostasis. This deficiency of anti-inflammatory lipids is due to their precursor (GLA, DGLA, AA, EPA, DPA and DHA) deficiency. It is paradoxical to know that corticosteroids inhibit both LXA4 and LTB4 synthesis but have a much lower effect on LTB4 that results in a pro-inflammatory status [86]. This proposal is further supported by the observation that supplementation of AA during active inflammatory process when PGs, LTs and TXs are being synthesized in excess, actually results in an increase in the formation of LXA4 (and possibly, resolvins, protectins and maresins) with little change in the concentrations of PGE2, tilting the balance more towards an anti-inflammatory status that results in suppression of the inflammation [2, 55, 87, 88]. However, unlike IL-6 and TNF-α that activate PLA2 and COX-2 and thus, enhance the formation of pro-inflammatory PG2, LTs and TXs, corticosteroids block the expression of PLA2 and COX-2 and thereby block the formation of PGE2, LTs and TXs that may explain their (corticosteroids) anti-inflammatory action compared to the pro-inflammatory actions of IL-6 and TNF-α (Figs. 3.7 and 3.8). These results imply that EFAs, PUFAs, and other bioactive lipids are the mediators of the actions of corticosteroids and IL-6 and TNF-α and paradoxically both corticosteroids and IL-6 and TNF-α induce an EFA (PUFA)-deficiency state by their ability to block the activities of desaturases. These results imply that co-administration of PUFAs along with corticosteroids may sustain their anti-inflammatory actions (by enhancing the formation of lipoxins, resolvins, protectins and maresins) and, when PUFAs are administered in conjunction with IL-6 and TNF-α, may potentiate their anti-cancer action by augmenting ROS generation in tumor cells (Fig. 3.8) [2, 61, 62]. It is interesting that corticosteroids and pro-inflammatory cytokines that have physiologically opposite actions seem to mediate their actions through the same molecules, namely BALs. This speaks of the pleiotropic actions of BALs.

It is noteworthy that IL-1β that is markedly increased during inflammation is capable of inducing PG biosynthesis and also up regulating the formation of LXA4 and maresins that are necessary for the inflammation resolution process [55]. LXA4, resolvins, protectins and maresins are potent down-regulators of PGE2 production. Increased 15-prostaglandin dehydrogenase (15-PGDH) expression enhances the formation of LXA4, resolvins, protectins and maresins and augments the regeneration of tissues to reestablish tissue homeostasis [2, 23, 55, 57, 72, 89–93]. Thus,

IL-1β and PGE2 have both pro- and anti-inflammatory actions depending on the context (Fig. 3.8). This suggests that in order to suppress both acute and chronic inflammation and inhibit the production of pro-inflammatory IL-6 and TNF-α, one needs to employ AA/EPA/DPA/DHA, LXA4, resolvins, protectins and maresins in combination with corticosteroids. Similarly, when IL-6 and TNF-α are co-administered along with GLA, DGLA, AA, EPA, DPA and DHA it could be possible to eliminate tumor cells selectively with little or no side effects of cytokines on normal cells since BALs suppress inappropriate production and action of pro-inflammatory cytokines [61, 62]. The relationship between bioactive lipids and corticosteroids suggests that Cushing's syndrome that is due to excess production of cortisol can be considered as an EFA deficiency state since it inhibits desaturase, PLA2, COX and LOX enzymes resulting in low plasma and tissue concentrations of GLA, DGLA, AA, EPA, DPA and DHA, and altered levels of PGs, LTs, TXs, LXA4, resolvins, protectins and maresins. Since BALs have a role in obesity, hypertension, type 2 diabetes mellitus, inflammation and immune function., it is reasonable to suggest that several features seen in Cushing's disease can be considered as a disorder of altered bioactive lipids and this offers a critical insight into the actions of BALs (Fig. 3.11). This also explains the Cushingoid-like features seen in many patients with metabolic syndrome implying that there could be a relative cortisol excess in these subjects.

7 Bioactive Lipids Modulate Immune Response

Aging is associated with a decrease in immunity and increased susceptibility to infections that could lead to sepsis. This increased susceptibility to infections can be ascribed to increased generation of pro-inflammatory PGE2 and LTs and decreased production of LXA4 with advancing age [52–54]. PGE2 suppresses the proliferation of T cells, immunosuppressive in nature, and inhibits the production of IL-6 and TNF-α that are needed to induce the generation of ROS by leukocytes and macrophages to kill bacteria and other invading organisms. Furthermore, lipoxins, resolvins, protectins and maresins are capable of enhancing the anti-bacterial action of leukocytes and macrophages and possibly that of other immunocytes [94–98]. Hence, their deficiency due to corticosteroid therapy and in aging may lead to increased incidence of infections and sepsis. Previously, it was also shown that several PUFAs and EFAs such as LA and ALA have anti-microbial actions [99–104]. This suggests that leukocytes, macrophages, T cells, NK cells and other immunocytes including endothelial cells may release EFAs, PUFAs, lipoxins, resolvins, protectins and maresins on exposure to microorganisms and tumor cells to inactive the microbes and kill tumor cells, respectively. The ability of EFAs and PUFAs and their metabolites to selectively induce apoptosis of tumor cells is particularly interesting since the incidence of cancer increases with age.

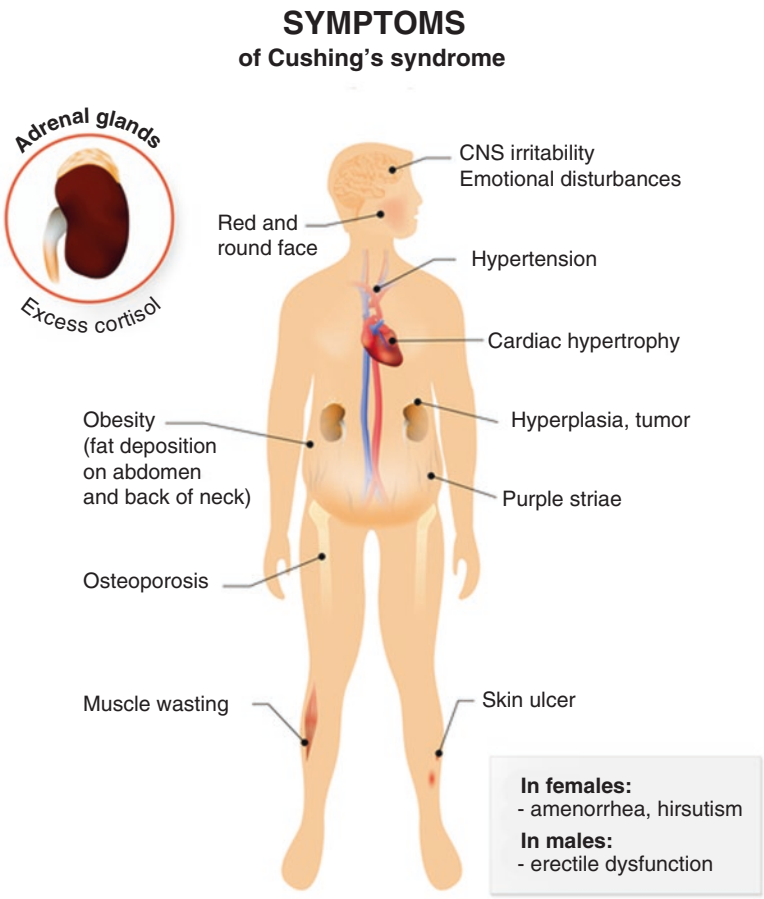


Fig. 3.11 The various symptoms of Cushing's disease are shown. Most of these can occur as a result of an EFA/PUFA deficient state. The development of hypertension, type 2 diabetes mellitus features, obesity, osteoporosis, cardiac hypertrophy, depression and irritability, and erective dysfunction, may all occur due to EFA/PUFA deficiency [3, 4, 6, 7, 10, 11, 13, 32, 33, 63, 64, 66–68, 70, 71]

8 Bioactive Lipids in the Immune Response and Cancer

Antigen-presenting cells (APCs) present antigen on their class II MHC molecules (MHC2s). Helper T cells recognize these, with the help of their expression of CD4 co-receptor (CD4+). The activation of the helper T cell causes it to release cytokines and other stimulatory signals that stimulate the activity of macrophages, killer T cells and B cells. The stimulation of B cells and macrophages drives the proliferation of T helper cells. The activated T cells, B cells and macrophages produce various BALs including PGE2, LTs, LXA4, resolvins, protectins and maresins, ROS, NO and cytokines that ultimately either eliminate the invading microorganisms,

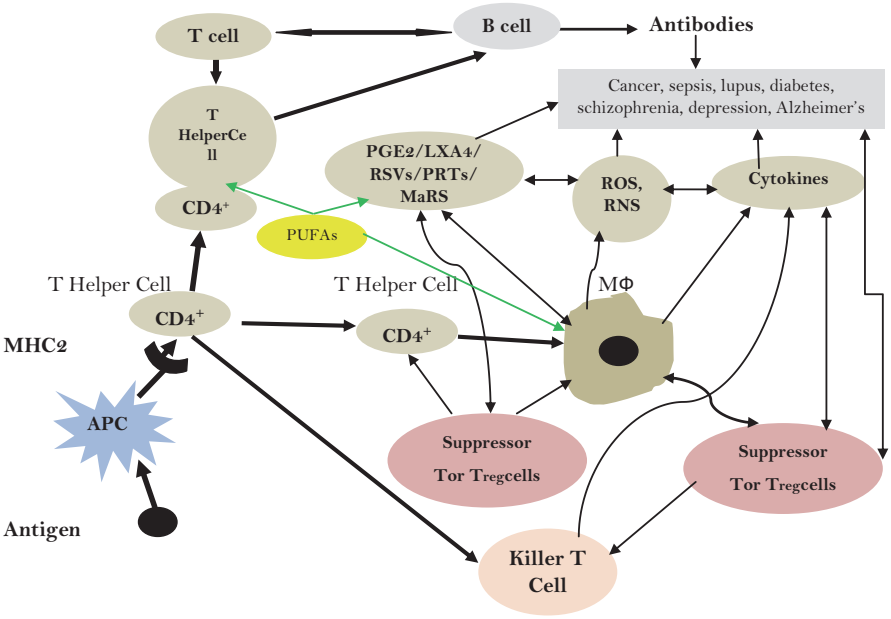


Fig. 3.12 Scheme showing interactions of various T and B cells and macrophages and their association with various diseases. The possible role of PUFAs in these events is outlined. PUFAs and their metabolites PGE2, LXA4, resolvins (RSVs), protectins (PRTs), and maresins (MaRs) may activate/inhibit macrophages and other immunocytes depending on the type of metabolite formed, as well as the context, concentration and duration of exposure to the target

intracellular pathogens and/or cause autoimmune diseases depending on the regulation or inappropriate function of T suppressor cells. This is an over-simplification of the events that occur when the immunocytes are exposed to various antigens. The actual interactions are much more complex compared to what has been described in Fig. 3.12.

Whenever there is tissue injury due to endogenous or exogenous agents, close interactions occur among various immunocytes and macrophages and their products and growth factors (including cytokines) that is modified by BALs, as shown in Fig. 3.13. The importance of the immune system is evident when its optimal function is needed to prevent cancer and autoimmune diseases. Thus, immunosurveillance and immunoediting become important in the context of cancer and autoimmune diseases. It is noteworthy that aging is associated with decreased immunosurveillance and increased incidence of cancer. An increase in PGE2 and a decrease in LXA4 (and possibly, that of resolvins, protectins and maresins) levels, defective immunosurveillance due to an increase in exhausted $CD8^+$ T cells that show increased expression of Tim-3 (T-cell immunoglobulin mucin domain-3, an exhaustion marker) on aged T cells, especially CD8(+) T cells, and increased expression of inhibitory receptors, such as programmed cell death protein 1 (PD-1), in the T cells of aged subjects may explain the decreased immunosurveillance seen with aging [105–108].

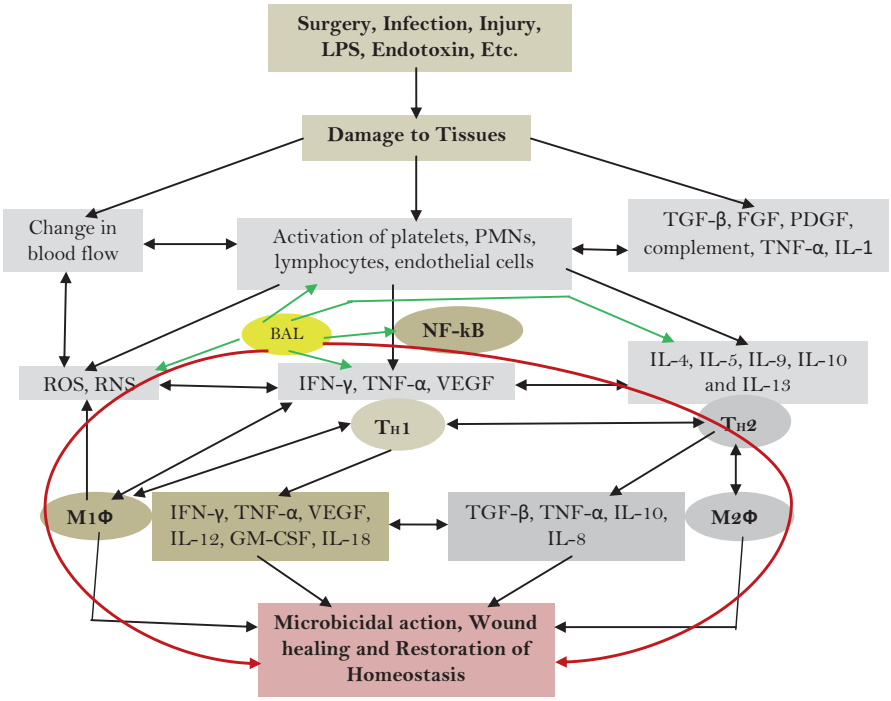


Fig. 3.13 Scheme showing interactions among various immunocytes and macrophages and their products and growth factors (including cytokines) in response to both endogenous and exogenous stimuli and insults. Most of these events could be modified by BALs. The modulatory actions of BALs on various events depicted in the figure include their ability to influence TH1 and TH2 cells, macrophages, NF-kB and the capacity of T cells and macrophages to secrete their respective cytokines or other soluble mediators. Thus, BALs may have both positive and negative influences on various immunocytes and their actions

In this context it is noteworthy that PGE2 plays a critical role in the development of TH17 cells and impair CTL function in co-ordination with PD-1. PGE2 is a pro-inflammatory molecule but is also a potent immunosuppressor [13, 109–121]. The immunosuppressive action of PGE2 may be responsible for the immunosuppression seen in cancer and its ability to limit the functions of NK cells, CD4 and CTLs [122, 123]. PGE2 induces the generation of IL-10, Treg cells and myeloid-derived suppressor cells and suppresses the proliferation and cytotoxicity of CTLs and their ability to produce IFN-γ [11, 110, 124–126]. In view of these immunosuppressive actions of PGE2, it is likely that increased production of PGE2 and a simultaneous decrease in LXA4, resolvins, protectins and maresins generation seen with aging may be responsible for the increase in the incidence of infections, persistence of infections, inflammatory events and high incidence of cancer in aged subjects (Figs. 3.14 and 3.15). Cancer may be considered as a non-resolving/non-healing wound that could be due to increased production of PGE2 and decreased levels of LXA4 [127–145]. Hence, the increased production of PGE2 by tumor cells and

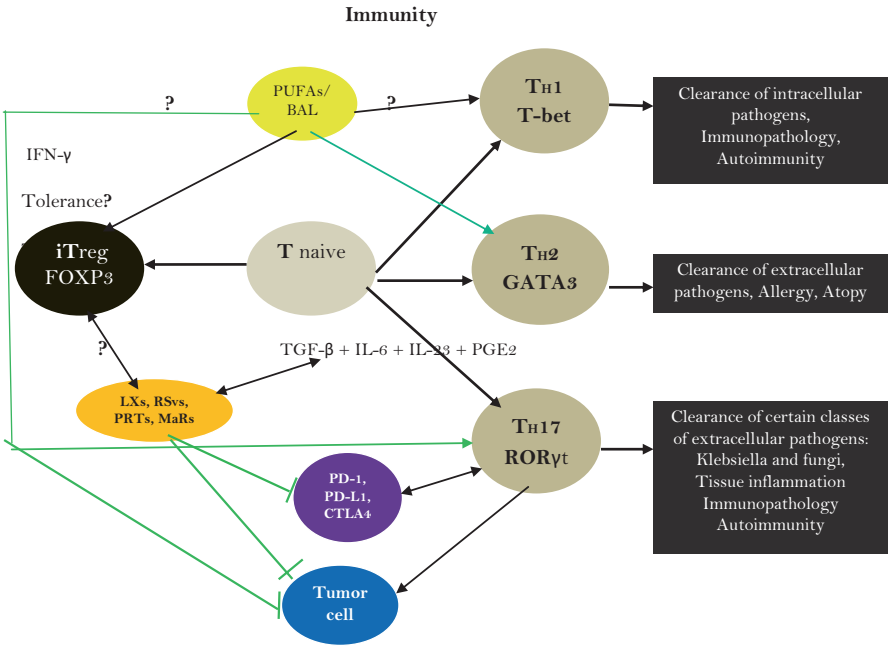


Fig. 3.14 Factors controlling formation of different subsets of T helper cells. LXs lipoxins, RSvs resolvins, PRTs protectins, MaRs maresins. Naive CD41T cells differentiate into subsets of T helper cells: TH1, TH2 and TH17. TGF-β, converts naive T cells into FOXP3-expressing induced Treg (iTreg) cells. Each T helper cell differentiation programme needs specific transcription factors as master regulators (T-bet, GATA3 and ROR-γt). Terminally differentiated T helper cells produce specific combinations of effector cytokines that bring about specific and distinct effector functions of the adaptive immune system. TGF-β, retinoic acid or cytokines (IL-6, IL-1, IL-23 or IL-27) provided by cells of the innate immune system (immature or activated dendritic cells (DCs), respectively) dictate whether a naive T cell develops into a FOXP31 Treg cell, a TH17cell or otherwise. Prostaglandin E2 (PGE2), through its receptor EP4 on T cells and dendritic cells, facilitates TH1 cell differentiation and amplifies IL-23–mediated TH17 cell expansion and EP4-selective antagonists decrease accumulation of both TH1 and TH17 cells and suppress progression of autoimmune encephalomyelitis or contact hypersensitivity in experimental animals. Although the role of PUFAs and their various metabolites is not discussed in detail, it is known that GLA, AA, EPA, DHA, lipoxins, resolvins, protectins, maresins and prostaglandins, leukotrienes and thromboxanes can influence macrophage and other immunocytes' phagocytosis, motility and ability to alter ROS generation and the final outcome of the inflammation and immune response

infiltrating macrophages will enable tumor cells to avoid immune surveillance, enhance their proliferation, augment tumor angiogenesis and ultimately enable them to grow faster and also metastasize. Furthermore, PGE2 is an inhibitor of TNF-α and IL-6 production [146–153], and also that of IFN-γ [153], which are pro-inflammatory molecules and known to possess tumoricidal actions. This is yet another action of PGE2 that help tumor cells to avoid immune surveillance. In addition, PGE2 modulates NO generation [154] and NO, in turn, alters PGE2 synthesis [154–163]. PGE2 enhances IL-10 production [164, 165], which is an anti-inflammatory cytokine.

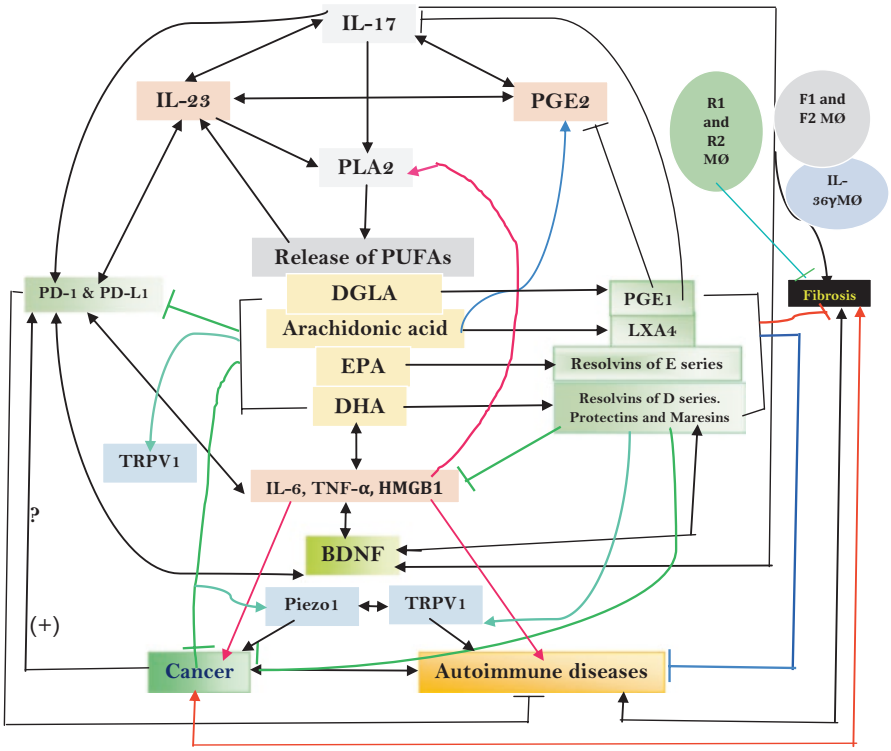


Fig. 3.15 Scheme showing potential relationship and interactions among cytokines, bioactive lipids, BDNF and PD-1 and PD-L1 and their potential role in cancer and autoimmune diseases. IL-17, IL-23 and PGE2 act together to induce a pro-inflammatory status in autoimmune diseases. Cytokines IL-17, IL-23, IL-6, TNF-α and HMGB1 activate phospholipase A2 to induce the release of PUFAs (especially DGLA, AA, EPA and DHA) that form precursors to PGE1, PGE2/LXA4, resolvins, protectins and maresins as shown in the figure. DGLA, AA, EPA and DHA suppress the production of IL17, IL-23, IL-6, TNF-α and HMGB1 and thus have a negative feedback control on the formation of pro-inflammatory cytokines. IL-17 enhances resistance to PD-1 and PD-L1 blockade. LXA4, resolvins, protectins and maresins inhibit inflammatory processes and thus, are useful in protection against autoimmune diseases such as RA, lupus, inflammatory bowel disease and multiple sclerosis. In addition, LXA4, resolvins, protectins and maresins inhibit proliferation of tumor cells. Similar and more potent anti-cancer action is shown by DGLA, AA, EPA and DHA and these induce apoptosis of various types of tumor cells. PUFAs may also suppress the expression of PD-1 and PD-LI and thus, may assist in overcoming immunosuppression seen in cancer. Furthermore, these PUFAs can act on Piezo1 channel which is capable of mediating mechanoelectrical transduction that, in turn, regulates several crucial cellular processes including cell migration. This action of PUFAs on Piezo1 could be attributed to their ability to change cell membrane fluidity. Similarly, PUFAs can regulate the other ion channel, namely TRPV1. There seems to be an interaction between Piezo1 and TRPV1 channels. Thus, PUFAs by their ability to alter the properties of Piezo1 and TRPV1 channels, can regulate membrane voltage changes which can alter cell adhesion, cell volume, apoptosis and angiogenesis. Since many cancer cells over-express K+, Na+, Ca2+ and Cl- channels, it is likely that incorporation of various PUFAs into the cell membrane can effectively alter these channels leading to changes in their mitotic and other properties. This could be one of the many actions of PUFAs/BALs to result in the arrest of growth of cancer cells and their eventual apoptosis.

Thus, PGE2 has actions on IL-17, TNF-α, IL-6, IFN-γ, Treg cells, CTL and NO, and may mediate the resistance of tumor cells to anti-VEGF therapy through its ability to enhance IL-17 secretion [164–178]. This may ultimately result in tumor cell proliferation, angiogenesis and metastasis (Figs. 3.14 and 3.15). Our studies have revealed that PGE1, PGE2, LTD4, LXA4, resolvins and protectins inhibit growth of IMR-32 cancer cells [179]. These and other studies have led to the suggestion that the balance between various metabolites formed from PUFAs and the cellular content and the surrounding milieu content of various PUFAs, determines the final outcome of whether tumor cells are induced to proliferate or inhibited from further growth. Consistent with this, we and others have noted that GLA, DGLA, AA, EPA and DHA have potent growth inhibitory action on several types of tumor cells both in vitro and in vivo [180–197]. Based on these findings, it is reasonable to propose that altered EFA/PUFAs metabolism can usher in a low-grade systemic inflammatory status, impair the immune surveillance system and thereby lead to higher incidence of cancer, type 2 diabetes mellitus, hypertension, osteoporosis, sarcopenia and accumulation of abdominal fat with advancing age. This implies that aging is an inflammatory condition [47].

9 Cancer and Auto-immune Diseases Are Two Sides of the Same Coin

Both cancer and autoimmune diseases are pro-inflammatory conditions although there are some distinct differences between them. Each autoimmune disease has its own distinct features despite the fact that the underlying mechanism(s) may be similar if not identical. For instance, bones and synovial membranes are predominantly involved in RA (rheumatoid arthritis), skin, blood vessels and kidney (sometimes brain) are involved in lupus and neurons in MS (e.g., multiple sclerosis). It is not known why joint deformities occur in RA but not in lupus, or why renal involvement is common in lupus but not in RA and why only brain and other neurological structures are involved in MS with no involvement of other tissues. This suggests that local inflammatory events are more important than systemic inflammatory changes despite the presence of systemic signs and symptoms such as fever, leukocytosis, loss of appetite, etc., in all of these diseases. On the other hand, in cancer both local and systemic manifestations are not uncommon and sometimes systemic events such as cachexia and immunosuppression are more dominant and can result in morbidity and mortality. But, paradoxically, in both autoimmune diseases and

Fig. 3.15 (continued) Not many studies have been performed on the action of PGs, LTs, TXs, lipoxin A4, resolvins, protectins, and maresins on ion channels, especially on Trpv 1 and Piezo1. However, it is likely that these bioactive lipids can also alter the behavior of various ion channels. For instance, it has been shown that PGE2 activates Ca^{2+} channels. It is likely that other bioactive lipids may also have similar actions on various ion channels and Trpv1 and Piezo1

cancer, inflammation is present. In autoimmune diseases, the local inflammatory events are more dominant as a result of recognition of self as foreign whereas, in cancer the immune system fails to recognize cancer cells as foreign. Despite the failure of recognition of cancer cells as foreign, some amount of inflammation occurs at the site of cancer. Despite these seemingly striking differences between autoimmune diseases and cancer, it is noteworthy that cancer is not uncommon in subjects with autoimmune diseases. With the recent development of immune check point inhibitor (ICI) therapy for cancer, it has been recognized that patients treated with this can develop autoimmune diseases. Thus, both autoimmune diseases and cancer can be considered as two sides of the same coin.

IL-17, IL-6, TNF-α and PGE2, LTs and TXs seem to have a role in the autoimmune diseases RA and lupus. Similarly, there is a significant role for IL-17, IL-6, TNF-α and other pro-inflammatory cytokines and PGE2 in cancer. Thus, these same molecules seem to participate both in cancer and autoimmune diseases suggesting that similar approaches in their management can be attempted. In Table 3.1, similarities and contrasting features between cancer and autoimmune diseases are given. It is evident from this table that some overlapping features can be seen between autoimmune diseases and cancer. In both cancer and autoimmune diseases, increased levels of IL-6, TNF-α and IL-17 are seen although, in autoimmune diseases an increase in the plasma levels of these cytokines is more common whereas in cancer they are predominantly seen at the site of the malignancy. This suggests that autoimmune diseases are predominantly systemic diseases whereas cancer is a more localized disease (at least in the initial stages). However, it needs to be noted that lupus, RA and other autoimmune diseases may start locally in a specific tissue or organ and later spread to the whole organ, system or body. For instance, RA may start in one joint and later may involve several other joints. Similarly, lupus may start as non-specific skin rash, or arthralgia and later show more systemic manifestations. Thus, at the molecular/biochemical level there seem to be a role for the same cytokines in both these diseases. One would expect decreased expression of PD-1 and PD-L1 in autoimmune diseases whereas in cancer their expressions are increased to escape the immune surveillance. It is evident from the details given in Table 3.1 that there are many similarities between autoimmune diseases and cancer, implying that same therapeutic strategies could be useful in the prevention and management of both diseases.

Plasma, synovial fluid and urinary levels of IL-6, TNF-α and IL-17 are increased with low plasma concentrations of anti-inflammatory cytokine IL-10 in those with active RA and lupus [198, 199]. In addition, RA and lupus patients have increased plasma, urinary and synovial fluid levels of PGE2 and TXA2 levels and decreased plasma levels of DGLA, AA, EPA and DHA [200–209]. Recent studies have shown that patients with lupus and RA and other rheumatological (and autoimmune) conditions have low plasma and urinary levels of lipoxin A4 (LXA4) [13, 72, 210–214]. Restoring LXA4 levels and COX-2 activity to normal may resolve arthritis, especially in RA. Blocking COX-2 activity and consequently reducing PGE2 levels seems to perpetuate inflammation in contrast to the expectation that reducing PGE2 levels is needed for resolution of inflammation. Subsequently it was reported that

Table 3.1 Comparison between autoimmune diseases and cancer with regard to their biochemical, immunological and management aspects

Parameter	Auto-immune diseases	Cancer
Systemic inflammation	More common↑↑↑	Less common - expect in late stages↑
Local inflammation	Common↑↑↑	More common than systemic inflammation↑
Systemic manifestations of disease such as loss of appetite, loss of weight, fever, etc.	More common↑↑↑	Less common but seen in late stages of disease↑
Plasma PGE2 levels	↑↑↑	↑
Plasma IL-17	↑↑↑	↑
Plasma IL-6	↑↑↑	↑
Plasma TNF-α	↑↑↑	↑
Plasma LXA4	↓↓	↓local levels at the site of cancer is more common than systemic levels
Plasma PUFAs (especially AA, EPA and DHA)	↓↓↓	↓
Autoantibodies	+++	±
Immunosuppression	Unlikely except when administered immunosuppressive drugs as part of treatment	Common
Self and non-self-recognition: PD-1 and PD-L1 expression	Immune system attacks self-antigens and produces disease. PD1 and PD-L1 expression decreased (abnormal)	Immune surveillance fails. PD-1 and PD-L1 expression is increased. immune check point inhibitor use may lead to the development of autoimmune diseases
Management	Immunosuppressive drugs used include anti-cancer drugs methotrexate, cyclophosphamide, etc.	Most anti-cancer drugs are immunosuppressors

repletion of PGE2 attenuated inflammation by enhancing the formation of LXA4, a lipoxygenase metabolite formed from AA, implying that PGE2 may actually trigger initiation of the inflammation resolution process. These results also indicate that there is a close relationship between COX-LOX pathways and PGE2 has a negative feedback control on the inflammation process. This is supported by the observation that inhibition of 15-PGDH that results in a two-fold increase in PGE2 levels in several tissues such as bone marrow, colon, and liver, gives a response to partial hepatectomy with a greater than two-fold increase in hepatocyte proliferation and are resistance to chemical-induced colitis. 15-PGDH inhibition also accelerated recovery of erythropoiesis after bone marrow transplantation [91] suggesting that this enzyme, and possibly PGE2, may have a regulatory role in regeneration and

repair in several tissues including bone marrow, colon and liver. It is possible that 15-PGDH inhibition and the consequent increase in PGE2 levels may induce increased formation of LXA4, possibly by redirecting AA metabolism towards LXA4 formation. These results raise the interesting possibility that depending on the context, PGE2 may have both pro- and anti-inflammatory actions. Based on these findings, it is proposed that enhanced levels of PGE2 may serve as a signal for redirecting AA metabolism towards increased formation of LXA4. Thus, both PGE2 and LXA4, derived from AA, seem to be critical not only in resolving inflammation but also by enhancing tissue regeneration. In this context, it is important to note that oral supplementation of AA does not affect PGE2 levels but enhances LXA4 formation [87, 88]. We observed that oral supplementation of AA suppresses inflammation by inhibiting the formation of IL-6, TNF-α and the expression of NF-kB [32, 33]. The anti-inflammatory cytokines IL-4 and IL-10 seem to trigger the conversion of AA, EPA and DHA to lipoxins, resolvins, protectins and maresins, suggesting a mechanism by which they are able to suppress inflammation [72, 215].

Both in autoimmune diseases and cancer, an increase in IL-17 levels have been described [216–224]. It is noteworthy that IL-17 not only promoted lung cancer growth but also contributed to the resistance to PD-1 blockade and promoted inflammation, factors that worsen prognosis of cancer [224]. IL-17 interacts with PGE2, IL-23, IL-6, TNF-α and the immune check point inhibitors (PD-1 and PD-L1) and, thereby, may facilitate tumor cell growth.

Thus, there are many overlapping features between autoimmune diseases (especially RA and lupus) and cancer implying that both could be managed by same, if not identical, therapeutic strategies. In view of these observations, it is tempting to propose that oral or intravenous administration of AA, EPA, DHA, GLA, DGLA, vitamin C, B1, B6, B12 in conjunction with immunosuppressive drugs such as corticosteroids and cyclophosphamide, methotrexate, and cyclosporine may be effective against RA, lupus and cancer. Both PUFAs and vitamin C may serve as antioxidants with regard to autoimmune diseases and as pro-oxidants in cancer to eliminate tumor cells as shown in Fig. 3.16. The big question is why the same compounds, BALs, vitamin C and anti-cancer drugs such as cyclophosphamide, methotrexate, and cyclosporine when given together serve as pro-oxidants in tumor cells but as antioxidants in normal cells. This could be attributed to the differences in antioxidant defences of the cells. When normal cells are exposed to vitamin C and BALs, the pro-oxidant action of these compounds stimulates their antioxidant defences, whereas tumor cells fail to do so since they have a defective antioxidant system. Thus, normal cells are able to protect themselves whereas tumor cells fail to do so and undergo apoptosis. Taken together, these findings support the idea that the same regimen of administering vitamin C and BALs with or without immunosuppressive drugs would be useful in cancer and lupus and RA.

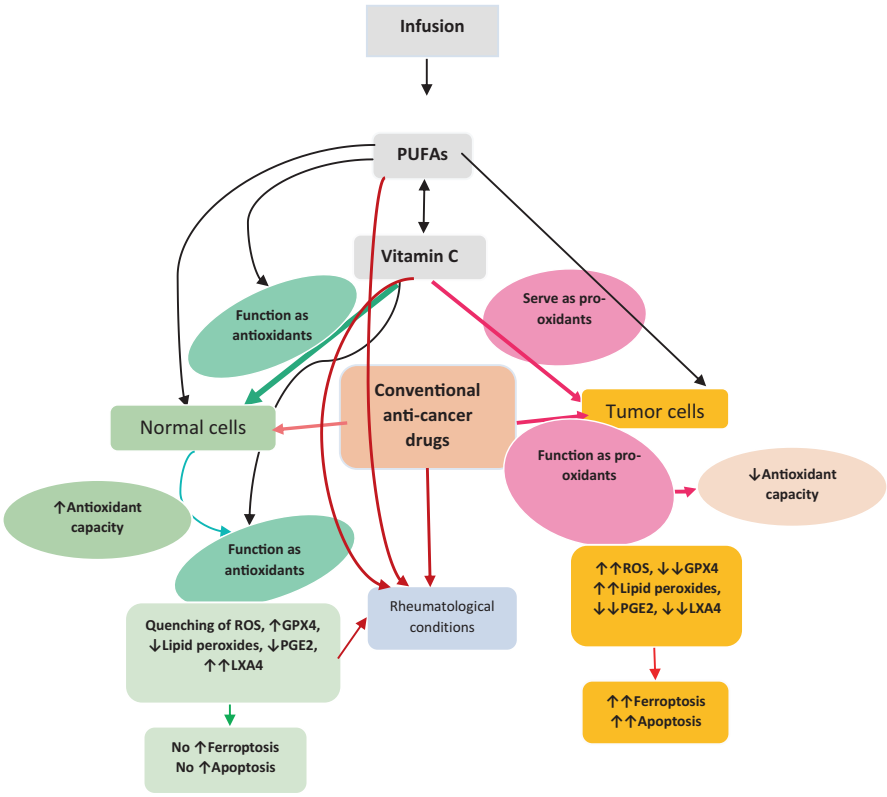


Fig. 3.16 An overview of the actions of PUFAs, vitamin C and conventional anti-cancer drugs on ROS generation, GPX4 activity and accumulation of lipid peroxides and ferroptosis/apoptosis in normal and tumor cells and their possible role in rheumatological conditions. When normal cells and tumor cells are exposed to chemotherapy and radiation there will be increased generation of free radicals (ROS), accumulation of lipid peroxides and decreases in the activity of the potent endogenous antioxidant GPX4. This leads to death (apoptosis and ferroptosis) of both normal and tumor cells. However, when vitamin C and PUFAs are administered to normal cells, they function as antioxidants and so quench ROS and prevent accumulation of lipid peroxides and protect the cells. In the case of tumor cells, both vitamin C and PUFAs act in conjunction with chemotherapy and radiation to generate more ROS and enhance accumulation of toxic lipid peroxides and decrease GPX4 that ultimately leads to their apoptosis and ferroptosis. This causes the elimination of the cancer cells. Our studies have shown that these differential actions of vitamin C and PUFAs in normal and tumor cells are a result of changes in the synthesis of PGE2 and LXA4 as shown in the figure. Vitamin C, PUFAs/BALs and immunosuppressive drugs are given in rheumatological conditions, which may lead to elimination of diseased cells and protection of normal cells and increase the generation of LXA4/resolvins/protectins/maresins and decrease in PGE2/LTs/TXs. This can result in the remission of diseases such as RA and lupus

10 Ion Channels and Bioactive Lipids

Another action of BALs that is relevant to their tumoricidal activities is their ability to modulate the properties of ion channels by altering cell membrane fluidity when they are incorporated into the membrane as shown in Fig. 3.17. PUFAs can modify the properties of the TRPV group of transient receptor potential family of ion channels and Piezo1 and Piezo2 channels. There is a close interaction between Piezo channels and TRPV ion channels. It is possible that this property of PUFAs (and possibly for various PGs, LTs, TXs, LXA4, resolvins, protectins and maresins including lipid peroxides) on various ion channels may explain many of the BAL actions including their role in inflammation, resolution of inflammation, immune response, fibrosis, tissue regeneration, epithelial to mesenchymal transition, and induction of apoptosis, ferroptosis and necrosis of tumor cells.

Depending on the type and amount of fatty acids in the cell membrane, the membrane can be fluid or rigid. The nature of the cell membrane determines the expression and function of various membrane receptors. If the cell membrane is fluid, due to the incorporation of higher amounts of PUFAs, the number of receptors, such as the insulin receptor, will be higher. In contrast, if the membrane is rigid due to higher amounts of saturated fatty acids the number of insulin receptors will decrease. The cell membrane also contains several ion channel voltage-gated ion channels (VGIC) that allow the diffusion of ions such as K^+, Ca^{2+}, Cl^-, Na^+. These ion channels control rapid bioelectrical signaling including action potentials and/or contraction, cell mitotic signaling, cell cycle progression, as well as cell volume regulation. Thus, they play a critical role in cancer cell proliferation. In addition to the VGIC, there are two other ion channels namely TrpV1 and Piezo1.

Phosphatidylserine (PS) is a phospholipid and an important constituent of the cell membrane. It plays a key role in cell cycle signaling including the apoptosis pathway. PS consists of two fatty acids attached via an ester linkage to the first and second carbon (C) of glycerol and serine attached through a phosphodiester linkage to the third carbon of the glycerol. Most phospholipids have a saturated fatty acid on C-1 and an unsaturated fatty acid on the C-2 position of the glycerol backbone. The fatty acid distribution at the C-1 and C-2 positions of glycerol within phospholipids is continually in flux, owing to its continuous degradation and remodeling. PS carries a net charge of −1 at physiological pH. PS mostly has palmitic or stearic acid on C-1 and a long chain unsaturated fatty acid (such as 18:2, 20:4 and 22:6) on C-2. However, this composition of PS is amenable to alteration depending on the diet, supplementation, state of the cell, environment and stimuli to which the cell is exposed.

TrpV1 is a member of the TRPV group of transient receptor potential family of ion channels. The function of TRPV1 is detection and regulation of body temperature and provision of a sensation of scalding heat and pain. Piezo1 and Piezo2 are nonselective Ca^{2+}-permeable cation channels that interact with Trpv1 [225]. Changes in the cell membrane lipid composition leads to alterations in the activities of all of these channels which, in turn, can affect cell proliferation, volume and

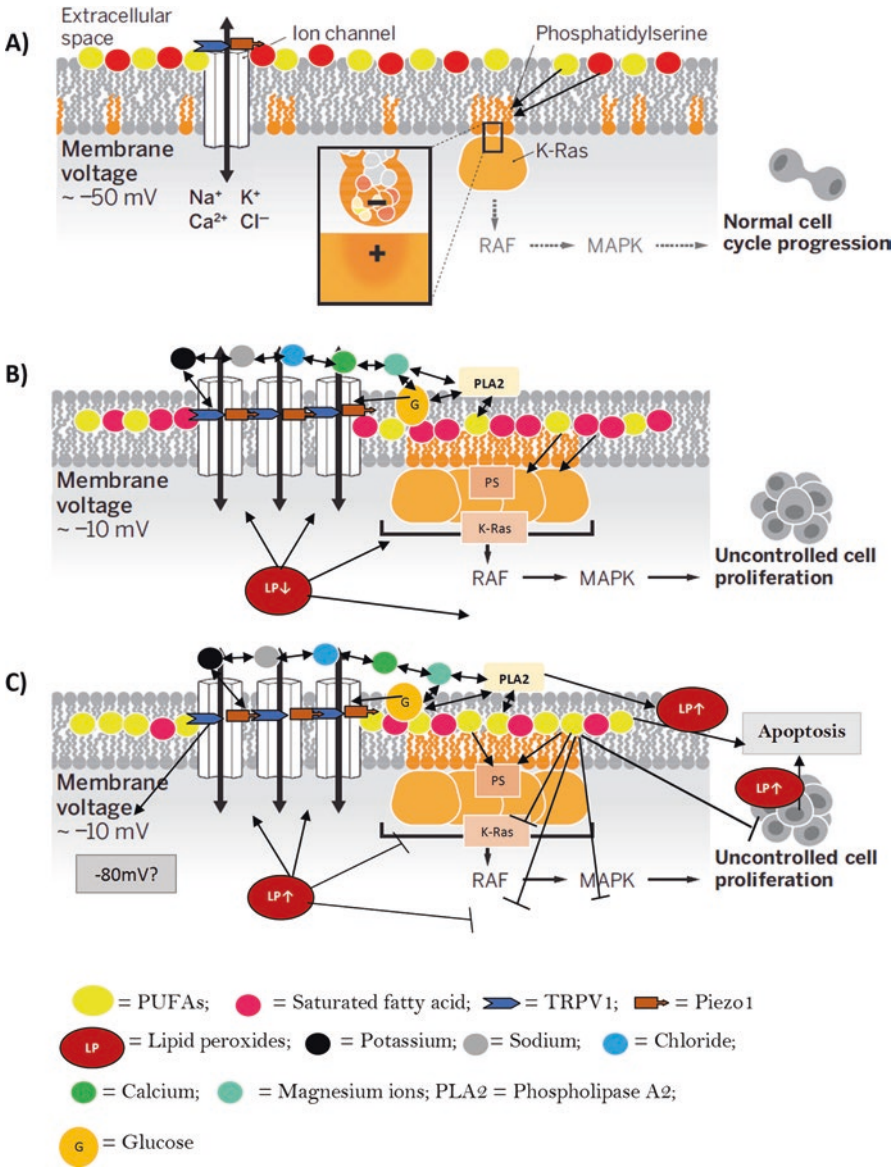


Fig. 3.17 Scheme showing possible relationship among ion channels, fatty acids and cell proliferation or apoptosis. (Modified from Accardi [227])

motility and, thus, metastasis in cancer. Plasma membrane depolarization can induce reorganization of PS and phosphatidylinositol 4,5-bisphosphate that can lead to amplification of K-Ras–dependent mitogen-activated protein kinase (MAPK) signaling. In contrast, plasma membrane repolarization disrupts K-Ras

nanoclustering and inhibits MAPK signaling. Thus, changes in cell membrane composition can induce changes in VGIC, TrpV1 and Piezo1, which can either enhance or suppress cellular mitosis or cause apoptosis [226, 227]. It is envisaged that under normal physiological conditions, the cell membrane will contain a balanced ratio between saturated fatty acids and PUFAs (Fig. 3.17) resulting in PS appearing in small clusters that localize to K-Ras and so low activation of RAF-MAPK pathway occurs. Cancer cells contain more saturated fatty acids and lower amounts of polyunsaturated fatty acids that results in an increase in the rigidity of the cell membrane leading to clustering of PS and K-Ras such that promotion of RAF-MAPK signaling occurs. This leads to uncontrolled proliferationof cancer cells. When tumor cells are supplemented with PUFAs, the cell membrane becomes more fluid and accumulation of excess of toxic lipid peroxides occurs, which results in disruption of PS and K-Ras clustering and its inactivation which results in their mitotic arrest and apoptosis. Changes in cell membrane fluidity and composition can affect PS composition, and changes in the expression and function of various ion channels including Trpv1 and Piezo1 as shown in Fig. 3.17. In turn, this results in perturbation of ion transmission across the channels and the membrane leading to cell apoptosis. Lipid peroxides that accumulate in the cell as a result of PUFA supplementation may inactivate various ion channel receptors, block K-Ras and the MAPK pathway or suppress it. Furthermore, changes in lipid composition of the cell membrane can also alter T cell proliferation, activation, and local response of T cells to the tumor cells [228].

It is possible that K^+ and other ions leak from the cancer cells into the surrounding milieu and act on infiltrating macrophages, T cells and suppress the immune response and, thus, aid in the escape of tumor cells from immune surveillance system [229]. AA and other PUFAs activate potassium channels [230, 231] and thereby enhance the T cell responses by removing excess potassium from the tumor cell milieu. In addition, K_{ATP} channels are inactivated by high glucose concentrations [232] that may explain why tumor cells have aerobic glycolysis. GABA (gamma-aminobutyric acid) inhibits K_{ATP} channels [232] and therefore neurons and local nerves may regulate tumor growth [233, 234]. Cancer cells form synaptic connections with neurons facilitated by cell adhesion proteins neurexins and neuroligins [235]. Through these synaptic connections neurotransmitters such as glutamate may be released that bind and activate AMPA and NMDA receptors that facilitates positively charged ions to enter the cells through the receptors to cause depolarization leading to a rise in intracellular positive charge. As a result, cancer cell migration and proliferation may occur [229–234]. Potassium leakage from cells activates Ca^{2+}-independent phospholipase A2, which enhances cleavage of pro-IL-1β by the IL-1 converting enzyme capsase-1 [236, 237]. This action of potassium on the IL-1 converting enzyme can be prevented by other monovalent cations such as sodium. High intracellular concentrations of potassium suppress apoptosis [238]. Thus, higher potassium concentration seen in the tumor microenvironment may suppress the immune response [229] such that immunosuppression against tumor cells may persist for a longer time. In addition, it will also lead to apoptosis of T cells since the concentration of potassium is higher in the tumor microenvironment compared to

intracellular levels of T cells. Furthermore, phospholipase A2 induces the release of PUFAs from the cell membrane lipid pool and PUFAs activate potassium channels [230, 231]. Thus, there is a close interaction of local and intracellular concentrations of $Ca^{2+}$and other ions such as K^+, Ca^{2+}, Cl^-, Na^+; phospholipase A2 activity, IL-1 and possibly other cytokines, glutamate, GABA and other neurotransmitters, with tumor growth. Thus, the tumor milieu, including the intra- and extracellular glucose concentration, contributes to tumor cell proliferation. Furthermore, there could be a close interaction among various ions within themselves and with intracellular and extracellular glucose concentrations. Glucose can activate or suppress the activity of phospholipase A2 depending upon its local concentration and thereby influence lipid peroxide formation.

11 Connecting the Cell Membrane to the Nucleus

All of the stimuli to which the cell gets exposed need to be transmitted to the respective genes to elicit an adequate and appropriate cellular response. How this occurs is not precisely known. One possibility is that membrane fluidity can influence the structure and composition of intermediate filaments and their multiple binding partners to regulate both cellular mechanics and gene(s) expression. The intermediate filaments, actin and microtubules form distinct cytoskeletal systems, and are critical in the dynamic interplay between these networks. Intermediate filaments provide structural support for the cells, and play a major role in cellular responses to external mechanical forces. It is known that tensional force-induced reinforcement of actin stress fibers requires the interaction of the RhoA-targeting Rho-guanine nucleotide exchange factors Solo/ARHGEF40 with keratin intermediate filaments to activate RhoA signaling, which promotes stress fiber formation and keratin network organization. These results illustrate the importance of keratins to enable cells to adapt to mechanical stress [239]. The interaction of desmoplakin with keratin filaments at desmosomes supports intercellular force transmission, traction force generation, and cell stiffness that ultimately alters the expression of several genes concerned with mitosis and apoptosis [240–242].

2-methoxy-oestradiol (2-ME), PUFAs, thalidomide, TNF, ILs and many anticancer drugs, radiation and protoporphyrin derivatives (used in photodynamic therapy) enhance free radical generation and augment the lipid peroxidation process. This leads to accumulation of lipid peroxides in the cells, resulting in apoptosis of tumor cells [243–250]. PUFAs are cytotoxic to tumor cells, possess anti-angiogenic action and enhance free radical generation in the tumor cells and regress the growth of human gliomas with few side-effects [180–188, 245–252]. It is likely that PUFAs and lipid peroxides alter or disrupt the intermediate filaments, actin and microtubules and cytoskeletal filament systems partly by altering cell membrane fluidity and to some extent by their direct action on the cytoskeleton.

12 Polyunsaturated Fatty Acids and Bioactive Lipids Are Involved in Mitochondrial Processes

Dietary or supplementation of PUFAs are absorbed from the gut and then distributed to cells where they enrich various cellular membranes. This influences not only cell metabolic processes and survival but also modulates mitochondrial processes [253, 254]. In addition, n-3 PUFAs protect ischemic myocardium [255] especially against oxidative-induced damage due to their ability to modulate mitochondrial ROS production [256].

These results are supported by the observation that fat-1 transgenic mice which synthesize n-3 fatty acids at the cost of AA showed a decrease in ROS production from electron transport complex (ETC)-I suggesting that EPA and DHA are able to reduce oxidative stress in the mammary tissue when exposed to the carcinogen 7,12-dimethyl benz(α)anthracene (DMBA) [257].

In contrast, tumor cells exposed to PUFAs were found to produce enhanced amounts of ROS and accumulation of toxic lipid peroxides leading to apoptosis in a caspase-dependent manner, involving both the intrinsic and extrinsic pathways [179, 258–262].

On the other hand, we observed that GLA, AA, EPA and DHA can protect pancreatic β cells against alloxan and streptozotocin-induced cytotoxicity and prevent the development of both type 1 and type 2 diabetes mellitus in experimental animals by suppressing free radical generation, and of NF-kB, IL-6 and TNF-α [32, 33].

Based on this data, it can be suggested that PUFAs seem to be metabolized differently by normal and tumor cells such that normal cells are protected and tumor cells get exposed to increased oxidative stress [183]. This differential action and metabolism of PUFAs by normal and tumor cells implies that PUFAs can be employed to selectively eliminate tumor cells and may also be useful to prevent diabetes mellitus [5, 11, 32, 33, 183, 246–250, 263–285]. This cytoprotective action of PUFAs is possibly mediated by their products: PGE_1, lipoxins, resolvins, protectins and maresins [11, 263, 266, 267]. Thus, the beneficial actions of PUFAs can be ascribed to their products such as PGE_1, PGI_2, lipoxins, resolvins, protectins and maresins and their ability to enhance NO and alter the expression of NF-kB, IkB, caspases, cytochrome C, Ras, Myc, Fos, Fas, p53, COX-2, and LOX, and by alteration of telomerase activity (Figs. 3.7, 3.10 and 3.16).

13 Bioactive Lipids Modulate G-Protein-Mediated Signals

BALs modulate G-protein–mediated signal transduction [286] and mobilize Ca^{2+} from intracellular stores [287]. This can induce apoptosis [288] especially of tumor cells, activate PKC and augment NADPH oxidase activity in macrophages [289], which can result in enhanced $O_2^{-\cdot}$ generation. GLA, AA, EPA and DHA decreased Bcl-2 and increased Bax in tumor cells [33, 290] in addition to their action on p53

[291]. DHA has been reported to enhance p27, inhibit cyclin-associated kinase, reduce pRb phosphorylation and induce apoptosis of melanoma cells [291]. BALs such as PUFAs inhibit cell division by blocking translation initiation [292]. PUFAs were found to induce free radicals in tumor cells that can directly activate heterodimeric G_i and G_0 (small G proteins) [293], which are critical signaling molecules. Thus, BALs such as PUFAs and their metabolites have actions that are detrimental to the survival of tumor cells.

14 Age-Related Disorders Are Inflammatory Conditions and Can Be Modulated by Bioactive Lipids

With advancing age, there is a tendency to accumulate abdominal fat, decrease in muscle and skeletal mass (osteoporosis), with development of insulin resistance, type 2 diabetes mellitus, hypertension, and an increase in the incidence of cancer, CHD and atherosclerosis, Alzheimer's disease and depression, and increased chances of having disc prolapse, osteoarthritis, and tendon tears. These are all inflammatory conditions. There is reasonable evidence to suggest that in all these conditions, there is a critical role for BALs (Fig. 3.2). Based on the preceding discussion it is evident that efforts directed to restore the altered BAL abnormalities to normality could be of benefit in all these disorders. The various actions of BALs as outlined above, such as the ability to alter cell membrane fluidity, influence ion channels, act on G protein coupled receptors, regulate inflammation, immune response and stem cell biology, telomerase activity, mitochondrial processes, cytoskeletal system and participate in resolution of inflammation and wound healing, are some of the crucial actions that are relevant to their involvement in these age-related disorders. Hence, analyzing the plasma and tissue concentrations of various BALs in these conditions may give clues as to the type of abnormalities that need to be corrected. In general, it is likely that the plasma tissue concentrations of GLA, DGLA, AA, EPA, DPA, DHA, lipoxins, resolvins, protectins and maresins and anti-inflammatory cytokines are likely to be low and accompanied by a deficiency of NO, H_2S, CO and an increase in pro-inflammatory PGs, LTs, TXs and cytokines with a concomitant decrease in antioxidants. Although it is unlikely that all of these molecules will be abnormal in these disorders, measuring all of them together may give clues to the specific alterations in their concentrations so that the underlying pathophysiology could be deciphered to plan relevant interventions.

15 Conclusions and Future Perspectives

One of the questions that should be answered is how BALs could have a role in many conditions. It should be mentioned here that it is the local actions of BALs that make them suitable candidates for a critical role in these conditions. Thus, it is

suggested that abnormalities in the BAL system in vascular endothelial cells may lead to hypertension, in the pancreatic β cells to diabetes mellitus, in adipose tissue to obesity, in the skeletal muscles to sarcopenia, in the osteoclasts and osteoblasts to osteoporosis, in the coronary vascular endothelial cells to atherosclerosis, in neuronal cells to Alzheimer's disease and depression, in the intervertebral disc to prolapsed, herniated, or extruded intervertebral disc (PIVD) and in specific cells to relevant cancers. If this proposal is true, it implies that administration of various PUFAs and/or lipoxins, resolvins, protectins and maresins in appropriate amounts and in a timely manner will lead to relief from these age-related disorders. Since lipoxins, resolvins, protectins and maresins are highly unstable and have short half-lives, they may not be suitable for clinical use. I propose that oral or intravenous administration of GLA, DGLA, AA, EPA, DPA, DHA and various co-factors such as vitamins B1, B6, B12, and C, zinc, magnesium, and folic acid should be provided to achieve their beneficial actions (Fig. 3.2). It should be noted that it may be necessary to administer other co-factors to optimize the synthesis and action of other relevant endogenous molecules such as NO. This could include provision of L-arginine, tetrahydrobiopterin and other minerals and trace elements to obtain the much-needed beneficial actions. By providing all these precursors, it is presumed that cells and tissues will utilize these raw materials to form the much needed and relevant BALs, NO, H_2S, CO and anti-inflammatory cytokines to boost the antioxidant defences. Since all these above-mentioned factors are endogenous to natural substances in our bodies, it is anticipated that their administration is unlikely to have any side effects. In some conditions such as tendon tears and PIVD, perhaps it is relevant to administer BALs locally, either using a transdermal approach or via local injections. For patients with cancer, BALs could be administered in conjunction with conventional anti-cancer drugs and immune check point inhibitors, as proposed previously [61, 62]. In our preliminary study, it was noted that administration of PUFAs along with conventional chemotherapeutic drugs can induce remission with few side effects, and reverse drug resistance to standard chemotherapy. In fact, it was noticed that co-administration of BALs and high doses of vitamin C blunted the side effects of chemotherapeutic drugs and induced full remission in one of our stage IV drug-resistant Hodgkin's disease patients. We observed that BALs can be administered with corticosteroids and other immunosuppressive drugs in cases of lupus and RA, to induce full remission in some cases with no recurrence of the disease. Some of these patients have been on follow up for more than 10 years and are still in full remission despite stoppage of all drugs. These preliminary results are encouraging and are in support of the proposals made here. Obviously, more thorough and in-depth studies are needed to bring BAL-based therapeutics to the mainstream, but are certainly encouraging. Based on these results, it is tempting to suggest that prophylactic administration of various PUFAs and their co-factors may aid in the prevention, postponement or delay of the aging process itself. In this context, it is noteworthy that exercise and calorie restriction, the two interventions that are known to delay aging, also modulate EFA/PUFA metabolism [47].

In general, it is believed that stem cells are essential for healing and regeneration of tissues and thus have a critical role in recovery from various diseases. In this

context, it is interesting to note that stem cells seem to bring about their beneficial actions by secreting LXA4 [294], which appears to regulate stem cell proliferation and differentiation [295]. Therefore, it is proposed that BALs may serve as the mediators of the beneficial actions of stem cells. Perhaps, a combination of stem cells and BALs may form a new therapeutic approach to several disorders associated with aging.

References

1. Le Gall JY, Ardaillou R (2009) The biology of aging. Bull Acad Natl Med 193:365–402
2. Poorani R, Bhatt AN, Dwarakanath BS, Das UN (2016) COX-2, aspirin and metabolism of arachidonic, eicosapentaenoic and docosahexaenoic acids and their physiological and clinical significance. Eur J Pharmacol 785:116–132
3. Das UN (1985) Minerals, trace elements and vitamins interact with essential fatty acids and prostaglandins to prevent hypertension, thrombosis, hypercholesterolemia and atherosclerosis and their attendant complications. IRCS J Med Sci 13:684–687
4. Das UN (1987) Magnesium, essential fatty acids and cardiovascular diseases. J Assoc Physicians India 35:171
5. Das UN, Ramadevi G, Rao KP, Rao MS (1989) Prostaglandins can modify gamma-radiation and chemical induced cytotoxicity and genetic damage in vitro and in vivo. Prostaglandins 38:689–716
6. Das UN (1989) Nutrients, essential fatty acids and prostaglandins interact to augment immune responses and prevent genetic damage and cancer. Nutrition 5:106–110
7. Das UN (2000) Interaction(s) between nutrients, essential fatty acids, eicosanoids, free radicals, nitric oxide, anti-oxidants and endothelium and their relationship to human essential hypertension. Med Sci Res 28:75–83
8. Das UN (2006) Essential fatty acids: biochemistry, physiology, and pathology. Biotechnol J 1:420–439
9. Das UN (2006) Biological significance of essential fatty acids. J Assoc Physicians India 54:309–319
10. Das UN (2008) Essential fatty acids and their metabolites could function as endogenous HMG-CoA reductase and ACE enzyme inhibitors, anti-arrhythmic, anti-hypertensive, anti-atherosclerotic, anti-inflammatory, cytoprotective, and cardioprotective molecules. Lipids Health Dis 7:37. https://doi.org/10.1186/1476-511X-7-37
11. Das UN (2011) Molecular basis of health and disease. Springer, New York. ISBN-10: 9400704941
12. Das UN (1991) Interaction(s) between essential fatty acids, eicosanoids, cytokines, growth factors and free radicals: relevance to new therapeutic strategies in rheumatoid arthritis and other collagen vascular diseases. Prostaglandins Leukot Essent Fatty Acids 44:201–210
13. Kumar GS, Das UN (1994) Effect of prostaglandins and their precursors on the proliferation of human lymphocytes and their secretion of tumor necrosis factor and various interleukins. Prostaglandins Leukot Essent Fatty Acids 50:331–334
14. Rotondo D, Earl CR, Laing KJ, Kaimakamis D (1994) Inhibition of cytokine-stimulated thymic lymphocyte proliferation by fatty acids: the role of eicosanoids. Biochim Biophys Acta 1223:185–194
15. Santoli D, Zurier RB (1989) Prostaglandin E precursor fatty acids inhibit human IL-2 production by a prostaglandin E-independent mechanism. J Immunol 143:1303–1309
16. Miles EA, Allen E, Calder PC (2002) In vitro effects of eicosanoids derived from different 20-carbon fatty acids on production of monocyte-derived cytokines in human whole blood cultures. Cytokine 20:215–223

17. Khalfoun B, Thibault F, Watier H, Bardos P, Lebranchu Y (1997) Docosahexaenoic and eicosapentaenoic acids inhibit in vitro human endothelial cell production of interleukin-6. Adv Exp Med Biol 400B:589–597
18. Czeslick EG, Simm A, Grond S, Silber RE, Sablotzki A (2003) Inhibition of intracellular tumour necrosis factor (TNF)-alpha and interleukin (IL)-6 production in human monocytes by iloprost. Eur J Clin Investig 33:1013–1017
19. Chen X, Wu S, Chen C, Xie B, Fang Z, Hu W et al (2017) Omega-3 polyunsaturated fatty acid supplementation attenuates microglial-induced inflammation by inhibiting the HMGB1/TLR4/NF-κB pathway following experimental traumatic brain injury. J Neuroinflammation 14:143. https://doi.org/10.1186/s12974-017-0917-3
20. Chen X, Chen C, Fan S, Wu S, Yang F, Fang Z et al (2018) Omega-3 polyunsaturated fatty acid attenuates the inflammatory response by modulating microglia polarization through SIRT1-mediated deacetylation of the HMGB1/NF-κB pathway following experimental traumatic brain injury. J Neuroinflammation 15:116. https://doi.org/10.1186/s12974-018-1151-3
21. Chang CS, Sun HL, Lii CK, Chen HW, Chen PY, Liu KL (2010) Gamma-linolenic acid inhibits inflammatory responses by regulating NF-kappaB and AP-1 activation in lipopolysaccharide-induced RAW 264.7 macrophages. Inflammation 33:46–57
22. Dooper MM, van Riel B, Graus YM, M'Rabet L (2003) Dihomo-gamma-linolenic acid inhibits tumour necrosis factor-alpha production by human leucocytes independently of cyclooxygenase activity. Immunology 110:348–357
23. Das UN (2010) Current and emerging strategies for the treatment and management of systemic lupus erythematosus based on molecular signatures of acute and chronic inflammation. J Inflammation Res 3:143–170
24. Menezes-de-Lima O Jr, Kassuya CA, Nascimento AF, Md H, Calixto JB (2006) Lipoxin A4 inhibits acute edema in mice: implications for the anti-edematogenic mechanism induced by aspirin. Prostaglandins Other Lipid Mediat 80:123–135
25. Benabdoun HA, Kulbay M, Rondon EP, Vallières F, Shi Q, Fernandes J et al (2019) In vitro and in vivo assessment of the proresolutive and antiresorptive actions of resolvin D1: relevance to arthritis. Arthritis Res Ther 21:72
26. Herrera BS, Ohira T, Gao L, Omori K, Yang R, Zhu M et al (2008) An endogenous regulator of inflammation, resolvin E1, modulates osteoclast differentiation and bone resorption. Br J Pharmacol 155:1214–1223
27. Wu L, Miao S, Zou LB, Wu P, Hao H, Tang K et al (2012) Lipoxin A4 inhibits 5-lipoxygenase translocation and leukotrienes biosynthesis to exert a neuroprotective effect in cerebral ischemia/reperfusion injury. J Mol Neurosci 48:185–200
28. Lee TH, Lympany P, Crea AE, Spur BW (1991) Inhibition of leukotriene B4-induced neutrophil migration by lipoxin A4: structure-function relationships. Biochem Biophys Res Commun 180:1416–1421
29. McMahon B, Mitchell D, Shattock R, Martin F, Brady HR, Godson C (2002) Lipoxin, leukotriene, and PDGF receptors cross-talk to regulate mesangial cell proliferation. FASEB J 16:1817–1819
30. Hudert CA, Weylandt KH, Lu Y, Wang J, Hong S, Dignass A et al (2006) Transgenic mice rich in endogenous omega-3 fatty acids are protected from colitis. Proc Natl Acad Sci U S A 103:11276–11281
31. Serhan CN, Dalli J, Karamnov S, Choi A, Park CK, Xu ZZ et al (2012) Macrophage proresolving mediator maresin 1 stimulates tissue regeneration and controls pain. FASEB J 26:1755–1765
32. Naveen KVG, Naidu VGM, Das UN (2017) Arachidonic acid and lipoxin A4 attenuate alloxan-induced cytotoxicity to RIN5F cells in vitro and type 1 diabetes mellitus in vivo. Biofactors 43:251–271
33. Naveen KVG, Naidu VGM, Das UN (2017) Arachidonic acid and lipoxin A4 attenuate streptozotocin-induced cytotoxicity to RIN5F cells in vitro and type 1 and type 2 diabetes mellitus in vivo. Nutrition 35:61–80

34. Das UN, Ells G, Begin ME, Horrobin DF (1986) Free radicals as possible mediators of the actions of interferon. J Free Rad Biol Med 2:183–188
35. Das UN, Padma M, Sangeetha P, Ramesh G, Koratkar R (1990) Stimulation of free radical generation in human leukocytes by various stimulants including tumor necrosis factor is a calmodulin dependent process. Biochem Biophys Res Commun 167:1030–1036
36. Tsujimoto M, Yokota S, Vilcek J, Weissman G (1986) Tumor necrosis factor provokes superoxide anion generation from neutrophils. Biochem Biophys Res Commun 137:1094–1100
37. Beton G, Zeni L, Casaatella MA, Rossi F (1986) Gamma-interferon is able to enhance the oxidative metabolism of human neutrophils. Biochem Biophys Res Commun 138:1276–1282
38. Das UN, Huang YS, Begin ME, Horrobin DF (1986) Interferons, phospholipid metabolism, immune responses and cancer. IRCS Med Sci 14:1069–1074
39. Bordoni A, Hrelia S, Lorenzini A, Bergami R, Cabrini L, Biagi PL et al (1998) Dual influence of aging and vitamin B6 deficiency on delta-6-desaturation of essential fatty acids in rat liver microsomes. Prostaglandins Leukot Essent Fatty Acids 58:417–420
40. Bordoni A, Biagi PL, Turchetto E, Hrelia S (1988) Aging influence on delta-6-desaturase activity and fatty acid composition of rat liver microsomes. Biochem Int 17:1001–1009
41. Biagi PL, Bordoni A, Hrelia S, Celadon M, Horrobin DF (1991) Gamma-linolenic acid dietary supplementation can reverse the aging influence on rat liver microsome delta 6-desaturase activity. Biochim Biophys Acta 1083:187–192
42. Lopez Jimenez JA, Bordoni A, Lorenzini A, Rossi CA, Biagi PL, Hrelia S (1997) Linoleic acid metabolism in primary cultures of adult rat cardiomyocytes is impaired by aging. Biochem Biophys Res Commun 237:142–145
43. Lorenzini A, Bordoni A, Spanò C, Turchetto E, Biagi PL, Hrelia S (1997) Age-related changes in essential fatty acid metabolism in cultured rat heart myocytes. Prostaglandins Leukot Essent Fatty Acids 57:143–147
44. Bourre JM, Piciotti M, Dumont O (1990) Delta 6 desaturase in brain and liver during development and aging. Lipids 25:354–356
45. Horrobin DF (1981) Loss of delta-6-desaturase activity as a key factor in aging. Med Hypotheses 7:1211–1220
46. Das UN (2007) A defect in the activity of Delta6 and Delta5 desaturases may be a factor in the initiation and progression of atherosclerosis. Prostaglandins Leukot Essent Fatty Acids 76:251–268
47. Das UN (2018) Ageing: is there a role for arachidonic acid and other bioactive lipids? A review. J Adv Res 11:67–79
48. Shim JH (2019) Prostaglandin E2 induces skin aging via E-prostanoid 1 in normal human dermal fibroblasts. Int J Mol Sci 20(22). pii: E5555. https://doi.org/10.3390/ijms20225555
49. Young MK, Bocek RM, Herrington PT, Beatty CH (1981) Ageing: effects on the prostaglandin production by skeletal muscle of male rhesus monkeys (Macaca mulatta). Mech Ageing Dev 16:345–353
50. Fraifeld V, Kaplanski J, Kukulansky T, Globerson A (1995) Increased prostaglandin E2 production by concanavalin A-stimulated splenocytes of old mice. Gerontology 41:129–133
51. Hayek MG, Meydani SN, Meydani M, Blumberg JB (1994) Age differences in eicosanoid production of mouse splenocytes: effects on mitogen-induced T-cell proliferation. J Gerontol 49:B197–B207
52. Wu D, Mura C, Beharka AA, Han SN, Paulson KE, Hwang D et al (1998) Age-associated increase in PGE2 synthesis and COX activity in murine macrophages is reversed by vitamin E. Am J Phys 275:C661–C668
53. Baek BS, Kim JW, Lee JH, Kwon HJ, Kim ND, Kang HS et al (2001) Age-related increase of brain cyclooxygenase activity and dietary modulation of oxidative status. J Gerontol A Biol Sci Med Sci 56:B426–B431
54. Gangemi S, Pescara L, D'Urbano E, Basile G, Nicita-Mauro V, Davì G et al (2005) Aging is characterized by a profound reduction in anti-inflammatory lipoxin A4 levels. Exp Gerontol 40:612–614

55. Das UN (2020) Molecular pathobiology of scleritis and its therapeutic implications. Int J Ophthalmol 13(1):163–175
56. Das UN (2019) Beneficial role of bioactive lipids in the pathobiology, prevention, and management of HBV, HCV and alcoholic hepatitis, NAFLD, and liver cirrhosis: a review. J Adv Res 17:17–29
57. Das UN (2019) Polyunsaturated fatty acids and sepsis. Nutrition 65:39–43
58. Das UN (2019) Bioactive lipids in intervertebral disc (IVD) degeneration and its therapeutic implications. BioSci Rep 39(10). pii: BSR20192117. https://doi.org/10.1042/BSR20192117
59. Das UN (2019) Bioactive lipids in shoulder tendon tears. Am J Pathol 189:2149–2153
60. Dakin SG, Colas RA, Wheway K, Watkins B, Appleton L, Rees J et al (2019) Proresolving mediators LXB_4 and RvE1 regulate inflammation in stromal cells from patients with shoulder tendon tears. Am J Pathol 189:2258–2268
61. Das UN (2019) Can bioactive lipid(s) augment anti-cancer action of immunotherapy and prevent cytokine storm? Arch Med Res 50:342–349
62. Das UN (2020) Bioactive lipids as modulators of immune check point inhibitors. Med Hypotheses 135:109473. https://doi.org/10.1016/j.mehy.2019.109473
63. Das UN (2018) Arachidonic acid in health and disease with focus on hypertension and diabetes mellitus. J Adv Res 11:43–55
64. Das UN (2010) Essential fatty acids and their metabolites in the context of hypertension. Hypertens Res 33:782–785
65. Inoue K, Kishida K, Hirata A, Funahashi T, Shimomura I (2013) Low serum eicosapentaenoic acid/arachidonic acid ratio in male subjects with visceral obesity. Nutr Metab (Lond) 10:25. https://doi.org/10.1186/1743-7075-10-25
66. Yagi S, Aihara K, Fukuda D, Takashima A, Bando M, Hara T et al (2015) Reduced ratio of eicosapentaenoic acid and docosahexaenoic acid to arachidonic acid is associated with early onset of acute coronary syndrome. Nutr J 14:111. https://doi.org/10.1186/s12937-015-0102-4
67. Yagi S, Hara T, Ueno R, Aihara K, Fukuda D, Takashima A et al (2014) Serum concentration of eicosapentaenoic acid is associated with cognitive function in patients with coronary artery disease. Nutr J 13:112. https://doi.org/10.1186/1475-2891-13-112
68. Das UN (2013) Arachidonic acid and lipoxin A4 as possible endogenous anti-diabetic molecules. Prostaglandins Leukot Essent Fatty Acids 88:201–210
69. Das UN (2007) Vagus nerve stimulation, depression and inflammation. Neuropsychopharmacology 32:2053–2054
70. Das UN (2017) Is there a role for bioactive lipids in the pathobiology of diabetes mellitus? Front Endocrinol (Lausanne) 8:182. https://doi.org/10.3389/fendo.2017.00182
71. Börgeson E, McGillicuddy FC, Harford KA, Corrigan N, Higgins DF et al (2012) Lipoxin A4 attenuates adipose inflammation. FASEB J 26:4287–4294
72. Das UN (2011) Lipoxins as biomarkers of lupus and other inflammatory conditions. Lipids Health Dis 10:76. https://doi.org/10.1186/1476-511X-10-76
73. Das UN (2016) Renin-angiotensin-aldosterone system in insulin resistance and metabolic syndrome. J Transl Int Med 4:66–72
74. Kain V, Ingle KA, Colas RA, Dalli J, Prabhu SD, Serhan CN et al (2015) Resolvin D1 activates the inflammation resolving response at splenic and ventricular site following myocardial infarction leading to improved ventricular function. J Mol Cell Cardiol 84:24–35
75. Mai J, Liu W, Fang Y, Zhang S, Qiu Q, Yang Y et al (2018) The atheroprotective role of lipoxin A_4 prevents oxLDL-induced apoptotic signaling in macrophages via JNK pathway. Atherosclerosis 278:259–268
76. Kain V, Liu F, Kozlovskaya V, Ingle KA, Bolisetty S, Agarwal A et al (2017) Resolution agonist 15-epi-lipoxin A_4 programs early activation of resolving phase in post-myocardial infarction healing. Sci Rep 7:9999. https://doi.org/10.1038/s41598-017-10441-8
77. Schnittert J, Heinrich MA, Kuninty PR, Storm G, Prakash J (2018) Reprogramming tumor stroma using an endogenous lipid lipoxin A4 to treat pancreatic cancer. Cancer Lett 420:247–258

78. Simões RL, De-Brito NM, Cunha-Costa H, Morandi V, Fierro IM, Roitt IM et al (2017) Lipoxin A_4 selectively programs the profile of M2 tumor-associated macrophages which favour control of tumor progression. Int J Cancer 140:346–357
79. Wang Z, Cheng Q, Tang K, Sun Y, Zhang K, Zhang Y et al (2015) Lipid mediator lipoxin A4 inhibits tumor growth by targeting IL-10-producing regulatory B (Breg) cells. Cancer Lett 364:118–124
80. Xu F, Zhou X, Hao J, Dai H, Zhang J, He Y et al (2018) Lipoxin A_4 and its analog suppress hepatocarcinoma cell epithelial-mesenchymal transition, migration and metastasis via regulating integrin-linked kinase axis. Prostaglandins Other Lipid Mediat 137:9–19
81. Liu C, Guan H, Cai C, Li F, Xiao J (2017) Lipoxin A4 suppresses osteoclastogenesis in RAW264.7 cells and prevents ovariectomy-induced bone loss. Exp Cell Res 352:293–303
82. Banu J, Bhattacharya A, Rahman M, Kang JX, Fernandes G (2010) Endogenously produced n-3 fatty acids protect against ovariectomy induced bone loss in fat-1 transgenic mice. J Bone Miner Metab 28:617–626
83. Sun D, Krishnan A, Zaman K, Lawrence R, Bhattacharya A, Fernandes G (2003) Dietary n-3 fatty acids decrease osteoclastogenesis and loss of bone mass in ovariectomized mice. J Bone Miner Res 18:1206–1216
84. Wei J, Chen S, Guo W, Feng B, Yang S, Huang C, Chu J (2018) Leukotriene D4 induces cellular senescence in osteoblasts. Int Immunopharmacol 58:154–159
85. Bhattacharya A, Rahman M, Banu J, Lawrence RA, McGuff HS, Garrett IR et al (2005) Inhibition of osteoporosis in autoimmune disease prone MRL/Mpj-Fas(lpr) mice by N-3 fatty acids. J Am Coll Nutr 24:200–209
86. Bhavsar PK, Levy BD, Hew MJ, Pfeffer MA, Kazani S, Israel E et al (2010) Corticosteroid suppression of lipoxin A4 and leukotriene B4 from alveolar macrophages in severe asthma. Respir Res 11:71. https://doi.org/10.1186/1465-9921-11-71
87. Tateishi N, Kakutani S, Kawashima H, Shibata H, Morita I (2014) Dietary supplementation of arachidonic acid increases arachidonic acid and lipoxin A_4 contents in colon but does not affect severity or prostaglandin E_2 content in murine colitis model. Lipids Health Dis 13:30. https://doi.org/10.1186/1476-511X-13-30
88. Tateishi N, Kaneda Y, Kakutani S, Kawashima H, Shibata H, Morita I (2015) Dietary supplementation with arachidonic acid increases arachidonic acid content in paw, but does not affect arthritis severity or prostaglandin E2 content in rat adjuvant-induced arthritis model. Lipids Health Dis 14:3. https://doi.org/10.1186/1476-511X-14-3
89. Das UN (2019) Circulating microparticles in septic shock and sepsis-related complications. Minerva Anestesiol. (in press)
90. Dakin SG, Ly L, Colas RA, Oppermann U, Wheway K, Watkins B et al (2017) Increased 15-PGDH expression leads to dysregulated resolution responses in stromal cells from patients with chronic tendinopathy. Sci Rep 7:11009. https://doi.org/10.1038/s41598-017-11188-y
91. Zhang Y, Desai A, Yang SY, Bae KB, Antczak MI, Fink SP et al (2015) Inhibition of the prostaglandin-degrading enzyme 15-PGDH potentiates tissue regeneration. Science 348:aaa2340. https://doi.org/10.1126/science.aaa2340
92. FitzGerald GA (2015) Bringing PGE2 in from the cold. Science 348:1208–1209
93. Duffin R, O'Connor RA, Crittenden S, Forster T, Yu C, Zheng X et al (2016) Prostaglandin E2 constrains systemic inflammation through an innate lymphoid cell–IL-22 axis. Science 351:1333–1338
94. Ueda T, Fukunaga K, Seki H, Miyata J, Arita M, Miyasho T et al (2014) Combination therapy of 15-epi-lipoxin A4 with antibiotics protects mice from Escherichia coli-induced sepsis. Crit Care Med 42:e288–e295
95. Walker J, Dichter E, Lacorte G, Kerner D, Spur B, Rodriguez A et al (2011) Lipoxin a4 increases survival by decreasing systemic inflammation and bacterial load in sepsis. Shock 36:410–416
96. Wu B, Walker J, Spur B, Rodriguez A, Yin K (2015) Effects of Lipoxin A4 on antimicrobial actions of neutrophils in sepsis. Prostaglandins Leukot Essent Fatty Acids 94:55–64

97. Wu B, Capilato J, Pham MP, Walker J, Spur B, Rodriguez A et al (2016) Lipoxin A4 augments host defense in sepsis and reduces Pseudomonas aeruginosa virulence through quorum sensing inhibition. FASEB J 30:2400–2410
98. Spite M, Norling LV, Summers L, Yang R, Cooper D, Petasis NA et al (2009) Resolvin D2 is a potent regulator of leukocytes and controls microbial sepsis. Nature 461:1287–1291
99. Desbois AP, Lawlor KC (2013) Antibacterial activity of long-chain polyunsaturated fatty acids against Propionibacterium acnes and Staphylococcus aureus. Mar Drugs 11:4544–4557
100. Das UN (2018) Arachidonic acid and other unsaturated fatty acids and some of their metabolites function as endogenous antimicrobial molecules: a review. J Adv Res 11:57–66
101. Zheng CJ, Yoo JS, Lee TG, Cho HY, Kim YH, Kim WG (2005) Fatty acid synthesis is a target for antibacterial activity of unsaturated fatty acids. FEBS Lett 579:5157–5162
102. Le PNT, Desbois AP (2017) Antibacterial effect of eicosapentaenoic acid against Bacillus cereus and Staphylococcus aureus: killing kinetics, selection for resistance, and potential cellular target. Mar Drugs 15: pii: E334. https://doi.org/10.3390/md15110334
103. Giamarellos-Bourboulis EJ, Grecka P, Dionyssiou-Asteriou A, Giamarellou H (1998) In vitro activity of polyunsaturated fatty acids on Pseudomonas aeruginosa: relationship to lipid peroxidation. Prostaglandins Leukot Essent Fatty Acids 58:283–287
104. Das UN (1985) Antibiotic-like action of essential fatty acids. Can Med Assoc J 132:1350
105. Lee KA, Shin KS, Kim GY, Song YC, Bae EA, Kim IK et al (2016) Characterization of age-associated exhausted $CD8^+$ T cells defined by increased expression of Tim-3 and PD-1. Aging Cell 15:291–300
106. Lages CS, Lewkowich I, Sproles A, Wills-Karp M, Chougnet C (2010) Partial restoration of T-cell function in aged mice by in vitro blockade of the PD-1/PD-L1 pathway. Aging Cell 9:785–798
107. Shimada Y, Hayashi M, Nagasaka Y, Ohno-Iwashita Y, Inomata M (2009) Age-associated up-regulation of a negative co-stimulatory receptor PD-1 in mouse CD4+ T cells. Exp Gerontol 44:517–522
108. Fukushima Y, Minato N, Hattori M (2018) The impact of senescence-associated T cells on immunosenescence and age-related disorders. Inflamm Regen 38:24. https://doi.org/10.1186/s41232-018-0082-9
109. Harris SG, Padilla J, Koumas L, Ray D, Phipps RP (2002) Prostaglandins as modulators of immunity. Trends Immunol 23:144–150
110. Kalinski P (2012) Regulation of immune responses by prostaglandin e2. J Immunol 188:21–28
111. Das UN, Padma MC (1978) Prostaglandins in lymphocyte transformation. J Assoc Physicians India 26:503–506
112. Das UN (1980) Prostaglandins and immune response in cancer. Int J Tiss Reac 2:233–236
113. Das UN (1981) Inhibition of sensitized lymphocyte response to sperm antigen(s) by prostaglandins. IRCS Med Sci 9:1087
114. Kumar GS, Das UN, Kumar KV, Madhavi DNP, Tan BKH (1992) Effect of n-6 and n-3 fatty acids on the proliferation and secretion of TNF and IL-2 by human lymphocytes in vitro. Nutr Res 12:815–823
115. Das UN (2014) HLA-DR expression, cytokines and bioactive lipids in sepsis. Arch Med Sci 10:325–335
116. Narumiya S (2007) Physiology and pathophysiology of prostanoid receptors. Proc Jpn Acad Ser B 83:296–319
117. Goodwin JS, Ceuppens J (1983) Regulation of the immune response by prostaglandins. J Clin Immunol 3:295–315
118. Betz M, Fox BS (1991) Prostaglandin E2 inhibits production of TH1 lymphokines but not of Th2 lymphokines. J Immunol 146:108–113
119. Gold KN, Weyand CM, Goronzy JJ (1994) Modulation of helper T cell function by prostaglandins. Arthritis Rheum 37:925–933
120. Hilkens CM, Vermeulen H, van Neerven RJ, Snijdewint FG, Wierenga EA, Kapsenberg ML (1995) Differential modulation of T helper type 1 (TH1) and T helper type 2 (TH2) cytokine secretion by prostaglandin E2 critically depends on interleukin-2. Eur J Immunol 25:59–63

121. Yao C, Sakata D, Esaki Y, Li Y, Matsuoka T, Kuroiwa K et al (2009) Prostaglandin E2–EP4 signaling promotes immune inflammation through TH1 cell differentiation and TH17 cell expansion. Nat Med 15:633–640
122. Linnemeyer PA, Pollack SB (1993) Prostaglandin E2-induced changes in the phenotype, morphology, and lytic activity of IL-2-activated natural killer cells. J Immunol 150:3747–3754
123. Sreeramkumar V, Fresno M, Cuesta N (2012) Prostaglandin E_2 and T cells: friends or foes? Immunol Cell Biol 90:579–586
124. Strassmann G, Patil-Koota V, Finkelman F, Fong M, Kambayashi T (1994) Evidence for the involvement of interleukin 10 in the differential deactivation of murine peritoneal macrophages by prostaglandin E2. J Exp Med 180:2365–2370
125. Demeure CE, Yang LP, Desjardins C, Raynauld P, Delespesse G (1997) Prostaglandin E2 primes naive T cells for the production of anti-inflammatory cytokines. Eur J Immunol 27:3526–3531
126. Gabrilovich DI, Nagaraj S (2009) Myeloid-derived suppressor cells as regulators of the immune system. Nat Rev Immunol 9:162–174
127. Owen K, Gomolka D, Droller MJ (1980) Production of prostaglandin E2 by tumor cells in vitro. Cancer Res 40:3167–3171
128. Young MR, Knies S (1984) Prostaglandin E production by Lewis lung carcinoma: mechanism for tumor establishment in vivo. J Natl Cancer Inst 72:919–922
129. Balch CM, Dougherty PA, Cloud GA, Tilden AB (1984) Prostaglandin E2-mediated suppression of cellular immunity in colon cancer patients. Surgery 95:71–77
130. Murray JL, Kollmorgen GM (1983) Inhibition of lymphocyte response by prostaglandin-producing suppressor cells in patients with melanoma. J Clin Immunol 3:268–276
131. Passwell J, Levanon M, Davidsohn J, Ramot B (1983) Monocyte PGE2 secretion in Hodgkin's disease and its relation to decreased cellular immunity. Clin Exp Immunol 51:61–68
132. Chiabrando C, Broggini M, Castagnoli MN, Donelli MG, Noseda A, Visintainer M et al (1985) Prostaglandin and thromboxane synthesis by Lewis lung carcinoma during growth. Cancer Res 45:3605–3608
133. McLemore TL, Hubbard WC, Litterst CL, Liu MC, Miller S, McMahon NA et al (1988) Profiles of prostaglandin biosynthesis in normal lung and tumor tissue from lung cancer patients. Cancer Res 48:3140–3247
134. Fulton AM (1988) Inhibition of experimental metastasis with indomethacin: role of macrophages and natural killer cells. Prostaglandins 35:413–425
135. Maxwell WJ, Kelleher D, Keating JJ, Hogan FP, Bloomfield FJ, MacDonald GS et al (1990) Enhanced secretion of prostaglandin E2 by tissue-fixed macrophages in colonic carcinoma. Digestion 47:160–166
136. Baxevanis CN, Reclos GJ, Gritzapis AD, Dedousis GV, Missitzis I, Papamichail M (1993) Elevated prostaglandin E2 production by monocytes is responsible for the depressed levels of natural killer and lymphokine-activated killer cell function in patients with breast cancer. Cancer 72:491–501
137. Alleva DG, Burger CJ, Elgert KD (1994) Tumor-induced regulation of suppressor macrophage nitric oxide and TNF-alpha production. Role of tumor-derived IL-10, TGF-beta, and prostaglandin E2. J Immunol 153:1674–1686
138. Liu XH, Connolly JM, Rose DP (1996) Eicosanoids as mediators of linoleic acid-stimulated invasion and type IV collagenase production by a metastatic human breast cancer cell line. Clin Exp Metastasis 14:145–152
139. Li S, Xu X, Jiang M, Bi Y, Xu J, Han M (2015) Lipopolysaccharide induces inflammation and facilitates lung metastasis in a breast cancer model via the prostaglandin E2-EP2 pathway. Mol Med Rep 11:4454–4462
140. Kim MJ, Kim HS, Lee SH, Yang Y, Lee MS, Lim JS (2014) NDRG2 controls COX-2/PGE_2-mediated breast cancer cell migration and invasion. Mol Cells 37:759–765
141. Zhang M, Zhang H, Cheng S, Zhang D, Xu Y, Bai X et al (2006) Prostaglandin E2 accelerates invasion by upregulating Snail in hepatocellular carcinoma cells. Tumour Biol 35:7135–7145

142. Han C, Michalopoulos GK, Wu T (2006) Prostaglandin E2 receptor EP1 transactivates EGFR/MET receptor tyrosine kinases and enhances invasiveness in human hepatocellular carcinoma cells. J Cell Physiol 207:261–270
143. Han C, Wu T (2005) Cyclooxygenase-2-derived prostaglandin E2 promotes human cholangiocarcinoma cell growth and invasion through EP1 receptor-mediated activation of the epidermal growth factor receptor and Akt. J Biol Chem 280:24053–24063
144. Xu L, Han C, Wu T (2006) A novel positive feedback loop between peroxisome proliferator-activated receptor-delta and prostaglandin E2 signaling pathways for human cholangiocarcinoma cell growth. J Biol Chem 281:33982–33996
145. Misra UK, Pizzo SV (2013) Evidence for a pro-proliferative feedback loop in prostate cancer: the role of Epac1 and COX-2-dependent pathways. PLoS One 8:e63150. https://doi.org/10.1371/journal.pone
146. Evans DB, Thavarajah M, Kanis JA (1990) Involvement of prostaglandin E2 in the inhibition of osteocalcin synthesis by human osteoblast-like cells in response to cytokines and systemic hormones. Biochem Biophys Res Commun 167:194–202
147. Hori T, Yamanaka Y, Hayakawa M, Shibamoto S, Tsujimoto M, Oku N et al (1991) Prostaglandins antagonize fibroblast proliferation stimulated by tumor necrosis factor. Biochem Biophys Res Commun 174:758–766
148. Kambayashi T, Alexander HR, Fong M, Strassmann G (1995) Potential involvement of IL-10 in suppressing tumor-associated macrophages. Colon-26-derived prostaglandin E2 inhibits TNF-alpha release via a mechanism involving IL-10. J Immunol 154:3383–3390
149. Takigawa M, Takashiba S, Takahashi K, Arai H, Kurihara H, Murayama Y (1994) Prostaglandin E2 inhibits interleukin-6 release but not its transcription in human gingival fibroblasts stimulated with interleukin-1 beta or tumor necrosis factor-alpha. J Periodontol 65:1122–1127
150. Fieren MW, van den Bemd GJ, Ben-Efraim S, Bonta IL (1992) Prostaglandin E2 inhibits the release of tumor necrosis factor-alpha, rather than interleukin 1 beta, from human macrophages. Immunol Lett 31:85–90
151. Vassiliou E, Jing H, Ganea D (2003) Prostaglandin E2 inhibits TNF production in murine bone marrow-derived dendritic cells. Cell Immunol 223:120–132
152. Stafford JB, Marnett LJ (2008) Prostaglandin E2 inhibits tumor necrosis factor-alpha RNA through PKA type I. Biochem Biophys Res Commun 366:104–109
153. Xu XJ, Reichner JS, Mastrofrancesco B, Henry WL Jr, Albina JE (2008) Prostaglandin E2 suppresses lipopolysaccharide-stimulated IFN-beta production. J Immunol 180:2125–2131
154. Huang CN, Liu KL, Cheng CH, Lin YS, Lin MJ, Lin TH (2005) PGE2 enhances cytokine-elicited nitric oxide production in mouse cortical collecting duct cells. Nitric Oxide 12:150–158
155. Gaillard T, Mülsch A, Klein H, Decker K (1992) Regulation by prostaglandin E2 of cytokine-elicited nitric oxide synthesis in rat liver macrophages. Biol Chem Hoppe Seyler 373:897–902
156. Stadler J, Harbrecht BG, Di Silvio M, Curran RD, Jordan ML, Simmons RL et al (1993) Endogenous nitric oxide inhibits the synthesis of cyclooxygenase products and interleukin-6 by rat Kupffer cells. J Leukoc Biol 53:165–172
157. Tetsuka T, Daphna-Iken D, Miller BW, Guan Z, Baier LD, Morrison AR (1996) Nitric oxide amplifies interleukin 1-induced cyclooxygenase-2 expression in rat mesangial cells. J Clin Invest 97:2051–2056
158. Wilson KT, Vaandrager AB, De Vente J, Musch MW, De Jonge HR, Chang EB (1996) Production and localization of cGMP and PGE2 in nitroprusside-stimulated rat colonic ion transport. Am J Phys 270(3 Pt 1):C832–C840
159. Sautebin L, Ialenti A, Ianaro A, Di Rosa M (1995) Endogenous nitric oxide increases prostaglandin biosynthesis in carrageenin rat paw oedema. Eur J Pharmacol 286:219–222
160. Biondi C, Fiorini S, Pavan B, Ferretti ME, Barion P, Vesce F (2003) Interactions between the nitric oxide and prostaglandin E2 biosynthetic pathways in human amnion-like WISH cells. J Reprod Immunol 60:35–52

161. Du Y, Sarthy VP, Kern TS (2004) Interaction between NO and COX pathways in retinal cells exposed to elevated glucose and retina of diabetic rats. Am J Physiol Regul Integr Comp Physiol 287:R735–R741
162. Chien CC, Shen SC, Yang LY, Chen YC (2012) Prostaglandins as negative regulators against lipopolysaccharide, lipoteichoic acid, and peptidoglycan-induced inducible nitric oxide synthase/nitric oxide production through reactive oxygen species-dependent heme oxygenase 1 expression in macrophages. Shock 38:549–558
163. Stæhr M, Hansen PB, Madsen K, Vanhoutte PM, Nüsing RM, Jensen BL (2013) Deletion of cyclooxygenase-2 in the mouse increases arterial blood pressure with no impairment in renal NO production in response to chronic high salt intake. Am J Physiol Regul Integr Comp Physiol 304:R899–R907
164. Harizi H, Norbert G (2004) Inhibition of IL-6, TNF-alpha, and cyclooxygenase-2 protein expression by prostaglandin E2-induced IL-10 in bone marrow-derived dendritic cells. Cell Immunol 228:99–109
165. Harizi H, Juzan M, Pitard V, Moreau JF, Gualde N (2002) Cyclooxygenase-2-issued prostaglandin e(2) enhances the production of endogenous IL-10, which down-regulates dendritic cell functions. J Immunol 168:2255–2263
166. Kanda N, Koike S, Watanabe S (2005) IL-17 suppresses TNF-alpha-induced CCL27 production through induction of COX-2 in human keratinocytes. J Allergy Clin Immunol 116:1144–1150
167. Khayrullina T, Yen JH, Jing H, Ganea D (2008) In vitro differentiation of dendritic cells in the presence of prostaglandin E2 alters the IL-12/IL-23 balance and promotes differentiation of Th17 cells. J Immunol 181:721–735
168. Chen H, Qin J, Wei P, Zhang J, Li Q, Fu L et al (2009) Effects of leukotriene B4 and prostaglandin E2 on the differentiation of murine Foxp3+ T regulatory cells and Th17 cells. Prostaglandins Leukot Essent Fatty Acids 80:195–200
169. Salvemini D, Misko TP, Masferrer JL, Seibert K, Currie MG, Needleman P (1993) Nitric oxide activates cyclooxygenase enzymes. Proc Natl Acad Sci U S A 90:7240–7244
170. Swierkosz TA, Mitchell JA, Warner TD, Botting RM, Vane JR (1995) Co-induction of nitric oxide synthase and cyclo-oxygenase: interactions between nitric oxide and prostanoids. Br J Pharmacol 114:1335–1342
171. Mollace V, Colasanti M, Muscoli C, Lauro GM, Iannone M, Rotiroti D et al (1998) The effect of nitric oxide on cytokine-induced release of PGE2 by human cultured astroglial cells. Br J Pharmacol 124:742–746
172. Marcinkiewicz J (1997) Regulation of cytokine production by eicosanoids and nitric oxide. Arch Immunol Ther Exp 45:163–167
173. Tanaka M, Ishibashi H, Hirata Y, Miki K, Kudo J, Niho Y (1996) Tumor necrosis factor production by rat Kupffer cells-regulation by lipopolysaccharide, macrophage activating factor and prostaglandin E2. J Clin Lab Immunol 48:17–31
174. Liu XH, Kirschenbaum A, Lu M, Yao S, Klausner A, Preston C et al (2002) Prostaglandin E(2) stimulates prostatic intraepithelial neoplasia cell growth through activation of the interleukin-6/GP130/STAT-3 signaling pathway. Biochem Biophys Res Commun 290:249–255
175. Reznikov LL, Kim SH, Westcott JY, Frishman J, Fantuzzi G, Novick D et al (2000) IL-18 binding protein increases spontaneous and IL-1-induced prostaglandin production via inhibition of IFN-gamma. Proc Natl Acad Sci U S A 97:2174–2179
176. Perkins DJ, Kniss DA (1999) Blockade of nitric oxide formation down-regulates cyclooxygenase-2 and decreases PGE2 biosynthesis in macrophages. J Leukoc Biol 65:792–799
177. Sakurai T, Tamura K, Kogo H (2004) Vascular endothelial growth factor increases messenger RNAs encoding cyclooxygenase-II and membrane-associated prostaglandin E synthase in rat luteal cells. J Endocrinol 183:527–533
178. Yao M, Kargman S, Lam EC, Kelly CR, Zheng Y, Luk P et al (2003) Inhibition of cyclooxygenase-2 by rofecoxib attenuates the growth and metastatic potential of colorectal carcinoma in mice. Cancer Res 63:586–592

179. Sailaja P, Mani AM, Naveen KVG, Anasuya DH, Siresha B, Das UN (2014) Effect of polyunsaturated fatty acids and their metabolites on bleomycin-induced cytotoxic action on human neuroblastoma cells in vitro. PLoS One 9:e114766. https://doi.org/10.1371/journal.pone.0114766
180. Booyens J, Englebrecht P, Le Roux S, Louwrens CC, Van der Merwe CF, Katzeff IE (1984) Some effects of the essential fatty acids linoleic acid, alpha-linolenic acid, and of their metabolites gamma-linolenic acid, arachidonic acid, eicosapentaenoic acid, and docosahexaenoic acid and of prostaglandins A and E on the proliferation of human osteogenic sarcoma cells in culture. Prostaglandins Leukot Med 15:15–33
181. Begin ME, Das UN, Ells G, Horrobin DF (1985) Selective killing of human cancer cells by polyunsaturated fatty acids. Prostaglandins Leukot Med 19:177–186
182. Begin ME, Ells G, Das UN, Horrobin DF (1986) Differential killing of human carcinoma cells supplemented with n-3 and n-6 polyunsaturated fatty acids. J Natl Cancer Inst 77:1053–1062
183. Das UN (1991) Tumoricidal action of cis-unsaturated fatty acids and their relationship to free radicals and lipid peroxidation. Cancer Lett 56:235–243
184. Sagar PS, Das UN, Koratkar R, Ramesh G, Padma M, Kumar GS (1992) Cytotoxic action of cis-unsaturated fatty acids on human cervical carcinoma (HeLa) cells: relationship to free radicals and lipid peroxidation and its modulation by calmodulin antagonists. Cancer Lett 63:189–198
185. Kumar GS, Das UN (1995) Free radical-dependent suppression of growth of mouse myeloma cells by α-linolenic and eicosapentaenoic acids in vitro. Cancer Lett 92:27–38
186. Padma M, Das UN (1996) Effect of cis-unsaturated fatty acids on cellular oxidant stress in macrophage tumor (AK-5) cells in vitro. Cancer Lett 109:63–75
187. Seigel I, Liu TL, Yaghoubzadeh E, Kaskey TS, Gleicher N (1987) Cytotoxic effects of free fatty acids on ascites tumor cells. J Natl Cancer Inst 78:271–277
188. Tolnai S, Morgan JF (1962) Studies on the in vitro anti-tumor activity of fatty acids. V. Unsaturated fatty acids. Can J Biochem Physiol 40:869–875
189. Monjazeb AM, High KP, Connoy A, Hart LS, Koumenis C, Chilton FH (2006) Arachidonic acid-induced gene expression in colon cancer cells. Carcinogenesis 27:1950–1960
190. Monjazeb AM, High KP, Koumenis C, Chilton FH (2005) Inhibitors of arachidonic acid metabolism act synergistically to signal apoptosis in neoplastic cells. Prostaglandins Leukot Essent Fatty Acids 73:463–474
191. Canuto RA, Muzio G, Bassi AM, Maggiora M, Leonarduzzi G, Lindahl R et al (1995) Enrichment with arachidonic acid increases the sensitivity of hepatoma cells to the cytotoxic effects of oxidative stress. Free Radic Biol Med 18:287–293
192. Piazzi G, D'Argenio G, Prossomariti A, Lembo V, Mazzone G, Candela M et al (2014) Eicosapentaenoic acid free fatty acid prevents and suppresses colonic neoplasia in colitis-associated colorectal cancer acting on Notch signaling and gut microbiota. Int J Cancer 135:2004–2013
193. Sauer LA, Dauchy RT, Blask DE, Krause JA, Davidson LK, Dauchy EM (2005) Eicosapentaenoic acid suppresses cell proliferation in MCF-7 human breast cancer xenografts in nude rats via a pertussis toxin-sensitive signal transduction pathway. J Nutr 135:2124–2129
194. Gu Z, Wu J, Wang S, Suburu J, Chen H, Thomas MJ et al (2013) Polyunsaturated fatty acids affect the localization and signaling of PIP3/AKT in prostate cancer cells. Carcinogenesis 34:1968–1975
195. Wang S, Wu J, Suburu J, Gu Z, Cai J, Axanova LS et al (2012) Effect of dietary polyunsaturated fatty acids on castration-resistant Pten-null prostate cancer. Carcinogenesis 33:404–412
196. Blanckaert V, Ulmann L, Mimouni V, Antol J, Brancquart L, Chénais B (2010) Docosahexaenoic acid intake decreases proliferation, increases apoptosis and decreases the invasive potential of the human breast carcinoma cell line MDA-MB-231. Int J Oncol 36:737–742

197. Collett ED, Davidson LA, Fan YY, Lupton JR, Chapkin RS (2001) n-6 and n-3 polyunsaturated fatty acids differentially modulate oncogenic Ras activation in colonocytes. Am J Physiol Cell Physiol 280:C1066–C1075
198. Havemose-Poulsen A, Sørensen LK, Stoltze K, Bendtzen K, Holmstrup P (2005) Cytokine profiles in peripheral blood and whole blood cell cultures associated with aggressive periodontitis, juvenile idiopathic arthritis, and rheumatoid arthritis. J Periodontol 76:2276–2285
199. Guimarães PM, Scavuzzi BM, Stadtlober NP, Franchi Santos LFDR, Lozovoy MAB, Iriyoda TMV et al (2017) Cytokines in systemic lupus erythematosus: far beyond Th1/Th2 dualism lupus: cytokine profiles. Immunol Cell Biol 95:824–831
200. Yoshida T, Ichikawa Y, Tojo T, Homma M (1996) Abnormal prostanoid metabolism in lupus nephritis and the effects of a thromboxane A2 synthetase inhibitor, DP-1904. Lupus 5:129–138
201. Navarro E, Esteve M, Olivé A, Klaassen J, Cabré E, Tena X et al (2000) Abnormal fatty acid pattern in rheumatoid arthritis. A rationale for treatment with marine and botanical lipids. J Rheumatol 27:298–303
202. Suryaprabha P, Das UN, Ramesh G, Kumar KV, Kumar GS (1991) Reactive oxygen species, lipid peroxides and essential fatty acids in patients with rheumatoid arthritis and systemic lupus erythematosus. Prostaglandins Leukot Essent Fatty Acids 43:251–255
203. Mohan IK, Das UN (1997) Oxidant stress, anti-oxidants and essential fatty acids in systemic lupus erythematosus. Prostaglandins Leukot Essent Fatty Acids 56:193–198
204. Horrobin DF (1987) Low prevalences of coronary heart disease (CHD), psoriasis, asthma and rheumatoid arthritis in Eskimos: are they caused by high dietary intake of eicosapentaenoic acid (EPA), a genetic variation of essential fatty acid (EFA) metabolism or a combination of both? Med Hypotheses 22:421–428
205. Horrobin DF (1984) Essential fatty acid metabolism in diseases of connective tissue with special reference to scleroderma and to Sjogren's syndrome. Med Hypotheses 14:233–247
206. Laitinen O, Seppalä E, Nissilä M, Vapaatalo H (1983) Plasma levels and urinary excretion of prostaglandins in patients with rheumatoid arthritis. Clin Rheumatol 2:401–406
207. Trang LE, Granström E, Lövgren O (1977) Levels of prostaglandins F2 alpha and E2 and thromboxane B2 in joint fluid in rheumatoid arthritis. Scand J Rheumatol 6:151–154
208. Egg D (1984) Concentrations of prostaglandins D2, E2, F2 alpha, 6-keto-F1 alpha and thromboxane B2 in synovial fluid from patients with inflammatory joint disorders and osteoarthritis. Z Rheumatol 43:89–96
209. Egg D, Günther R, Herold M, Kerschbaumer F (1980) Prostaglandins E2 and F2 alpha concentrations in the synovial fluid in rheumatoid and traumatic knee joint diseases. Z Rheumatol 39:170–175
210. Das UN (2012) Is multiple sclerosis a proresolution deficiency disorder? Nutrition 28:951–958
211. Conte FP, Menezes-de-Lima O Jr, Verri WA Jr, Cunha FQ, Penido C, Henriques MG (2010) Lipoxin A(4) attenuates zymosan-induced arthritis by modulating endothelin-1 and its effects. Br J Pharmacol 161:911–924
212. Chan MM, Moore AR (2010) Resolution of inflammation in murine autoimmune arthritis is disrupted by cyclooxygenase-2 inhibition and restored by prostaglandin E2-mediated lipoxin A4 production. J Immunol 184:6418–6426
213. Hashimoto A, Hayashi I, Murakami Y, Sato Y, Kitasato H, Matsushita R et al (2007) Antiinflammatory mediator lipoxin A4 and its receptor in synovitis of patients with rheumatoid arthritis. J Rheumatol 34:2144–2153
214. Thomas E, Leroux JL, Blotman F, Chavis C (1995) Conversion of endogenous arachidonic acid to 5,15-diHETE and lipoxins by polymorphonuclear cells from patients with rheumatoid arthritis. Inflamm Res 44:121–124
215. Katoh T, Lakkis FG, Makita N, Badr KF (1994) Co-regulated expression of glomerular 12/15-lipoxygenase and interleukin-4 mRNAs in rat nephrotoxic nephritis. Kidney Int 46:341–349

216. Jiang C, Wang H, Xue M, Lin L, Wang J, Cai G et al (2019) Reprograming of peripheral Foxp3$^+$ regulatory T cell towards Th17-like cell in patients with active systemic lupus erythematosus. Clin Immunol 108267. https://doi.org/10.1016/j.clim.2019.108267
217. Mohammadi S, Sedighi S, Memarian A (2019) IL-17 is aberrantly overexpressed among under-treatment Systemic Lupus Erythematosus patients. Iran J Pathol 14:236–242
218. Nordin F, Shaharir SS, Abdul Wahab A, Mustafar R, Abdul Gafor AH, Mohamed Said MS et al (2019) Serum and urine interleukin-17A levels as biomarkers of disease activity in systemic lupus erythematosus. Int J Rheum Dis 22:1419–1426
219. Zhang Q, Liu S, Ge D, Cunningham DM, Huang F, Ma L et al (2017) Targeting Th17-IL-17 pathway in prevention of micro-invasive prostate cancer in a mouse model. Prostate 77:888–899
220. Zhang Q, Liu S, Parajuli KR, Zhang W, Zhang K, Mo Z et al (2017) Interleukin-17 promotes prostate cancer via MMP7-induced epithelial-to-mesenchymal transition. Oncogene 36:687–699
221. Wang B, Zhao CH, Sun G, Zhang ZW, Qian BM, Zhu YF et al (2019) IL-17 induces the proliferation and migration of glioma cells through the activation of PI3K/Akt1/NF-κB-p65. Cancer Lett 447:93–104
222. Zhang Y, Wang ZC, Zhang ZS, Chen F (2018) MicroRNA-155 regulates cervical cancer via inducing Th17/Treg imbalance. Eur Rev Med Pharmacol Sci 22:3719–3726
223. Changchun K, Pengchao H, Ke S, Ying W, Lei W (2017) Interleukin-17 augments tumor necrosis factor α-mediated increase of hypoxia-inducible factor-1α and inhibits vasodilator-stimulated phosphoprotein expression to reduce the adhesion of breast cancer cells. Oncol Lett 13:3253–3260
224. Akbay EA, Koyama S, Liu Y, Dries R, Bufe LE, Silkes M et al (2017) Interleukin-17A promotes lung tumor progression through neutrophil attraction to tumor sites and mediating resistance to PD-1 blockade. J Thorac Oncol 12:1268–1279
225. Borbiro I, Badheka D, Rohacs T (2015) Activation of TRPV1 channels inhibits mechanosensitive Piezo channel activity by depleting membrane phosphoinositides. Sci Signal 8:ra15. https://doi.org/10.1126/scisignal.2005667
226. Romero LO, Massey AE, Mata-Daboin AD, Sierra-Valdez FJ, Chauhan SC, Cordero-Morales JF et al (2019) Dietary fatty acids fine-tune Piezo1 mechanical response. Nat Commun 10(1):1200. https://doi.org/10.1038/s41467-019-09055-7
227. Accardi A (2015) Lipids link ion channels and cancer. Science 349:789–790
228. Liu CSC, Raychaudhuri D, Paul B, Chakrabarty Y, Ghosh AR, Rahaman O et al (2018) Piezo1 mechanosensors optimize human T cell activation. J Immunol 200:1255–1260
229. Chandy KG, Norton RS (2016) Channelling potassium to fight cancer. Nature 537:497–498
230. Ordway R, Walsh JV Jr, Singer JJ (1989) Arachidonic acid and other fatty acids directly activate potassium channels in smooth muscle cells. Science 244:1176–1179
231. Kim D, Clapham DE (1989) Potassium channels in cardiac cells activated by arachidonic acid and phospholipids. Science 244:1174–1176
232. Amoroso S, Schmid-Antomarchi H, Fosset M, Lazdunskit M (1990) Glucose, sulfonylureas, and neurotransmitter release: role of ATP-sensitive K+ channels. Science 247:852–854
233. Isaacs JT (2013) Prostate cancer takes nerve. Science 341:134–135
234. Hayakawa Y, Wang TC (2017) Nerves switch on angiogenic metabolism. Science 358:305–306
235. Barria A (2019) Dangerous liaisons as tumours form synapses. Nature 573:1–2
236. Walev I, Klein J, Husmann M, Valeva A, Strauch S, Wirtz H et al (2000) Potassium regulates IL-1β processing via calcium-independent phospholipase A2. J Immunol 164:5120–5124
237. Walev I, Reske K, Palmer M, Valeva A, Bhakdi S (1995) Potassium-inhibited processing of IL-1β in human monocytes. EMBO J 14:1607–1614
238. Hughes FM Jr, Bortner CD, Purdy GD, Cidlowski JA (1997) Intracellular K$^+$ suppresses the activation of apoptosis in lymphocytes. J Biol Chem 272:30567–30576

239. McGowan SE, Jackson SK, Doro MM, Olson PJ (1997) Peroxisome proliferators alter lipid acquisition and elastin gene expression in neonatal rat lung fibroblasts. Am J Physiol 273:L1249–L1257
240. Das UN (1993) Oxy radicals and their clinical implications. Curr Sci 65:964–968
241. Lin HL, Liu TY, Chau GY, Lui WY, Chi CW (2000) Comparison of 2-methoxyestradiol-induced, docetaxel-induced, and paclitaxel-induced apoptosis in hepatoma cells and its correlation with reactive oxygen species. Cancer 89:983–994
242. Huang P, Feng L, Oldham EA, Keating MJ, Plunkett W (2000) Superoxide dismutase as a target for the selective killing of cancer cells. Nature 407:390–395
243. Das UN (2002) A radical approach to cancer. Med Sci Monit 8:RA79–RA92
244. Ge Y, Byun JS, De Luca P, Gueron G, Yabe IM, Sadiq-Ali SG et al (2008) Combinatorial antileukemic disruption of oxidative homeostasis and mitochondrial stability by the redox reactive thalidomide 2-(2,4-difluoro-phenyl)-4,5,6,7-tetrafluoro-1H-isoindole-1,3(2H)-dione (CPS49) and flavopiridol. Mol Pharmacol 74:872–883
245. Colquhoun A (2009) Mechanisms of action of eicosapentaenoic acid in bladder cancer cells in vitro: alterations in mitochondrial metabolism, reactive oxygen species generation and apoptosis induction. J Urol 181:1885–1893
246. Naidu MR, Das UN, Kishan A (1992) Intratumoral gamma-linoleic acid therapy of human gliomas. Prostaglandins Leukot Essent Fatty Acids 45:181–184
247. Das UN, Prasad VV, Reddy DR (1995) Local application of gamma-linolenic acid in the treatment of human gliomas. Cancer Lett 94:147–155
248. Bakshi A, Mukherjee D, Bakshi A, Banerji AK, Das UN (2003) Gamma-linolenic acid therapy of human gliomas. Nutrition 19:305–309
249. Das UN (2007) Gamma-linolenic acid therapy of human glioma-a review of in vitro, in vivo, and clinical studies. Med Sci Monit 13:RA119–RRA31
250. Reddy DR, Prasad VS, Das UN (1998) Intratumoural injection of gamma linolenic acid in malignant gliomas. J Clin Neurosci 5:36–39
251. Smith DL, Willis AL, Mahmud I (1984) Eicosanoid effects on cell proliferation in vitro: relevance to atherosclerosis. Prostaglandins Leukot Med 16:1–10
252. Sakai T, Yamaguchi N, Shiroko Y, Sekiguchi M, Fujii G, Nishino H (1984) Prostaglandin D_2 inhibits the proliferation of human malignant tumor cells. Prostaglandins 27:17–26
253. Rohrbach S (2009) Effects of dietary polyunsaturated fatty acids on mitochondria. Curr Pharm Des 15:4103–4116
254. Tuo Y, Wang D, Li S, Chen C (2011) Long-term exposure of INS-1 rat insulinoma cells to linoleic acid and glucose in vitro affects cell viability and function through mitochondrial-mediated pathways. Endocrine 39:128–138
255. Zeghichi-Hamri S, de Lorgeril M, Salen P, Chibane M, de Leiris J, Boucher F et al (2010) Protective effect of dietary n-3 polyunsaturated fatty acids on myocardial resistance to ischemia-reperfusion injury in rats. Nutr Res 30:849–857
256. Hagopian K, Weber KL, Hwee DT, Van Eenennaam AL, López-Lluch G, Villalba JM et al (2010) Complex I-associated hydrogen peroxide production is decreased and electron transport chain enzyme activities are altered in n-3 enriched fat-1 mice. PLoS One 5:e12696. https://doi.org/10.1371/journal.pone.0012696
257. Kansal S, Negi AK, Kaur R, Sarotra P, Sharma G, Aggarwal R et al (2011) Evaluation of the role of oxidative stress in chemopreventive action of fish oil and celecoxib in the initiation phase of 7,12-dimethyl benz(α)anthracene-induced mammary carcinogenesis. Tumour Biol 32:167–177
258. Virgili F, Santini MP, Canali R, Polakowska RR, Haake A, Perozzi G (1998) Bcl-2 overexpression in the HaCaT cell line is associated with a different membrane fatty acid composition and sensitivity to oxidative stress. Free Radic Biol Med 24:93–101
259. Sailaja P, Dwarakanath BS, Das UN (2018) Arachidonic acid activates extrinsic apoptotic pathway to enhance tumoricidal action of bleomycin against IMR-32 cells. Prostaglandins Leukot Essen Fatty Acids 132:16–22

260. Dymkowska D, Wojtczak L (2009) Arachidonic acid-induced apoptosis in rat hepatoma AS-30D cells is mediated by reactive oxygen species. Acta Biochim Pol 56:711–715
261. Ribeiro G, Benadiba M, de Oliveira SD, Colquhoun A (2010) The novel ruthenium-gamma-linolenic complex [Ru(2)(aGLA)(4)Cl] inhibits C6 rat glioma cell proliferation and induces changes in mitochondrial membrane potential, increased reactive oxygen species generation and apoptosis in vitro. Cell Biochem Funct 28:15–23
262. Giros A, Grzybowski M, Sohn VR, Pons E, Fernandez-Morales J, Xicola RM et al (2009) Regulation of colorectal cancer cell apoptosis by the n-3 polyunsaturated fatty acids Docosahexaenoic and Eicosapentaenoic. Cancer Prev Res (Phila) 2:732–742
263. Das UN (2011) Essential fatty acids enhance free radical generation and lipid peroxidation to induce apoptosis of tumor cells. Clin Lipidol 6:463–489
264. Halliwell BA (2000) Superway to kill cancer cells? Nature Med 6:1105–1106
265. Ponnala S, Rao KP, Chaudhury JR, Ahmed J, Rama Rao B, Kanjilal S et al (2009) Effect of polyunsaturated fatty acids on diphenyl hydantoin-induced genetic damage in vitro and in vivo. Prostaglandins Leukot Essent Fatty Acids 80:43–50
266. Das UN, Rao KP (2006) Effect of gamma-linolenic acid and prostaglandins E1 on gamma-radiation and chemical-induced genetic damage to the bone marrow cells of mice. Prostaglandins Leukot Essent Fatty Acids 74:165–173
267. Das UN, Ramadevi G, Rao KP, Rao MS (1985) Prostaglandins and their precursors can modify genetic damage-induced by gamma-radiation and benzo(a)pyrene. Prostaglandins 29:911–920
268. Das UN (2006) Tumoricidal and anti-angiogenic actions of gamma-linolenic acid and its derivatives. Curr Pharm Biotechnol 7:457–466
269. Dhayal S, Morgan NG (2011) Pharmacological characterization of the cytoprotective effects of polyunsaturated fatty acids in insulin-secreting BRIN-BD11 cells. Br J Pharmacol 162:1340–1350
270. Suresh Y, Das UN (2003) Long-chain polyunsaturated fatty acids and chemically induced diabetes mellitus: effect of omega-6 fatty acids. Nutrition 19:93–114
271. Suresh Y, Das UN (2003) Long-chain polyunsaturated fatty acids and chemically induced diabetes mellitus. Effect of omega-3 fatty acids. Nutrition 19:213–228
272. Bazan NG (2007) Omega-3 fatty acids, pro-inflammatory signaling and neuroprotection. Curr Opin Clin Nutr Metab Care 10:136–141
273. Sangeetha PS, Das UN (1993) Gamma-linolenic acid and eicosapentaenoic acid potentiate the cytotoxicity of anti-cancer drugs on human cervical carcinoma (HeLa) cells in vitro. Med Sci Res 21:457–459
274. Madhavi N, Das UN (1994) Reversal of KB-3-1 and KB-Ch-8-5 tumor cell drug-resistance by cis-unsaturated fatty acids in vitro. Med Sci Res 22:689–692
275. Madhavi N, Das UN (1994) Effect of n-6 and n-3 fatty acids on the survival of vincristine sensitive and resistant human cervical carcinoma cells in vitro. Cancer Lett 84:31–41
276. Das UN, Madhavi N, Sravan Kumar G, Padma M, Sangeetha P (1998) Can tumour cell drug resistance be reversed by essential fatty acids and their metabolites? Prostaglandins Leukot Essent Fatty Acids 58:39–54
277. Germain E, Chajès V, Cognault S, Lhuillery C, Bougnoux P (1998) Enhancement of doxorubicin cytotoxicity by polyunsaturated fatty acids in the human breast tumor cell line MDA-MB-231: relationship to lipid peroxidation. Int J Cancer 75:578–583
278. Mahéo K, Vibet S, Steghens JP, Dartigeas C, Lehman M, Bougnoux P et al (2005) Differential sensitization of cancer cells to doxorubicin by DHA: a role for lipoperoxidation. Free Radic Biol Med 39:742–751
279. Ilc K, Ferrero JM, Fischel JL, Formento P, Bryce R, Etienne MC et al (1999) Cytotoxic effects of two gamma linoleic salts (lithium gammalinolenate or meglumine gammalinolenate) alone or associated with a nitrosourea: an experimental study on human glioblastoma cell lines. Anticancer Drugs 10:413–417

280. Menendez JA, Ropero S, Lupu R, Colomer R (2004) Omega-6 polyunsaturated fatty acid gamma-linolenic acid (18:3n-6) enhances docetaxel (Taxotere) cytotoxicity in human breast carcinoma cells: Relationship to lipid peroxidation and HER-2/neu expression. Oncol Rep 11:1241–1252
281. Menéndez JA, Ropero S, del Barbacid MM, Montero S, Solanas M, Escrich E et al (2002) Synergistic interaction between vinorelbine and gamma-linolenic acid in breast cancer cells. Breast Cancer Res Treat 72:203–219
282. Menendez JA, Ropero S, Mehmi I, Atlas E, Colomer R, Lupu R (2004) Overexpression and hyperactivity of breast cancer-associated fatty acid synthase (oncogenic antigen-519) is insensitive to normal arachidonic fatty acid-induced suppression in lipogenic tissues but it is selectively inhibited by tumoricidal alpha-linolenic and gamma-linolenic fatty acids: a novel mechanism by which dietary fat can alter mammary tumorigenesis. Int J Oncol 24:1369–1383
283. Kong X, Ge H, Chen L, Liu Z, Yin Z, Li P et al (2009) Gamma-linolenic acid modulates the response of multidrug-resistant K562 leukemic cells to anticancer drugs. Toxicol In Vitro 23:634–639
284. Buckingham LE, Balasubramanian M, Safa AR, Shah H, Komarov P, Emanuele RM et al (1998) Reversal of multi-drug resistance in vitro by fatty acid-PEG-fatty acid diesters. Int J Cancer 65:74–79
285. Ramesh G, Das UN, Koratkar R, Padma M, Sagar PS (1992) Effect of essential fatty acids on tumor cells. Nutrition 8:343–347
286. Huang ZH, Hii CS, Rathjen DA, Poulos A, Murray AW, Ferrante A (1997) N-6 and N-3 polyunsaturated fatty acids stimulate translocation of protein kinase C alpha, beta I, beta II and –epsilon and enhance agonist-induced NADPH oxidase in macrophages. Biochem J 325:553–557
287. Peterson DA, Mehta N, Butterfield J, Husak M, Christopher MM, Jagarlapudi S et al (1988) Polyunsaturated fatty acids stimulate superoxide formation in tumor cells: a mechanism for specific cytotoxicity and a model for tumor necrosis factor? Biochem Biophys Res Commun 155:1033–1037
288. Chiu LC, Wan JM (1999) Induction of apoptosis in HL-60 cells by eicosapentaenoic acid (EPA) is associated with downregulation of bcl-2 expression. Cancer Lett 145:17–27
289. Albino AP, Juan G, Traganos F, Reinhart L, Connolly J, Rose DP et al (2000) Cell cycle arrest and apoptosis of melanoma cells by docosahexaenoic acid: association with decreased pRb phosphorylation. Cancer Res 60:4139–4145
290. Chen ZY, Istfan NW (2001) Docosahexaenoic acid, a major constituent of fish oil diets, prevents activation of cyclin-dependent kinases and S-phase entry by serum stimulation in HT-29 cells. Prostaglandins Leukot Essen Fatty Acids 64:67–73
291. Anasuya HD, Naidu VGM, Das UN (2018) n-6 and n-3 Fatty acids and their metabolites augment inhibitory action of doxorubicin on the proliferation of human neuroblastoma (IMR-32) cells by enhancing lipid peroxidation and suppressing Ras, Myc, and Fos. Biofactors 44:387–401
292. Palakurthi SS, Fluckiger R, Aktas H, Changolkar AK, Shahsafaei A, Harneit S et al (2006) Inhibition of translation initiation mediates the anticancer effect of the n-3 polyunsaturated fatty acid eicosapentaenoic acid. Cancer Res 60:2919–2925
293. Nishida M, Maruyama Y, Tanaka R, Kontani K, Nagao T, Kurose H (2000) $G\alpha_i$ and $G\alpha_o$ are target proteins of reactive oxygen species. Nature 408:492–495
294. Bai Y, Wang J, He Z, Yang M, Li L, Jiang H (2019) Mesenchymal stem cells reverse diabetic nephropathy disease via lipoxin A4 by targeting transforming growth factor β (TGF-β)/smad pathway and pro-inflammatory cytokines. Med Sci Monit 25:3069–3076
295. Wada K, Arita M, Nakajima A, Katayama K, Kudo C, Kamisaki Y et al (2006) FASEB J 2:1785–1792

Chapter 4
Effect of Short Chain Fatty Acids on Age-Related Disorders

Mariane Font Fernandes, Sarah de Oliveira, Mariana Portovedo, Patrícia Brito Rodrigues, and Marco Aurélio Ramirez Vinolo

1 Introduction

The microbiota is formed by an astonishing variety of microorganisms including bacteria, fungi, archaea, virus and some protozoa that colonize host tissues, mainly at sites in direct contact with the external environment such as skin, gastrointestinal, respiratory and urogenital tracts. These microorganisms contribute to the maintenance of host homeostasis by different mechanism such as providing nutrients and defense against pathogens [1].

Our comprehension of the microbiota relevance, composition and of its mechanisms of interactions with host cells have drastically changed over the last 20 years with the use of next-generation sequencing technologies, metabolomic analyses, gnotobiotic models and several other tools [1, 2]. These approaches have led to the discovery of new functions of microbiota such as biotransformation of drugs and food contaminants [3, 4], and to extension of the knowledge regarding the type of molecules produced by the microbiota and the molecular targets activated in the host [5].

The intestinal tract is the most densely colonized site of our body. Studies have estimated that there are between 10^{13} and 10^{14} bacteria in the human gastrointestinal tract with the highest density of these microbes in the colon [6]. Most of the bacteria in the human intestine belong to *Firmicutes* and *Bacteroidetes* phyla, but other phyla such as *Proteobacteria*, *Verrumicrobia*, *Actinobacteria*, *Fusobacteria* and

Mariane Font Fernandes, Sarah de Oliveira, Mariana Portovedo, Patrícia Brito Rodrigues and Marco Aurélio Ramirez Vinolo contributed equally to the work.

M. F. Fernandes · S. de Oliveira · M. Portovedo · P. B. Rodrigues · M. A. R. Vinolo (✉)
Laboratory of Immunoinflammation, Department of Genetics, Evolution, Microbiology, and Immunology, Institute of Biology, University of Campinas, Campinas, SP, Brazil
e-mail: mvinolo@unicamp.br

P. C. Guest (ed.), *Reviews on New Drug Targets in Age-Related Disorders*,
Advances in Experimental Medicine and Biology 1260,
https://doi.org/10.1007/978-3-030-42667-5_4

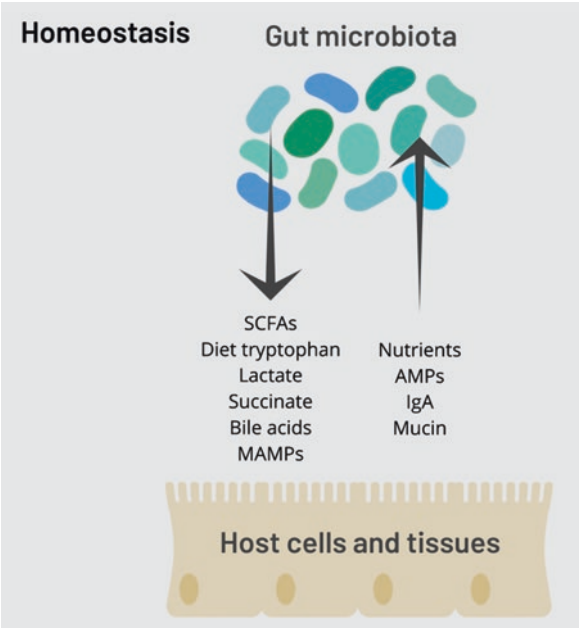


Fig. 4.1 Microbiota and host interactions are important for the maintenance of homeostasis. Microbiota derived signals including short-chain fatty acids (SCFAs), succinate, tryptophan metabolites, secondary bile acids and microbial-associated molecular patterns (MAMPs) are examples of molecules that shape the host immune system, which actively produce a broad range of molecules that contribute to the compartmentalization and regulation of the microbiota composition including mucin, antibodies such as immunoglobulin A (IgA) and antimicrobial peptides (i.e. defensins and cathelicidins) that regulate the composition of the microbiota

Cyanobacteria are also relevant in this site [7]. The composition of the microbiota varies within and between individuals. Factors affecting individual composition of the microbiota include the anatomical site of analysis, the type of food ingested and intake of antibiotics [8, 9]. The inter-individual variation of microbiota is dependent on the host genetics, which surprisingly seems to shape only a minor part of the microbiome, and environmental factors including the diet, drug intake, exercise and anthropometric parameters [9, 10].

The mechanisms by which the microbiota and host cells communicate are starting to be deciphered. The bi-directional and complex host-microbiota interactions depend on several signals derived from microbes including microbe-associated molecular patterns (MAMPs), modified host molecules and microbe metabolites that can interact with components of the microbiota itself and with host cells [11]. The host produces and secretes several types of molecules that regulate microbiota numbers and composition such as antimicrobial peptides and immunoglobulins. These bi-directional interactions are essential for the establishment of the individual's equilibrium between host and microbiota and may be a key factor influencing health and disease in the host (Fig. 4.1).

One group of microbe-derived metabolites that participate in host-microbe interactions are short-chain fatty acids (SCFAs). These are small carboxylic acids produced by bacterial components of the microbiota, mainly in the colon, from the process of fermentation of complex carbohydrates that reach this site almost undigested [12, 13]. The main SCFAs found in the intestinal tract are acetic, propionic and butyric acids, which are normally found in their deprotonated forms (acetate, propionate and butyrate) [12, 13]. A recent study indicated that pentanoate, a SCFA found in lower concentrations compared to other SCFAs in the colon, may also have relevance in the context of immunomodulation by the microbiota [14].

Short-chain fatty acids are abundant in the colon (mM range). Epithelial cells take these metabolites up and use some of them (especially, butyrate) for generation of adenosine triphosphate (ATP). In addition, SCFAs also reach the blood circulation from where they can interact and mediate systemic effects of the microbiota. These molecules have an important homeostatic role in the gut but also may be important in disease states. In this context, we and other research groups have demonstrated that they have an impact in both intestinal [15–17] and extraintestinal infections [18–20].

Some of the most described effects of these metabolites include their capacity to regulate host metabolism and immune cells, as previously reviewed [11, 12, 21]. These actions are attributed to their ability to activate different cellular pathways including: (i) G-protein-coupled receptors (GPCRs) - the free fatty acid receptors (FFAR), FFAR2 (also known as GPR43) and FFAR3 (or GPR41) and the hydroxycarboxylic acid receptor 2 (HCA2), also known as niacin receptor 1 (NIACR1) or GPR109A [12, 21]; (ii) regulation of protein acylation state, an effect mainly linked to their inhibitory effect on histone deacetylases (HDACs) [12, 21, 22]; (iii) modifications (direct or indirect) of the cellular metabolism [23, 24]; and (iv) regulation of the activity/stability of transcription factors such as peroxisome proliferator–activated receptor (PPAR-y) and hypoxia inducible factor-1 (HIF-1) [25, 26].

Considering the effects of microbiota-derived SCFAs on metabolism and immune cell function, it is not surprising that several research groups have tested their association with the development of age-related diseases. In the next sections, we will discuss recent evidence of this relationship for some age-related diseases including metabolic diseases and type 2 diabetes mellitus (T2DM), hypertension, cardiovascular and neurodegenerative disease. We also highlight their impact on the development of cancer.

2 SCFAs and Metabolic Diseases

The prevalence of metabolic diseases such as T2DM and obesity are rising worldwide, especially in middle- and low-income countries [27]. T2DM occurs when the tissues become insulin resistant or the pancreas does not produce enough insulin to prevent hyperglycemia. This disruption in glucose homeostasis is a major risk factor to development of comorbidities such as cardiovascular diseases (CVDs) [28].

Changes in lifestyle and dietary patterns are major factors behind the increased prevalence of T2DM and its related metabolic disorders [29]. Currently, around 8.8% of adults have T2DM and this disease is responsible for 9.9% of all-cause mortality globally [27]. Low-grade systemic inflammation is considered the hallmark of metabolic diseases and many therapeutic interventions targeting inflammatory pathways are currently under investigation [30, 31].

Evidence has led to the suggestion that the human microbiota and its metabolites contribute to obesity since fecal transplantation of gut microbiota from obese individuals leads to increased adiposity in germ-free mice and reduced content of fecal SCFA [32]. On the other hand, fecal transplantation of gut microbiota from lean humans exerts beneficial effects in individuals with metabolic syndrome and its effects are associated with increased content of butyrate-producing bacteria [33]. Furthermore, increased production of butyrate is associated with improved insulin sensitivity in humans [34].

Mechanistic studies in mice have revealed that butyrate supplementation improves body weight loss by increasing energy expenditure and fat oxidation [35]. Butyrate has also been found to reduce recruitment of inflammatory cells for white adipose tissue leading to an attenuation of inflammation in this tissue and the liver [36]. Acetate and propionate also act in high-fat diet-fed mice improving body weight loss and insulin sensitivity [37]. In obese humans, propionate supplementation also leads to weight loss [38]. Although it is well established that dietary fiber consumption is associated with several health benefits such as improved insulin sensitivity and body weight control, the mechanism of action of the microbiota in this context has still not been elucidated. Several of these mechanisms are associated with the action of SCFA in host cells, like appetite control, increased energy expenditure and improved metabolic function [35, 38, 39]. The concentration of SCFAs in plasma is a marker of metabolic health, since it is directly associated with insulin sensitivity and inversely correlated with body mass index (BMI) [40].

SCFAs are capable of stimulating production of satiety hormones such as glucagon-like peptide-1 (GLP-1) and peptide YY (PYY) by intestinal enteroendocrine cells in a FFAR2-dependent manner [38, 39]. The secretion of these hormones results in the stimulation of proopiomelanocortin (POMC)-secreting neurons and suppression of neuropeptide Y (PPY)-producing neurons, therefore promoting satiety signals [41].

Interestingly, the effects of SCFA in appetite are not restricted to their direct effects in intestinal epithelial cells since acetate is capable of crossing the blood-brain barrier [42]. Also, propionate stimulates leptin production by adipose tissue [43]. Besides acting in appetite control, SCFA increases thermogenesis-related genes in brown adipose tissue, therefore increasing energy expenditure [35].

Another important aspect of SCFAs in metabolic diseases is the prevention of low-grade inflammation through different mechanisms, including decreased production of inflammatory cytokines, improvement of the intestinal barrier and prevention of endotoxemia and upregulation of regulatory T (T_{REG}) cells [44–46]. Although the link between the modulation of the immune system and inflammatory response during metabolic diseases by SCFAs is promising, most of the studies have

only been performed in murine models. In addition, there are controversial findings that need to be addressed. For example, although acetate can act in the brain to suppress food intake, its metabolism is dysregulated in high-fat diet-induced obese mice. There is an increased acetate turnover in this model which, in turn, leads to parasympathetic nervous system activation and insulin resistance [47]. Also, the FFAR2-mediated effect of acetate in pancreatic beta cells promotes inhibition of insulin secretion. Interestingly, this effect does not affect glucose homeostasis in healthy mice. On the contrary, in diabetic mice with increased pancreatic and systemic acetate levels, the deletion of FFAR2 and FFAR3 enhances insulin secretion and glucose tolerance [48].

In summary, SCFAs act in host cells to modulate its metabolism, immune function and to control appetite. SCFA supplementation can improve metabolic status especially in murine models by targeting inflammatory pathways and controlling food intake and energy metabolism. However, more clinical studies are needed to support the therapeutic findings of SCFAs in obesity, T2DM and other metabolic disorders.

3 SCFAs and Hypertension and CVDs

CVDs are disorders involving the heart or blood vessels and include high blood pressure (AH), stroke, peripheral vascular disease, coronary heart disease, and other heart diseases [49]. For the past 15 years, CVD has been the leading cause of death worldwide. In 2016, CVDs were responsible for 15.2 million deaths [50]. Moreover, CVDs contributed significantly to increased health care costs by causing physical disability and invalidity.

Therapy through dietary and lifestyle modifications is an important tool used in clinical practice for cardiovascular and metabolic diseases. These interventions are known to impact the gut microbiota composition and function. There are many interactions between the changes in the intestinal microbiota and its metabolites with susceptibility to CVD, which makes the microbiome a potential new therapeutic target [51, 52].

The intestinal microbiota may act to promote or prevent CVD. Atherosclerotic patients have intestinal dysbiosis with a characteristic increase of *Enterobacteriaceae* and *Streptococcus* spp., which has been suggested to be associated with inflammatory status, thus contributing to atherosclerosis [53–55]. However, due to the use of different drugs during CVD, it is still unknown whether or not these associations are secondary to the use of medications and further investigation in cohort studies is required.

Data from experimental and human studies have demonstrated that increased blood pressure levels are associated with intestinal microbiota dysbiosis. In the analysis of the microbiota richness, diversity and uniformity of spontaneously hypertensive rats, an increase in the *Firmicutes*/*Bacteroidetes* ratio was observed with a reduction in acetate and butyrate luminal levels. In the same study, the authors

found the same dysbiotic pattern in hypertensive patients and antibiotic treatment rebalanced the dysbiotic hypertension gut microbiota by reducing the *Firmicutes/Bacteroidetes* ratio and attenuating high blood pressure [56].

Consumption of probiotics in combination with standard drugs could offer additional benefits for patients with heart failure, for example by reducing the severity of heart failure after a heart attack. In a classic murine model for CVD, the spontaneously hypertensive rat (SHR), the treatment with different *Lactobacillus* strains reduced the number of *Bacteroidetes* spp. and *Clostridium* spp. in the cecum, and attenuated systolic pressure levels and the vascular inflammatory state [56]. In humans with stable coronary artery disease, oral supplementation with *Lactobacillus plantarum* 299v improved vascular endothelial function and inflammation by mediating changes in circulating metabolites leading to increased bioavailability of nitric oxide, an important vasodilator [57].

The intestinal microbiota has been considered a new endocrine organ for the host due to its important role in converting nutritional signals into hormone-like signals that affect both normal physiology and host disease [58]. SCFAs from intestinal microbial metabolism, as well as the host receptors that recognize them, are directly involved in this communication, which has been identified as an important factor in the control and prevention of CVD [59–61].

Butyrate may play an important role in regulating blood pressure. In a study with the SHR rat, not only butyrate plasmatic levels were reduced, the expression of receptors activated by this metabolite in the region of the central nervous system responsible for regulating blood pressure was also decreased [61]. In this study, butyrate administration directly in the hypothalamus led to a reduction in blood pressure in the hypertensive rats [61]. Propionate and acetate have been appointed as blood pressure regulators. The activation of GPR41 has been shown to reduce blood pressure, while the activation of Olfr78 antagonizes this effect [62]. Further advances in the development of strategies that combine classic therapies in the treatment of CVD and the regulation of microbiota and its metabolites require well-controlled intervention studies in humans.

4 SCFAs and Neurodegenerative Disease

The diversity of the intestinal microbiota is known to be essential not only for the homeostasis of tissues but also for the correct physiological functioning of the nervous system. Dysbiosis can have a major impact on brain development and function, especially if we consider that the brain is dependent on metabolic products of the intestinal microbiota, like SCFAs [63, 64]. Changes in intestinal bacterial niches may alter the blood-brain barrier permeability, which contributes to a chronic neuroinflammatory state, increasing the risk of developing neurodegenerative diseases (NDs) [65–67].

NDs are disorders that are becoming increasingly prevalent in the population due to the increasing proportion of the older population. Examples of major diseases are

Parkinson's disease (PD), Alzheimer's disease (AD), Huntington's disease (HD), Amyotrophic lateral sclerosis (ALS) and spinocerebellar ataxias (SCA) [68]. These diseases have unique pathophysiological mechanisms, in part because some may cause memory loss and cognitive impairment, and others may affect a person's ability to move, speak, and breathe. The prevalence of such diseases is noticeable between 55 and 80 years old with an average of 3 to 17 cases per 100,000 people. The most common diseases are AD and PD, In the case of AD, the incidence rate doubles every 5 years, with the yearly risk ranging from 0.5% in individuals between 65 and 69 years old to 6% in those more than 85 years old [69]. In PD, the incidence is high with an average of 8 to 18 cases per 100,000 persons and curiously men have 1.5–2.0 times higher prevalence and incidence than women. After diagnosis PD patients have a mean survival of 15 years [69].

A dysbiotic change can be observed in patients with PD and this fact could trigger inflammation-induced misfolding of α-synuclein (Syn), contributing to the development of PD pathology. One of the links between dysbiosis and inflammation is the impairment of intestinal barrier function, which may initiate immune activation [70]. These changes in microbiota can also be associated with PD symptoms, since the abundance of *Enterobacteriaceae* has been linked with the motor phenotype in these patients [71].

Some fecal markers can be used to identify intestinal inflammation and increased intestinal permeability such as calprotectin for inflammation and alpha-1-antitrypsin and zonulin for increased intestinal permeability. The levels of these are significantly elevated in PD patients, although they are not disease-specific [72].

A significant reduction of acetate, propionate and butyrate levels in fecal samples of PD patients have been reported [73]. Interestingly, sodium butyrate, a histone deacetylase inhibitor (HDACi) can protect dopaminergic neurons through the upregulation of genes related to DNA damage response, thus preventing motor impairment when used in a toxin-induced Drosophila model of PD [74–76]. Other effects attributed to sodium butyrate are the attenuation of motor deficits, oxidative stress and neuroinflammation markers, and it has been found to increase striatal dopamine levels [77]. Gut microbiota signals have also been associated with microglia activation and α-Syn aggregation. A recent study showed that SCFAs can accelerate both processes impacting on neuroinflammation and motor dysfunction [78]. Thus more studies are needed to explore and understand the role of microbiota in PD.

In AD, tau hyperphosphorylation and amyloid β-peptide overproduction are key aspects of the pathology. A study with AD mouse model showed that valproic acid, a molecule that has a structure similar to butyrate and which also acts as inhibitor of HDAC, attenuated senile plaques and neuronal loss, improved behavioral deficits, modified synaptic structure and accelerated neurite outgrowth by inhibiting the activity of GSK-3b [79]. It has been suggested that the dysregulation of histone acetylation is involved in the onset of age-associated memory impairment in AD, so prolonged treatment with the HDAC inhibitor sodium butyrate improved associative memory, even when administered at an advanced stage of pathology [80].

In addition to mouse models, some studies have employed Drosophila models for the study of neurogenerative diseases like AD. Results obtained using this model indicate that gut microbiota dysregulation may participate in AD pathogenesis. The diversity of gut microbiota was increased in an AD Drosophila model with a decrease in the proportion of *Acetobacter* and *Lactobacillus* spp., thus resulting in a marked decrease of acetate levels [81]. However, in a specific model of AD (APPSWE/PS1ΔE9) the ABX antibiotic cocktail treatment appeared to alter the microbiome in ways to attain states of host-microbiome interactions that affect immune response systemically, preventing the natural progression of disease by regulating β amyloid deposition [82].

HD is a genetic disorder caused by a trinucleotide repeat expansion in the Huntingtin (HTT) gene. The elongated huntingtin protein accumulates within cells forming aggregates that are toxic and cause dysfunction and death of specific neurons. This neurodegenerative disease is clinically associated with motor, cognitive and psychiatric disturbances [83]. As described for other diseases, there is also some evidence indicating that the changes in intestinal microbiota are present in HD. A recent study identified a significant difference in intestinal microbiota composition in HD mice at 12 weeks of age. More specifically, the authors found an increase in *Bacteriodetes* and a proportional decrease in *Firmicutes*, suggesting that microbiota may also influence the development of this disorder [84].

An impaired gut-neuromuscular crosstalk may actively contribute to progression and pathogenesis of neurodegenerative diseases, such as ALS. In a mouse G39A model that presents motor neuron degeneration, the mice showed an imbalance in the gut bacterial profile with a reduced population of butyrate-producing bacteria and increased intestinal permeability (leaky gut). Remarkably, after administration of 2% butyrate, the mice with ALS exhibited a delay in the onset of symptoms and a prolonged life span. This suggests that butyrate administration can be used for restoration of the microbiota and gut homeostasis [85].

In the case of SCA type 3, it has been suggested that butyrate alleviates the symptoms of transgenic mice by inhibiting HDAC activity. One problem of this disease is the cerebellar transcriptional repression by the hypoacetylation of histones H3 and H4. However, through the intraperitoneal administration of sodium butyrate, it was possible to delay the onset of ataxic symptoms and to ameliorate various phenotypes through HDAC inhibition [86].

In summary, research related to brain-gut microbiome interactions is still scarce but already indicates the existence of significant crosstalk between these components. These interactions may be relevant for the development of several pathologies and also present the possibility that by changing the microbiota or avoiding the "pathological" shift of the microbiota, we may be able to delay the development of neurodegenerative diseases.

5 SCFAs and Cancer

Cancer is a disease promoted by genetic and epigenetic changes that may evolve depending on environmental signals [87, 88]. The numbers of cases and deaths associated with cancer worldwide are high. In 2018, 18.1 million cases were diagnosed and there were 9.6 million deaths associated with cancer [89]. Aging is a well-known risk factor for cancer development. Indeed, more than 50% of the cases appeared in individuals older than 70 years [90]. Other factors that may contribute to the increasing numbers of cases involve unhealthy lifestyle including physical inactivity, excess alcohol consumption and poor diet [91], all of which are known modifiers of microbiota-composition. There are numerous reports on microbiota changes associated with cancer development and even cancer therapy. These topics are beyond the scope of the chapter and will not be discussed, although we highlight recent reviews on this subject [92, 93].

Fiber consumption and SCFAs have been studied in the context of different types of tumoral diseases, especially, colorectal cancer (CRC). This type of cancer is one of the main causes of death by cancer, presenting a high worldwide incidence of almost 1.8 million cases each year [89]. The progression of this disease is marked by disturbed innate immunity responses [94], dysbiotic microbiota [95], decreased SCFA concentrations and increased pH of the faeces [96].

Despite the controversies in the literature, epidemiological and experimental data indicate that increased fiber consumption is associated with prevention and suppression of colorectal cancer development. The mechanisms proposed for these effects are variable and dependent on the type of fiber. For insoluble fiber, a general protective effect is the increased mobility of colon, which helps to minimize the exposure of colonocytes to ingested carcinogens [97]. For soluble fibers, the increase in SCFA-producing bacteria and SCFA colonic levels appear to be relevant [97, 98].

Some reports suggest the use of butyric acid derivatives or other molecules that have the same mechanisms of action, as therapeutic interventions for colon cancer treatment and prevention [99–102]. Butyrate administered via enema is known to ameliorate inflammatory bowel diseases (IBD) symptoms *in vivo* [97] and could be tested for CRC therapy. However, considering the point that SCFAs are quickly metabolized by the colonocytes [100] and that long term application of this treatment is not viable for prevention, it would be better to develop approaches involving prodrugs of butyrate or synthetic molecules that have the same effect of butyrate but with increased half-life.

The use of tributyrin, a prodrug of butyrate, is an option that has already been explored by different groups [103]. Tributyrin is composed of glycerol and three molecules of butyrate that can reach the colon tumour and release butyrate for a considerable time after metabolism [104]. *In vivo* treatment with tributyrin or a high-fibre diet was found to reduce DNA damage, tumour growth and pro-inflammatory cytokine production, in addition to increased apoptosis and hyper-acetylation of histone 3 via HDAC inhibition [105–107]. Positive results were also reported with the use of tributyrin in experimental studies involving other types of

cancer including liver [108] and prostate [109]. However, it is important to mention that there are some studies which have indicated that tributyrin or fiber supplementation do not have any effect on tumour development or can even make it worse [110].

Several different mechanisms have been described for the effect of SCFAs, especially butyrate, on tumoural cells including the inhibition of HDAC, activation of GPCRs, induction of autophagy and expression of miRNAs, as discussed below.

Some cells acquire resistance and restore a normal cell cycle progression in an environment with chronic exposure to butyrate [111]. Cancer cells capable of metabolizing butyrate, similar to normal colonocytes, are butyrate-resistant and protected against its effect as an HDAC inhibitor. Once butyrate does not accumulate in the cytoplasm or nucleus, aggressive cancer cells are selected since they are able to maintain use of butyrate as a source of carbon to incorporate into long chains fatty acids and provide energy [112]. This effect can also be related to different degrees of Wnt attenuation [104] or regulation of the Bcl-2 family of proteins, cyclin D1 and p21Waf1/Cip1 [111], once ERK1/2 activation results in Bim degradation via the proteasome, protecting cells from death [113]. When ERK is also followed by protein kinase D activation, SphK2 is translocated from the nuclei to the cytoplasm to promote survival of CRC cells [114, 115]. Butyrate-resistant cells express high levels of matrix metallopeptidase (MMP)-2, MMP-9, and the α2 and α3 integrins, and low levels of E-cadherin, suggesting invasive and metastatic behaviour that confirms their aggressive profile [112].

Another way to promote cancer cell protection is through autophagy [116]. This catabolic process transfers the cytoplasmic contents into vesicles, called autophagosomes, that fuse with a lysosome to break down and recycle old intracellular proteins or damaged organelles in order to maintain cellular homeostasis, sustain bioenergetics and promote cell survival [117]. In cancer, the degradation of defective mitochondria by autophagy could prevent the release of proapoptotic factors [117], impairing the efficacy of propionate and butyrate-induced cell death [118]. Autophagy promoted by propionate is dependent on reduction of mTOR activity, which is related to hyper-phosphorylation of the AMP-activated protein kinase (AMPK)-α activated by an increased AMP/ATP ratio and reactive oxygen species (ROS) accumulation [117]. This catabolic process can also be induced by butyrate via activation of the liver kinase B (LKB)-1-AMPK signalling pathway and is capable of resulting in cell cycle arrest and tumour growth inhibition [119]. At low doses (2 mM), butyrate promoted autophagy in HCT-116 tumour cells mediated by endoplasmic reticulum (ER) stress. However, at high doses (5 mM) butyrate induced-autophagy is reduced, while apoptosis occurs [116].

Acetate has been reported as one of the alternative fuels for cancer cells [120], being capable of maintaining neoplastic cell proliferation and survival in different types of cancer, including glioblastoma [121], hepatocellular carcinoma, prostate [122] and breast cancer [123]. However, depending on the local environmental conditions, acetate can also inhibit tumour cell survival [123, 124]. Similar to acetate, butyrate can act in different ways on the colon, providing energy for normal cells and also inhibiting proliferation of cancerous cells [104, 125–127]. This event, known as the “butyrate paradox”, can be explained by the availability of this SCFA

in the environment. The use of butyrate by colonocytes close to the lumen leads to reduction in butyrate levels at the crypts, where the stem cells are located, protecting the turnover in the deep base from suppression [128]. Patients with colorectal cancer have elevated pro-inflammatory cytokines including tumour necrosis factor-alpha (TNF-α) and IL-1β, which are capable of reducing the oxidation of butyrate [129]. In addition, the epithelium morphology of the patients is modified, thus contributing to higher concentrations of SCFAs reaching the bottom of the crypts and inhibiting stem cell proliferation [104].

Another possible explanation for butyrate paradox is the Warburg effect, in which cancerous cells preferentially carry out aerobic glycolysis to obtain the energy required [96, 104] and reduce the uptake of butyrate by the sodium-coupled monocarboxylate transporter 1 (SMCT-1), thus reducing the effect of this SCFA. However, the monocarboxylate transporter 1 (MCT-1), which is also involved in maintenance of glycolytic metabolism [130] can also mediate butyrate uptake, leading to SCFA accumulation within the cells [96]. As a result, butyrate triggers apoptosis and inhibits proliferation possibly by inhibiting HDAC activity [96].

Epigenetic mechanisms have an important role in carcinogenesis. As one of the effects of butyrate, the inhibition of HDAC3 can significantly block both activation of Akt1 and ERK1/2, which impairs metastatic properties of cancer, such as migration and invasion of CRC cells [131]. However, SCFAs not only act as HDACis at high concentrations [125], but are also metabolized to acetyl-CoA, which is a substrate for histone acetyltransferase (HAT), thus contributing to the increase in the histone acetylation [94, 132]. Histone hyperacetylation improves p21 expression in HCT116 cells, exacerbating the negative role of p21 as a tumour suppressor [133]. Another post-translational modification, known as DNA methylation, can be promoted in cancerous cells in the regulation of apoptosis. One factor that mediates this modification is the protein arginine methyltransferase 1 (PRMT1), which is upregulated in colon cancer at early stages [134]. Propionate treatment downregulates PRMT1 and induces apoptosis by inhibiting phospho-p70 S6 kinase in the HCT116 cell line [135]. Butyrate combined with docosahexaenoic acid (DHA) reduced promoter methylation of five proapoptotic genes (*CIDEB*, *DAPK1*, *TNFRSF25*, *BCL2l11*, and *LTBR*), and butyrate alone decreased global methylation and promoter methylation of *BCL2l11*, known as an apoptosis inducer [134].

Butyrate can also influence cancer development via alterations in microRNA (miRNA) profiles. MiRNAs are non-coding RNAs with approximately 22 nucleotides that can target tumour suppressors or oncogenes [136]. The suppression of the miR-106b family, which includes miR-17, miR-20a/b, miR-93, and miR-106a/b, and inhibition of HDAC can reduce cell proliferation through the expression of p21 induced by butyrate [136]. Butyrate can also decrease the expression of the oncogenic miR-17-92a cluster that promotes colon cancer cells, via reduction of the c-Myc protein and up-regulation of p57 [137]. Other studies have revealed that butyrate could upregulate the expression of miR-203 in CRC cells [96], as well as miR-200 family members, known as potential metastasis suppressors that downregulate *BMI-1*, *EZH2*, and *ZEB1* [138]. Both miRNAs consequently inhibit cell proliferation, invasion and growth.

Anticarcinogenic effects are also promoted by SCFAs through FFAR2 and GPR109A activation [104]. FFAR2 is highly expressed in normal colon cells, relatively reduced in benign colon tumours, but downregulated in colon carcinoma cells, suggesting that a decrease in expression of this receptor may contribute to cancer development [139]. Restoration of FFAR2 in cancer cells (HCT8 and SW480) results in more sensitivity to SCFAs (propionate and butyrate), triggering apoptosis with reduced anti-apoptotic proteins (Bcl-2 and survivin) and upregulated pro-apoptotic protein Bad, while p21 expression decreases cyclin D3, CDK1 and CDK2 levels and inhibits growth through G1/G0 cell cycle arrest in a p53-independent manner [139]. The SCFA-FFAR2 axis improves barrier functions via modulation of innate and adaptive immune responses, as well as increasing the expression of the genes for occludin (*OCLN*) and ZO-1 (*TJP1*) [140]. However, this receptor does not seem to have a significant role in controlling the microbiota in colorectal cancer [140].

GPR109A is also highly expressed in normal human colon tissue on the apical membrane, where it has access to luminal contents and can be activated by nicotinate (niacin) and butyrate without inducing cell death [100, 141]. In contrast, this receptor is silenced via DNA methylation by DNA (cytosine-5)-methyltransferase 1 (DNMT1) in colon cancer cell lines. When expressed and activated in these cells, GPR109A has a suppressor function, leading to apoptosis over the inhibition of Bcl-2, Bcl-xL, cyclin D1 and activation of the death receptor signalling pathway [141]. The same function can be seen in breast cancer cells, where the GPR109A-SCFA axis blocks colony formation and tumour growth in mice [142].

In addition to the signalling pathways already presented in this chapter, SCFAs can also cause apoptosis via the upregulated expression of metabolic enzymes isocitrate dehydrogenase 1 (IDH1) and pyruvate dehydrogenase (PDH), promoting the generation of α-ketoglutarate and acetyl-CoA that enter the nucleus and act as epigenetic modifiers, resulting in demethylation and acetylation of the mismatch repair genes, MLH1 and MSH2 [143]. Moreover, activation of the c-Jun N-terminal kinase (JNK) pathway promoted by butyrate causes reduction of mitochondrial transmembrane potential with decreased Bcl-2 and increased Bax translocated into the mitochondria by JNK promotion, resulting in the stimulation of caspase-3 and 9 [144]. The hyperactivation of Wnt signalling induced by butyrate also leads to enhanced transcription of proteins related to colon cancer cell apoptosis [125, 145]. At the same time, healthy colon cells that metabolize butyrate at higher rates as an energy source are usually less susceptible to apoptosis induction [96].

As presented in this section, the microbiota and their metabolites may be relevant for tumour development and progression. However, more studies are needed to clarify the differences observed in both epidemiological and experimental studies involving fiber consumption as well as those regarding other strategies that increase SCFA production and/or induce microbiota changes which may be beneficial for the host.

6 Conclusions

This chapter has described how changes in microbiota composition and function are associated with the development of different age-related diseases. We have also discussed how several prophylactic and therapeutic strategies used in these conditions such as changes in diet, chemotherapy and use of other types of drugs, are known to impact the microbiota and possibly the production of SCFAs. Therefore, further studies in this area are critical for understanding the relevance of the microbiota and their metabolites in these diseases and for assessing the effects of interventions that may block negative changes in the microbiome and, consequently, in host-microbiota interactions. Such studies may reveal new prophylactic and therapeutic interventions for attenuating the age-associated changes and reducing or delaying the development of metabolic, neurodegenerative and tumoral diseases which are commonly associated with aging.

Acknowledgements Sarah de Oliveira and Patrícia Brito Rodrigues are supported by fellowships from São Paulo Research Foundation (FAPESP #2019/11662-0 and 2019/14342-7). Mariana Portovedo and Mariane Fernandes Font are supported by fellowships from CAPES. This study is also supported by the National Council for Scientific and Technological Development (CNPq) (304433/2018-7) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior—Brasil (CAPES)—Finance Code 001.

References

1. Sekirov I, Russell SL, Caetano M, Antunes L, Finlay BB (2010) Gut microbiota in health and disease. Physiol Rev 90(3):859–904
2. Hooper DU, Adair EC, Cardinale BJ, Byrnes JEK, Hungate BA, Matulich KL et al (2012) A global synthesis reveals biodiversity loss as a major driver of ecosystem change. Nature 486(7401):105–108
3. Zimmermann M, Zimmermann-Kogadeeva M, Wegmann R, Goodman AL (2019) Mapping human microbiome drug metabolism by gut bacteria and their genes. Nature 570(7762):462–467
4. Wolf AR, Wesener DA, Cheng J, Houston-Ludlam AN, Beller ZW, Hibberd MC et al (2019) Bioremediation of a common product of food processing by a human gut bacterium. Cell Host Microbe 26(4):463–477.e8
5. Chen H, Nwe PK, Yang Y, Rosen CE, Bielecka AA, Kuchroo M et al (2019) A forward chemical genetic screen reveals gut microbiota metabolites that modulate host physiology. Cell 177(5):1217–1231.e18
6. Hillman ET, Lu H, Yao T, Nakatsu CH (2017) Microbial ecology along the gastrointestinal tract. Microbes Environ 32(4):300–313
7. Arumugam M, Raes J, Pelletier E, Le Paslier D, Yamada T, Mende DR et al (2011) Enterotypes of the human gut microbiome. Nature 473(7346):174–180
8. Johnson AJ, Vangay P, Al-Ghalith GA, Hillmann BM, Ward TL, Shields-Cutler RR et al (2019) Daily sampling reveals personalized diet-microbiome associations in humans. Cell Host Microbe 25(6):789–802.e5
9. Rinninella E, Raoul P, Cintoni M, Franceschi F, Miggiano GAD, Gasbarrini A et al (2019) What is the healthy gut microbiota composition? A changing ecosystem across age,

environment, diet, and diseases. Microorganisms 7(1). pii: E14. https://doi.org/10.3390/microorganisms7010014
10. Rothschild D, Weissbrod O, Barkan E, Kurilshikov A, Korem T, Zeevi D et al (2018) Environment dominates over host genetics in shaping human gut microbiota. Nature 555(7695):210–215
11. Nicolas GR, Chang PV (2019) Deciphering the chemical lexicon of host–gut microbiota interactions. Trends Pharmacol Sci 40(6):430–445
12. Koh A, De Vadder F, Kovatcheva-Datchary P, Bäckhed F (2016) From dietary fiber to host physiology: short-chain fatty acids as key bacterial metabolites. Cell 165(6):1332–1345
13. Vinolo MAR, Rodrigues HG, Nachbar RT, Curi R (2011) Regulation of inflammation by short chain fatty acids. Nutrients 3(10):858–876
14. Luu M, Pautz S, Kohl V, Singh R, Romero R, Lucas S et al (2019) The short-chain fatty acid pentanoate suppresses autoimmunity by modulating the metabolic-epigenetic crosstalk in lymphocytes. Nat Commun 10(1):760. https://doi.org/10.1038/s41467-019-08711-2
15. Fachi JL (2019) Role of microbiota-derived metabolites, the short-chain fatty acids, on innate lymphoid cells. Sci Rep Res Internships Abroad. https://bv.fapesp.br/en/bolsas/178988/role-of-microbiota-derived-metabolites-the-short-chain-fatty-acids-on-innate-lymphoid-cells/
16. Sorbara MT, Dubin K, Littmann ER, Moody TU, Fontana E, Seok R et al (2019) Inhibiting antibiotic-resistant Enterobacteriaceae by microbiota-mediated intracellular acidification. J Exp Med 216(1):84–98
17. Chun E, Lavoie S, Fonseca-Pereira D, Bae S, Michaud M, Hoveyda HR et al (2019) Metabolite-sensing receptor Ffar2 regulates colonic group 3 innate lymphoid cells and gut immunity. Immunity 51(5):871–884.e6
18. Galvão I, Tavares LP, Corrêa RO, Fachi JL, Rocha VM, Rungue M et al (2018) The metabolic sensor GPR43 receptor plays a role in the control of Klebsiella pneumoniae infection in the lung. Front Immunol 9:142. https://doi.org/10.3389/fimmu.2018.00142
19. Trompette A, Gollwitzer ES, Pattaroni C, Lopez-Mejia IC, Riva E, Pernot J et al (2018) Dietary fiber confers protection against flu by shaping Ly6c− patrolling monocyte hematopoiesis and CD8+ T cell metabolism. Immunity 48(5):992–1005.e8
20. Antunes KH, Fachi JL, de Paula R, da Silva EF, Pral LP, dos Santos AÁ et al (2019) Microbiota-derived acetate protects against respiratory syncytial virus infection through a GPR43-type 1 interferon response. Nat Commun 10(1):1–17
21. Corrêa-Oliveira R, Fachi JL, Vieira A, Sato FT, Vinolo MAR (2016) Regulation of immune cell function by short-chain fatty acids. Clin Transl Immunol 5(4):e73. https://doi.org/10.1038/cti.2016.17
22. Fellows R, Denizot J, Stellato C, Cuomo A, Jain P, Stoyanova E et al (2018) Microbiota derived short chain fatty acids promote histone crotonylation in the colon through histone deacetylases. Nat Commun 9(1):105. https://doi.org/10.1038/s41467-017-02651-5
23. Kim M, Qie Y, Park J, Kim CH (2017) Gut microbial metabolites fuel host antibody responses. Cell Host Microbe 20(2):202–214
24. Bachem A, Makhlouf C, Binger KJ, de Souza DP, Tull D, Hochheiser K et al (2019) Microbiota-derived short-chain fatty acids promote the memory potential of antigen-activated CD8+ T cells. Immunity 51(2):285–297.e5
25. Byndloss MX, Olsan EE, Rivera-Chávez F, Tiffany CR, Cevallos SA, Lokken KL et al (2017) Microbiota-activated PPAR-γ signaling inhibits dysbiotic Enterobacteriaceae expansion. Science 357(6351):570–575
26. Kelly CJ, Zheng L, Campbell EL, Saeedi B, Scholz CC, Bayless AJ et al (2015) Crosstalk between microbiota-derived short-chain fatty acids and intestinal epithelial HIF augments tissue barrier function. Cell Host Microbe 17(5):662–671
27. Cho NH, Shaw JE, Karuranga S, Huang Y, da Rocha Fernandes JD, Ohlrogge AW et al (2018) IDF Diabetes Atlas: global estimates of diabetes prevalence for 2017 and projections for 2045. Diabetes Res Clin Pract 138:271–281

28. Tuomi T, Santoro N, Caprio S, Cai M, Weng J, Groop L (2014) The many faces of diabetes: a disease with increasing heterogeneity. Lancet 383(9922):1084–1094
29. Blüher M (2019) Obesity: global epidemiology and pathogenesis. Nat Rev Endocrinol 15(5):288–298
30. Hotamisligil GS (2017) Inflammation, metaflammation and immunometabolic disorders. Nature 542(7640):177–185
31. Goldfine AB, Shoelson SE (2017) Therapeutic approaches targeting inflammation for diabetes and associated cardiovascular risk. J Clin Invest 127(1):83–93
32. Ridaura VK, Faith JJ, Rey FE, Cheng J, Alexis E, Kau AL et al (2014) Cultured gut microbiota from twins discordant for obesity modulate adiposity and metabolic phenotypes in mice. Science 341(6150):1241214. https://doi.org/10.1126/science.1241214
33. Vrieze A, Van Nood E, Holleman F, Salojärvi J, Kootte RS, Bartelsman JFWM et al (2012) Transfer of intestinal microbiota from lean donors increases insulin sensitivity in individuals with metabolic syndrome. Gastroenterology 143(4):913–916.e7
34. Sanna S, van Zuydam NR, Mahajan A, Kurilshikov A, Vich Vila A, Võsa U et al (2019) Causal relationships among the gut microbiome, short-chain fatty acids and metabolic diseases. Nat Genet 51(4):600–605
35. Gao Z, Yin J, Zhang J, Ward RE, Martin RJ, Lefevre M et al (2009) Butyrate improves insulin sensitivity and increases energy expenditure in mice. Diabetes 58(7):1509–1517
36. Vinolo MAR, Rodrigues HG, Festuccia WT, Crisma AR, Alves VS, Martins AR et al (2012) Tributyrin attenuates obesity-associated inflammation and insulin resistance in high-fat-fed mice. Am J Physiol Endocrinol Metab 303(2):E272–E282
37. Den Besten G, Van Eunen K, Groen AK, Venema K, Reijngoud DJ, Bakker BM (2013) The role of short-chain fatty acids in the interplay between diet, gut microbiota, and host energy metabolism. J Lipid Res 54(9):2325–2340
38. Chambers ES, Viardot A, Psichas A, Morrison DJ, Murphy KG, Zac-Varghese SEK et al (2015) Effects of targeted delivery of propionate to the human colon on appetite regulation, body weight maintenance and adiposity in overweight adults. Gut 64(11):1744–1754
39. Tolhurst G, Heffron H, Lam YS, Parker HE, Habib AM, Diakogiannaki E et al (2012) Short-chain fatty acids stimulate glucagon-like peptide-1 secretion via the G-protein-coupled receptor FFAR2. Diabetes 61(2):364–371
40. Müller M, Hernández MAG, Goossens GH, Reijnders D, Holst JJ, Jocken JWE et al (2019) Circulating but not faecal short-chain fatty acids are related to insulin sensitivity, lipolysis and GLP-1 concentrations in humans. Sci Rep 9(1):12515. https://doi.org/10.1038/s41598-019-48775-0
41. De Silva A, Bloom SR (2012) Gut hormones and appetite control: a focus on PYY and GLP-1 as therapeutic targets in obesity. Gut Liver 6(1):10–20
42. Frost G, Sleeth ML, Sahuri-Arisoylu M, Lizarbe B, Cerdan S, Brody L et al (2014) The short-chain fatty acid acetate reduces appetite via a central homeostatic mechanism. Nat Commun 5:3611. https://doi.org/10.1038/ncomms4611
43. Xiong Y, Miyamoto N, Shibata K, Valasek MA, Motoike T, Kedzierski RM et al (2004) Short-chain fatty acids stimulate leptin production in adipocytes through the G protein-coupled receptor GPR41. Proc Natl Acad Sci U S A 101(4):1045–1050
44. Smith PM, Howitt MR, Panikov N, Michaud M, Gallini CA, Bohlooly-Y M et al (2018) The microbial metabolites, short chain fatty acids, regulate colonic Treg cell homeostasis. Science 341(6145):569–573
45. Macia L, Tan J, Vieira AT, Leach K, Stanley D, Luong S et al (2015) Metabolite-sensing receptors GPR43 and GPR109A facilitate dietary fibre-induced gut homeostasis through regulation of the inflammasome. Nat Commun 6:6734. https://doi.org/10.1038/ncomms7734
46. Cani PD, Neyrinck AM, Fava F, Knauf C, Burcelin RG, Tuohy KM et al (2007) Selective increases of bifidobacteria in gut microflora improve high-fat-diet-induced diabetes in mice through a mechanism associated with endotoxaemia. Diabetologia 50(11):2374–2383

47. Perry RJ, Peng L, Barry NA, Cline GW, Zhang D, Cardone RL et al (2016) Acetate mediates a microbiome-brain-β-cell axis to promote metabolic syndrome. Nature 534(7606):213–217
48. Tang C, Ahmed K, Gille A, Lu S, Gröne HJ, Tunaru S et al (2015) Loss of FFA2 and FFA3 increases insulin secretion and improves glucose tolerance in type 2 diabetes. Nat Med 21(2):173–177
49. Word Health Organization (2015) About cardiovascular disease. In: Cardiovascular disease. https://www.who.int/cardiovascular_diseases/about_cvd/en/. Accessed 02 Nov 2019
50. Word Health Organization (2018) The top 10 causes of death. https://www.who.int/en/news-room/fact-sheets/detail/the-top-10-causes-of-death. Accessed 02 Nov 2019
51. Guimarães RM, De Araújo Andrade SSC, Bahia CA, Machado EL, De Oliveira MM, Jacques FVL (2015) Regional differences in cardiovascular mortality transition in Brazil, 1980 to 2012. Rev Panam Salud Publica 37(2):83–89
52. Tang WHW, Hazen SL (2014) The contributory role of gut microbiota in cardiovascular disease. J Clin Invest 124(10):4204–4211
53. Tang WHW, Kitai T, Hazen SL (2017) Gut microbiota in cardiovascular health and disease. Circ Res 120(7):1183–1196
54. Jie Z, Xia H, Zhong SL, Feng Q, Li S, Liang S et al (2017) The gut microbiome in atherosclerotic cardiovascular disease. Nat Commun 8(1):845. https://doi.org/10.1038/s41467-017-00900-1
55. Karlsson FH, Fåk F, Nookaew I, Tremaroli V, Fagerberg B, Petranovic D et al (2012) Symptomatic atherosclerosis is associated with an altered gut metagenome. Nat Commun 3:1245. https://doi.org/10.1038/ncomms2266
56. Yang T, Santisteban MM, Rodriguez V, Li E, Ahmari N, Sahay B et al (2016) Gut microbiota dysbiosis is linked to hypertension. Hypertension 65(6):1331–1340
57. Gómez-Guzmán M, Toral M, Romero M, Jiménez R, Galindo P, Sánchez M et al (2015) Antihypertensive effects of probiotics Lactobacillus strains in spontaneously hypertensive rats. Mol Nutr Food Res 59(11):2326–2336
58. Malik M, Suboc TM, Tyagi S, Salzman N, Wang J, Ying R et al (2018) Lactobacillus plantarum 299v supplementation improves vascular endothelial function and reduces inflammatory biomarkers in men with stable coronary artery disease. Circ Res 123(9):1091–1102
59. Brown JM, Hazen SL (2015) The gut microbial endocrine organ: bacterially derived signals driving cardiometabolic diseases. Annu Rev Med 66:343–359
60. Yang T, Magee KL, Colon-Perez LM, Larkin R, Liao YS, Balazic E et al (2019) Impaired butyrate absorption in the proximal colon, low serum butyrate and diminished central effects of butyrate on blood pressure in spontaneously hypertensive rats. Acta Physiol (Oxf) 226(2):e13256. https://doi.org/10.1111/apha.13256
61. Hsu CN, Chang-Chien GP, Lin S, Hou CY, Tain YL (2019) Targeting on gut microbial metabolite trimethylamine-N-oxide (TMAO) and short chain fatty acid to prevent maternal high-fructose diet-induced developmental programming of hypertension in adult male offspring. Mol Nutr Food Res 63(18):e1900073. https://doi.org/10.1002/mnfr.201900073
62. Pluznick JL, Protzko RJ, Gevorgyan H, Peterlin Z, Sipos A, Han J et al (2013) Olfactory receptor responding to gut microbiota derived signals plays a role in renin secretion and blood pressure regulation. Proc Natl Acad Sci U S A 110(11):4410–4415
63. Dinan TG, Cryan JF (2017) The microbiome-gut-brain axis in health and disease. Gastroenterol Clin N Am 46(1):77–89
64. Erny D, De Angelis ALH, Jaitin D, Wieghofer P, Staszewski O, David E et al (2015) Host microbiota constantly control maturation and function of microglia in the CNS. Nat Neurosci 18(7):965–977
65. Spielman LJ, Gibson DL, Klegeris A (2018) Unhealthy gut, unhealthy brain: the role of the intestinal microbiota in neurodegenerative diseases. Neurochem Int 120:149–163
66. Fung TC, Olson CA, Hsiao EY (2017) Interactions between the microbiota, immune and nervous systems in health and disease. Nat Neurosci 20(2):145–155

67. Braniste V, Al-Asmakh M, Kowal C, Anuar F, Abbaspour A, Tóth M et al (2014) The gut microbiota influences blood-brain barrier permeability in mice. Sci Transl Med 6(263):263ra158. https://doi.org/10.1126/scitranslmed.3009759
68. Gitler AD, Dhillon P, Shorter J (2017) Neurodegenerative disease: models, mechanisms, and a new hope. DMM Dis Model Mech 10(5):499–502
69. Erkkinen MG, Kim M, Geschwind MD (2018) Clinical neurology and epidemiology of the major neurodegenerative diseases. Cold Spring Harb Perspect Biol 10(4). pii: a033118. https://doi.org/10.1101/cshperspect.a033118
70. Keshavarzian A, Green SJ, Engen PA, Voigt RM, Naqib A, Forsyth CB et al (2015) Colonic bacterial composition in Parkinson's disease. Mov Disord 30(10):1351–1360
71. Scheperjans F, Aho V, Pereira PAB, Koskinen K, Paulin L, Pekkonen E et al (2015) Gut microbiota are related to Parkinson's disease and clinical phenotype. Mov Disord 30(3):350–358
72. Schwiertz A, Spiegel J, Dillmann U, Grundmann D, Bürmann J, Faßbender K et al (2018) Fecal markers of intestinal inflammation and intestinal permeability are elevated in Parkinson's disease. Park Relat Disord 50:104–107
73. Unger MM, Spiegel J, Dillmann KU, Grundmann D, Philippeit H, Bürmann J et al (2016) Short chain fatty acids and gut microbiota differ between patients with Parkinson's disease and age-matched controls. Park Relat Disord 32:66–72
74. St. Laurent R, O'Brien LM, Ahmad ST (2013) Sodium butyrate improves locomotor impairment and early mortality in a rotenone-induced Drosophila model of Parkinson's disease. Neuroscience 246:382–390
75. Kidd SK, Schneider JS (2010) Protection of dopaminergic cells from MPP+-mediated toxicity by histone deacetylase inhibition. Brain Res 1354:172–178
76. Paiva I, Pinho R, Pavlou MA, Hennion M, Wales P, Schütz AL et al (2017) Sodium butyrate rescues dopaminergic cells from alpha-synuclein-induced transcriptional deregulation and DNA damage. Hum Mol Genet 26(12):2231–2246
77. Sharma S, Taliyan R, Singh S (2015) Beneficial effects of sodium butyrate in 6-OHDA induced neurotoxicity and behavioral abnormalities: modulation of histone deacetylase activity. Behav Brain Res 291:306–314
78. Sampson TR, Debelius JW, Thron T, Janssen S, Shastri GG, Ilhan ZE et al (2016) Gut microbiota regulate motor deficits and neuroinflammation in a model of Parkinson's disease. Cell 167(6):1469–1480.e12
79. Long ZM, Zhao L, Jiang R, Wang KJ, Luo SF, Zheng M et al (2015) Valproic acid modifies synaptic structure and accelerates neurite outgrowth via the glycogen synthase kinase-3β signaling pathway in an Alzheimer's disease model. CNS Neurosci Ther 21(11):887–897
80. Govindarajan N, Agis-Balboa RC, Walter J, Sananbenesi F, Fischer A (2011) Sodium butyrate improves memory function in an Alzheimer's disease mouse model when administered at an advanced stage of disease progression. J Alzheimers Dis 26(1):187–197
81. Kong Y, Jiang B, Luo X (2018) Gut microbiota influences Alzheimer's disease pathogenesis by regulating acetate in Drosophila model. Future Microbiol 13:1117–1128
82. Minter MR, Zhang C, Leone V, Ringus DL, Zhang X, Oyler-Castrillo P et al (2016) Antibiotic-induced perturbations in gut microbial diversity influences neuro-inflammation and amyloidosis in a murine model of Alzheimer's disease. Sci Rep 6:30028. https://doi.org/10.1038/srep30028
83. Jones L, Hughes A (2011) Pathogenic mechanisms in Huntington's disease. Int Rev Neurobiol 98:373–418
84. Kong G, Cao K-AL, Judd LM, Li S, Renoir T, Hannan AJ (2018) Microbiome profiling reveals gut dysbiosis in a transgenic mouse model of Huntington's disease. Neurobiol Dis 5:104268. https://doi.org/10.1016/j.nbd.2018.09.001. [Epub ahead of print]
85. Zhang YG, Wu S, Yi J, Xia Y, Jin D, Zhou J et al (2017) Target intestinal microbiota to alleviate disease progression in amyotrophic lateral sclerosis. Clin Ther 39(2):322–336

86. Chou AH, Chen SY, Yeh TH, Weng YH, Wang HL (2011) HDAC inhibitor sodium butyrate reverses transcriptional downregulation and ameliorates ataxic symptoms in a transgenic mouse model of SCA3. Neurobiol Dis 41(2):481–488
87. Dzutsev A, Badger JH, Perez-Chanona E, Roy S, Salcedo R, Smith CK et al (2017) Microbes and Cancer. Annu Rev Immunol 35:199–228
88. Tsilimigras MCB, Fodor A, Jobin C (2017) Carcinogenesis and therapeutics: the microbiota perspective. Nat Microbiol 2:17008. https://doi.org/10.1038/nmicrobiol.2017.8
89. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A (2018) Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 68(6):394–424
90. Siegel RL, Miller KD, Jemal A (2015) Cancer statistics, 2015. CA Cancer J Clin 65(1):5–29
91. American Cancer Society (2019) Cancer facts & figures 2019. In: CA: A Cancer Journal for Clinicians. American Cancer Society. https://www.cancer.org/content/dam/cancer-org/research/cancer-facts-and-statistics/annual-cancer-facts-and-figures/2019/cancer-facts-and-figures-2019.pdf. Accessed 15 Oct 2019
92. Helmink BA, Khan MAW, Hermann A, Gopalakrishnan V, Wargo JA (2019) The microbiome, cancer, and cancer therapy. Nat Med 25(3):377–388
93. Vivarelli S, Salemi R, Candido S, Falzone L, Santagati M, Stefani S et al (2019) Gut microbiota and cancer: from pathogenesis to therapy. Cancers (Basel) 11(1). pii: E38. https://doi.org/10.3390/cancers11010038
94. Triff K, McLean MW, Callaway E, Goldsby J, Ivanov I, Chapkin RS (2018) Dietary fat and fiber interact to uniquely modify global histone post-translational epigenetic programming in a rat colon cancer progression model. Int J Cancer 143(6):1402–1415
95. Chen HM, Yu YN, Wang JL, Lin YW, Kong X, Yang CQ et al (2013) Decreased dietary fiber intake and structural alteration of gut microbiota in patients with advanced colorectal adenoma. Am J Clin Nutr 97(5):1044–1052
96. Wang G, Yu Y, Wang YZ, Wang JJ, Guan R, Sun Y et al (2019) Role of SCFAs in gut microbiome and glycolysis for colorectal cancer therapy. J Cell Physiol 234(10):17023–17049
97. Bultman SJ (2014) Molecular pathways: gene-environment interactions regulating dietary fiber induction of proliferation and apoptosis via butyrate for cancer prevention. Clin Cancer Res 20(4):799–803
98. Bishehsari F, Engen PA, Preite NZ, Tuncil YE, Naqib A, Shaikh M et al (2018) Dietary fiber treatment corrects the composition of gut microbiota, promotes SCFA production, and suppresses colon carcinogenesis. Genes (Basel) 9(2). pii: E102. https://doi.org/10.3390/genes9020102
99. Foglietta F, Serpe L, Canaparo R, Vivenza N, Riccio G, Imbalzano E et al (2014) Modulation of butyrate anticancer activity by solid lipid nanoparticle delivery: an in vitro investigation on human breast cancer and leukemia cell lines. J Pharm Pharm Sci 17(2):231–247
100. Pattayil L, Thampi H, Saraswathi B (2019) In vitro evaluation of apoptotic induction of butyric acid derivatives in colorectal carcinoma cells. Anticancer Res 39(7):3795–3801
101. Queirós O, Preto A, Pacheco A, Pinheiro C, Azevedo-Silva J, Moreira R et al (2012) Butyrate activates the monocarboxylate transporter MCT4 expression in breast cancer cells and enhances the antitumor activity of 3-bromopyruvate. J Bioenerg Biomembr 44(1):141–153
102. Yamamura T, Matsumoto N, Matsue Y, Okudera M, Nishikawa Y, Abiko Y et al (2014) Sodium butyrate, a histone deacetylase inhibitor, regulates lymphangiogenic factors in oral cancer cell line HSC-3. Anticancer Res 34(4):1701–1708
103. Sivaprakasam S, Prasad PD, Singh N (2016) Benefits of short-chain fatty acids and their receptors in inflammation and carcinogenesis. Pharmacol Ther 164:144–151
104. van der Beek CM, Dejong CHC, Troost FJ, Masclee AAM, Lenaerts K (2017) Role of short-chain fatty acids in colonic inflammation, carcinogenesis, and mucosal protection and healing. Nutr Rev 75(4):286–305

105. Chen J, Vitetta L (2018) Inflammation-modulating effect of butyrate in the prevention of colon cancer by dietary fiber. Clin Colorectal Cancer 17(3):e541–e544. https://doi.org/10.1016/j.clcc.2018.05.001
106. Heidor R, Silva K, Festa J, Franco T, Oliveira D, Eduardo P et al (2014) The chemopreventive activity of the histone deacetylase inhibitor tributyrin in colon carcinogenesis involves the induction of apoptosis and reduction of DNA damage. Toxicol Appl Pharmacol 276(2):129–135
107. Wei W, Sun W, Yu S, Yang Y, Ai L (2016) Butyrate production from high-fiber diet protects against lymphoma tumor. Leuk Lymphoma 57(10):2401–2408
108. Kuroiwa-Trzmielina J, De Conti A, Scolastici C, Pereira D, Horst MA, Purgatto E et al (2009) Chemoprevention of rat hepatocarcinogenesis with histone deacetylase inhibitors: efficacy of tributyrin, a butyric acid prodrug. Int J Cancer 124(11):2520–2527
109. Kuefer R, Hofer MD, Altug V, Zorn C, Genze F, Kunzi-Rapp K et al (2004) Sodium butyrate and tributyrin induce in vivo growth inhibition and apoptosis in human prostate cancer. Br J Cancer 90(2):535–541
110. Biondo LA, Teixeira AAS, Silveira LS, Souza CO, Costa RGF, Diniz TA et al (2019) Tributyrin in inflammation: does white adipose tissue affect colorectal cancer? Nutrients 11(1). pii: E110. https://doi.org/10.3390/nu11010110
111. Kang HR, Choi HG, Jeon CK, Lim SJ, Kim SH (2016) Butyrate-mediated acquisition of chemoresistance by human colon cancer cells. Oncol Rep 36(2):1119–1126
112. Serpa J, Caiado F, Carvalho T, Torre C, Gonçalves LG, Casalou C et al (2010) Butyrate-rich colonic microenvironment is a relevant selection factor for metabolically adapted tumor cells. J Biol Chem 285(5):39211–39223
113. Ley R, Balmanno K, Hadfield K, Weston C, Cook SJ (2003) Activation of the ERK1/2 signaling pathway promotes phosphorylation and proteasome-dependent degradation of the BH3-only protein, Bim. J Biol Chem 278(21):18811–18816
114. Xiao M, Liu YG, Zou MC, Zou F (2014) Sodium butyrate induces apoptosis of human colon cancer cells by modulating ERK and sphingosine kinase 2. Biomed Environ Sci 27(3):197–203
115. Xiao M, Liu Y, Zou F (2012) Sensitization of human colon cancer cells to sodium butyrate-induced apoptosis by modulation of sphingosine kinase 2 and protein kinase D. Exp Cell Res 318(1):43–52
116. Zhang J, Yi M, Zha L, Chen S, Li Z, Li C et al (2016) Sodium butyrate induces endoplasmic reticulum stress and autophagy in colorectal cells: implications for apoptosis. PLoS One 11(1):e0147218. https://doi.org/10.1371/journal.pone.0147218
117. Tang Y, Chen Y, Jiang H, Nie D (2011) Short-chain fatty acids induced autophagy serves as an adaptive strategy for retarding mitochondria-mediated apoptotic cell death. Cell Death Differ 18(4):602–618
118. Tang Y, Chen Y, Jiang H, Nie D (2011) The role of short-chain fatty acids in orchestrating two types of programmed cell death in colon cancer. Autophagy 7(2):235–237
119. Luo S, Li Z, Mao L, Chen S, Sun S (2019) Sodium butyrate induces autophagy in colorectal cancer cells through LKB1/AMPK signaling. J Physiol Biochem 75(1):53–63
120. Lyssiotis CA, Cantley LC (2014) Acetate fuels the cancer engine. Cell 159(7):1492–1494
121. Mashimo T, Pichumani K, Vemireddy V, Hatanpaa KJ, Singh DK, Sirasanagandla S et al (2014) Acetate is a bioenergetic substrate for human glioblastoma and brain metastases. Cell 159(7):1603–1614
122. Comerford SA, Huang Z, Du X, Wang Y, Cai L, Witkiewicz AK et al (2014) Acetate dependence of tumors. Cell 159(7):1591–1602
123. Marques C, Oliveira CSF, Alves S, Chaves SR, Coutinho OP, Côrte-Real M et al (2013) Acetate-induced apoptosis in colorectal carcinoma cells involves lysosomal membrane permeabilization and cathepsin D release. Cell Death Dis 4:e507. https://doi.org/10.1038/cddis.2013.29

124. Pandey SK, Yadav S, Temre MK, Singh SM (2018) Tracking acetate through a journey of living world: evolution as alternative cellular fuel with potential for application in cancer therapeutics. Life Sci 215:86–95
125. Zeng H, Umar S, Rust B, Lazarova D, Bordonaro M (2019) Secondary bile acids and short chain fatty acids in the colon: a focus on colonic microbiome, cell proliferation, inflammation, and cancer. Int J Mol Sci 20(5). pii: E1214. https://doi.org/10.3390/ijms20051214
126. Li Q, Cao L, Tian Y, Zhang P, Ding C, Lu W et al (2018) Butyrate suppresses the proliferation of colorectal cancer cells via targeting pyruvate kinase M2 and metabolic reprogramming. Mol Cell Proteomics 17(8):1531–1545
127. Singh V, Yeoh BS, Chassaing B, Xiao X, Saha P, Aguilera Olvera R et al (2018) Dysregulated microbial fermentation of soluble fiber induces cholestatic liver cancer. Cell 175(3):679–694.e22
128. Kaiko GE, Ryu SH, Koues OI, Collins PL, Solnica-Krezel L, Pearce EJ et al (2016) The colonic crypt protects stem cells from microbiota-derived metabolites. Cell 165(7):1708–1720
129. Johnstone M, Bennett N, Standifer C, Smith A, Han A, Bettaieb A et al (2017) Characterization of the pro-inflammatory cytokine IL-1β on butyrate oxidation in colorectal cancer cells. J Cell Biochem 118(6):1614–1621
130. Ferro S, Azevedo-Silva J, Casal M, Côrte-Real M, Baltazar F, Preto A (2016) Characterization of acetate transport in colorectal cancer cells and potential therapeutic implications. Oncotarget 7(43):70639–70653
131. Li Q, Ding C, Meng T, Lu W, Liu W, Hao H et al (2017) Butyrate suppresses motility of colorectal cancer cells via deactivating Akt/ERK signaling in histone deacetylase dependent manner. J Pharmacol Sci 135(4):148–155
132. Shi L, Tu BP (2015) Acetyl-CoA and the regulation of metabolism: mechanisms and consequences. Curr Opin Cell Biol 33:125–131
133. Zeng H, Taussig DP, Cheng WH, Johnson LAK, Hakkak R (2017) Butyrate inhibits cancerous HCT116 colon cell proliferation but to a lesser extent in noncancerous NCM460 colon cells. Nutrients 9(1). pii: E25. https://doi.org/10.3390/nu9010025
134. Cho Y, Turner ND, Davidson LA, Chapkin RS, Carroll RJ, Lupton JR (2014) Colon cancer cell apoptosis is induced by combined exposure to the n-3 fatty acid docosahexaenoic acid and butyrate through promoter methylation. Exp Biol Med 239(3):302–310
135. Ryu TY, Kim K, Son MY, Min JK, Kim J, Han TS et al (2019) Downregulation of PRMT1, a histone arginine methyltransferase, by sodium propionate induces cell apoptosis in colon cancer. Oncol Rep 41(3):1691–1699
136. Hu S, Dong TS, Dalal SR, Wu F, Bissonnette M, Kwon JH et al (2011) The microbe-derived short chain fatty acid butyrate targets miRNA-dependent p21 gene expression in human colon Cancer. PLoS One 6(1):e16221. https://doi.org/10.1371/journal.pone.0016221
137. Hu S, Liu L, Chang EB, Wang JY, Raufman JP (2015) Butyrate inhibits pro-proliferative miR-92a by diminishing c-Myc-induced miR-17-92a cluster transcription in human colon cancer cells. Mol Cancer 14:1–15
138. Xu Z, Tao J, Chen P, Chen L, Sharma S, Wang G et al (2018) Sodium butyrate inhibits colorectal cancer cell migration by downregulating Bmi-1 through enhanced miR-200c expression. Mol Nutr Food Res 62(6):e1700844. https://doi.org/10.1002/mnfr.201700844
139. Tang Y, Chen Y, Jiang H, Robbins GT, Nie D (2011) G-protein-coupled receptor for short-chain fatty acids suppresses colon cancer. Int J Cancer 128(4):847–856
140. Kim M, Friesen L, Park J, Kim HM, Kim CH (2018) Microbial metabolites, short-chain fatty acids, restrain tissue bacterial load, chronic inflammation, and associated cancer in the colon of mice. Eur J Immunol 48(7):1235–1247
141. Thangaraju M (2009) GPR109A is a G-protein-coupled receptor for the bacterial fermentation product butyrate and functions as a tumor suppressor in colon. Cancer Res 69(7):2826–2832
142. Elangovan S, Pathania R, Ramachandran S, Ananth S, Padia RN, Lan L et al (2014) The niacin/butyrate receptor GPR109A suppresses mammary tumorigenesis by inhibiting cell survival. Cancer Res 74(4):1166–1178

143. Sun X, Zhu MJ (2018) Butyrate inhibits indices of colorectal carcinogenesis via enhancing α-ketoglutarate-dependent DNA demethylation of mismatch repair genes. Mol Nutr Food Res 62(10):e1700932. https://doi.org/10.1002/mnfr.201700932
144. Zhang Y, Zhou L, Bao YL, Wu Y, Yu CL, Huang YX et al (2010) Butyrate induces cell apoptosis through activation of JNK MAP kinase pathway in human colon cancer RKO cells. Chem Biol Interact 185(3):174–181
145. Lazarova DL, Chiaro C, Wong T, Drago E, Rainey A, O'Malley S et al (2013) CBP activity mediates effects of the histone deacetylase inhibitor butyrate on WNT activity and apoptosis in colon cancer cells. J Cancer 4(6):481–490

Chapter 5
The Effects of Parabiosis on Aging and Age-Related Diseases

Vasily V. Ashapkin, Lyudmila I. Kutueva, and Boris F. Vanyushin

1 Introduction

Parabiosis, from the Greek 'para' (next to) and 'bios' (life), refers to the union of two living individuals that can occur either spontaneously as in the case of joined twins or may be produced by surgical operation in which two organisms are joined and develop a shared circulatory system. During the hundred and fifty years since the parabiosis technique was introduced as a research tool, it has been employed in many countries and for diverse experiments [1]. Most fruitful results have been in the fields of pituitary-gonad endocrinology, but it has generally proved to be a useful tool in any problem which involves humoral transmission. The most significant result obtained by the use of parabiosis in the study of pituitary-gonad relations is the demonstration of the existence of a feedback regulation loop: gonadectomy induces gonadotrophic hypersecretion and the administration of gonadal hormones can suppress this hypersecretion. The effect of constant hyperglycemia on pancreatic secretion was studied by making one of two parabiont rats diabetic with alloxan. Approximately 50% of the diabetic rats showed a reduction in hyperglycemia. Exogenous insulin given to the non-diabetic partner reduced the hyperglycemia of the diabetic one, indicating that insulin crosses between the parabiotic pair. The parabiosis technique has also been used to study the factors which control successful skin homografting. If a part of the skin of one animal remained attached to its partner after separation of a parabiont pair, it usually persisted. The success of skin homografts depended to a large extent on the genetic relationship between the host and recipient, with the best results being obtained between young littermates. Parabiosis has been used to determine whether or not resistance to transplanted

V. V. Ashapkin (✉) · L. I. Kutueva · B. F. Vanyushin
Belozersky Institute of Physico-Chemical Biology, Lomonosov Moscow State University, Moscow, Russia
e-mail: ashapkin@genebee.msu.ru

P. C. Guest (ed.), *Reviews on New Drug Targets in Age-Related Disorders*,
Advances in Experimental Medicine and Biology 1260,
https://doi.org/10.1007/978-3-030-42667-5_5

tumors is dependent upon the presence of circulating antibodies. The union of susceptible and resistant strains of rats or mice has generally shown no alteration in the rate of tumor growth. It was shown that the complete nephrectomy of one parabiont partner was enough to induce hypertension in that partner, whereas the blood pressure of the other remained normal. The parabiotic technique has been successfully used to determine the role of the bloodstream in transmitting the stimulus to the mitotic activity which initiates hepatic regeneration. A high rate of mitosis in the liver of partners of partially hepatectomized rats was observed, whereas a low rate of mitosis is characteristic of the normal adult liver. Numerous research publications describe the use of parabiosis for studies of allergy and immunity. In homogenous parabiont pairs, induced immunity in one partner was usually transferred readily to the other. Important contributions by the parabiosis method were obtained in irradiation studies and other fields.

The experimental technique to establish parabiosis in animals was first introduced by the French physiologist Paul Bert in the 1860s using white albino rats in an attempt to understand and facilitate organ transplantation. Although mice are being used preferentially today, the surgical procedure still follows Bert's initial descriptions. In general, skin incisions extending along the adjoining flanks of two mice are made, and adjacent skin flaps are sutured between the animals. In current protocols, the incisions typically extend along the whole body flank. The peritoneum is also incised and sutured together to form a common peritoneal cavity. As a result of revascularization of the injured tissue, blood vessels between the two animals join to form anastomoses and establish a joint vascular system. The animals used in the parabiosis experiment are genetically identical to preclude the 'tissue' rejection [2]. Parabiosis, therefore, enables researchers to ask whether or not transmissible factors in the blood of one parabiont, have physiological effects on its partner. In other words, parabiosis allows researchers to explore whether circulating factors in the bloodstream can alter tissue function. This procedure enables a holistic approach to study biological processes and diseases where there are known organism-wide changes, such as those associated with diet and aging [2]. Heterochronic parabiosis, pairing together a young and aged organism, provides a unique experimental design to assess the effects of the systemic environment on age-related processes and longevity [3]. In the first heterochronic grafting together of young and old rats, the older heterochronic parabiont had an extended life span compared with aged isochronic counterparts [4]. This effect, especially pronounced in female rats, provided the first evidence of the possible rejuvenation by the youthful systemic milieu. This experimental approach to study the aging processes at the whole organism level underwent a renaissance recently, with several studies demonstrating the rejuvenating effects of youthful systemic milieu on aging processes throughout the body.

2 Organ and Tissue Rejuvenation via Heterochronic Parabiosis

2.1 Liver

Heterochronic parabiosis, the parabiotic pairing of two animals of different ages, has been extensively used for the last 15 years as an experimental system to test the effects of systemic milieu on the process of aging at the cell, tissue, and organismal levels. In young isochronic parabionts, the levels of basal hepatocyte proliferation were two- to three-fold higher than in non-parabiosis controls [5]. The proliferation of hepatocytes in old isochronic parabionts was less than in young isochronic parabionts, consistent with the known age-related decline in the basal rate of hepatocyte proliferation. Parabiosis to young mice significantly increased hepatocyte proliferation in aged partners. Moreover, a small reduction of progenitor cell proliferation was detected in the livers of young parabiont partners. In addition, as in muscle, the enhancement of hepatocyte proliferation in aged mice was due to resident cells and not the engraftment of circulating cells from the young partner. The age-related decline in hepatocyte proliferation is due to the formation of an age-specific complex between the chromatin remodeling factor Brm and CCAAT/enhancer-binding protein alpha (cEBP-α) that inhibits E2F transcription factor-driven gene expression. This inhibitory complex was detected in liver extracts from old isochronic parabionts but not in young isochronic parabionts. The formation of the cEBPα–Brm complex was diminished in liver extracts from old heterochronic parabionts. The complex was present at elevated levels in young heterochronic parabionts compared with young controls, consistent with the modest inhibition of hepatocyte proliferation.

2.2 Skeletal Muscle

Heterochronic parabiosis was found to restore the activity of the Notch signaling pathway, as well as the proliferative and regenerative capacity of the aged skeletal muscle satellite cells [5]. After muscle injury in young mice, activation of satellite cells leads to proliferative myoblasts that ultimately fuse to form nascent myotubes. Robust regeneration of muscles in young mice have been found to occur 5 days after injury in both isochronic and heterochronic parabiont pairs. In contrast, in old isochronic parabionts, the injured muscle was found to regenerate poorly, typical of aged animals. This loss of muscle regeneration with age is due at least partially to an age-related impairment in the upregulation of the Notch ligand Delta after muscle injury. In heterochronic parabiosis with young mice, the regeneration of muscle in the old partners significantly increased. The appearance of nascent myotubes in these old mice was similar to that seen in young mice. Importantly, this increased regeneration was due to the activation of resident, aged satellite cells, not to the

engraftment of circulating progenitor cells from the young partner. Notably, satellite cells from the aged partners of heterochronic parabionts showed a marked upregulation of Delta, comparable to that found in their young partners and control young mice. There was also a weak inhibition of Delta in satellite cells from the young partners. Further studies of muscle aging have shown that decrease of the Notch-pathway activity and increase of the Wnt- and transforming growth factor beta (TGF-β) pathway activity play a significant role in phenotypic manifestations of aging and that the rejuvenating effects of heterochronic parabiosis may be due to restoration of a more "youthful" balance between their activities [5–7]. These data show that the young systemic environment restores a younger profile of molecular signaling to the aged progenitor cells. Likewise, the young stem cells adopt a more aged molecular and functional state in these heterochronic parabiotic pairings [6, 8]. Thus, age-related decline of progenitor cell activity could be modulated by systemic factors that change with age.

The reduced regenerative capacity of satellite cells in muscles of aged animals could be explained at least partially by the finding that in clonal myogenesis assays, these cells form fewer colonies by up to four-fold compared with cells from young animals [9]. In single-cell gel electrophoresis assays, freshly sorted satellite cells showed a marked increase in DNA damage with age; ~60% of the aged cells exhibited severely compromised DNA integrity. Likewise, ~60% of the satellite cells in aged muscle showed increased immunoreactivity for the phosphorylated form of histone H2AX – a known marker of DNA damage foci. In contrast, young satellite cells were mostly devoid of detectable DNA damage and rarely contained more than two phosphorylated H2AX foci. Satellite cells from aged (22 months-old) joined to young (2 months-old) mice in the heterochronic pairs showed improved colony-forming activity and restored genomic integrity, with DNA damage scores indistinguishable from those of young-isochronic mice and reduced numbers of phosphorylated H2AX foci, as compared with aged-isochronic mice. After 4-weeks of daily intraperitoneal injections of aged mice with recombinant growth differentiation factor 11 (rGDF11; 0.1 mg/kg), satellite cell frequency and the number of satellite cells with intact DNA were significantly increased compared with aged mice that received vehicle injections. The percentage of satellite cells with severely damaged DNA was reduced by a factor of 4.

In contrast, young mice similarly treated with rGDF11 showed no changes in satellite cell frequency, myogenic colony formation, or DNA damage. When a cohort of rGDF11-treated mice was subjected to cryoinjury to the anterior tibialis muscle, myofiber caliber in regenerating muscle in aged mice was increased to 92% of the level seen in young control mice. However, this rGDF11 supplementation did not alter the myofiber caliber of uninjured muscles in young or aged animals. Although no alterations in gross anatomy, body weight, fat mass, or muscle mass were seen in the GDF11-treated aged animals, immunofluorescence analysis demonstrated increases in the size of neuromuscular junctions, while electron microscopy of uninjured muscle revealed striking improvements of myofibrillar and mitochondrial morphology. Treated muscles showed a reduction of atypical

and swollen mitochondria, reduced accumulation of vacuoles, and restoration of regular sarcomeric and interfibrillar mitochondrial patterning. Consistent with these ultrastructural improvements, the levels of peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α), a master regulator of mitochondrial biogenesis, were increased in the muscle of aged GDF11-treated mice. In addition, increased basal levels of autophagosome markers were observed. Collectively, these data suggest that enhanced autophagy/mitophagy and mitochondrial biogenesis likely explain the cellular remodeling of muscle fibers in GDF11-treated aged mice. Furthermore, improvements in muscle ultrastructure and mitochondrial turnover in GDF11-treated aged mice correlate with improved physical function in exercise endurance and grip-strength analyses.

Since the testosterone level is known to decline with aging progressively and to be associated with loss of muscle mass strength, its possible role as a factor mediating effects of heterochronic parabiosis was investigated [10]. Serum testosterone level and gastrocnemius muscle mass were found to be significantly higher in young (5 months-old) male mice compared with their aged counterparts (22–23 months-old). Old mice from 'castrated young with testosterone implants' – old pairing had a significantly higher level of serum testosterone and increased gastrocnemius muscle mass compared with those from 'castrated young' – old pairing. Changes in gastrocnemius muscle mass were significantly ($r = 0.92$; $P < 0.02$) and positively correlated with changes in testosterone levels. Muscle fiber histology in old mice in the young-old or 'castrated young' – old pairing was indistinguishable from that in old controls. Notably, the 'castrated young with testosterone implants' – old pairing resulted in an increase in muscle fiber size in old mice to values similar to those in young controls. Gastrocnemius muscle from the young mice exhibited normal architecture under transmission electron microscopy with abundant normal-looking mitochondria, no intramyofibrillar lipid (IML) accumulation, and no tubular aggregation (TA). In contrast, varying degrees of abnormalities were noted in aged muscle, including mitochondrial swelling with broken cristae, mitochondrial vacuolization, increased IML accumulation, and presence of TA. The young-old pairing partially reversed these changes in old mice, whereas no evidence of such reversion was seen in the 'castrated young' – old pairing. In striking contrast, the 'castrated young with testosterone implants' – old pairing showed remarkable improvement in muscle ultrastructure. The ultrastructural appearance of muscle in these old parabionts was similar to that seen in young mice. Western blotting analysis showed a significant decrease in Notch-1 levels in gastrocnemius muscles from old mice when compared with young animals. The young-old or 'castrated young with testosterone implants' – old pairing muscle from old parabionts exhibited increased Notch-1 expression in comparison to old controls. No such upregulation was detected in old mice from 'castrated young' – old pairing. These results indicate that testosterone may be one of the serum factors necessary for muscle growth seen in aged mice in the heterochronic parabiosis model. It is tempting to speculate that testosterone may restore the aged systemic milieu to its youthful state via stimulation of Notch signaling.

2.3 Nervous System

Like most mammalian tissues, the central nervous system experiences a declined efficiency of regeneration with aging, including reduced remyelination. Partially it occurs due to changes in the environmental signals but also reflects epigenetic changes within aging oligodendrocyte precursor cells, which decrease their ability to differentiate into remyelinating oligodendrocytes. The impact of blood-borne factors on remyelination activity in aged mice was evaluated in a heterochronic parabiosis study [11]. The number of proliferating oligodendrocyte precursor cells was significantly increased, and remyelination improved significantly in heterochronic-old animals compared with isochronic-old controls. These data demonstrate that exposure of aged animals to a youthful systemic environment promotes oligodendrocyte precursor cell proliferation and restores their ability to form mature remyelinating oligodendrocytes to levels indistinguishable from those of young animals.

In mice, adult neurogenesis occurs in local neurogenic niches known as the subventricular zone of the lateral ventricles and the subgranular zone of the hippocampus. Importantly, these neurogenic niches are localized around blood vessels, allowing for potential communication with the systemic milieu. Therefore, the decline in neurogenesis and cognitive functions observed with aging could be due to changes in the systemic milieu composition [8]. In the heterochronic parabiosis pairs of mice, the number of newly born neurons and progenitors decreased in young and increase in old parabionts. Thus, global age-dependent changes of the systemic milieu can modulate neurogenesis in both the young and aged neurogenic niches, potentially contributing to the decline in regenerative capacity in the normal aging brain. When plasma isolated from young or old mice was intravenously injected into young mice, the number of newly born neurons in the dentate gyrus decreased in animals that received old plasma compared to animals that received young plasma. Therefore, some soluble factors present in the old blood inhibit neurogenesis. By a proteomic approach, seventeen proteins were identified whose levels increased with aging in plasma of normal mice and correlated with decreased neurogenesis. Six of these factors, CCL2, CCL11, CCL12, CCL19, haptoglobin, and β2-microglobulin (B2m), were also elevated in young heterochronic parabiont mice. CCL11 is a chemokine involved in allergic responses. It was not previously linked to aging, neurogenesis, or cognition. An age-related increase in CCL11 was detected in plasma and cerebrospinal fluid of healthy human individuals between 20 and 90 years of age. Intraperitoneal injection of CCL11 in young adult mice significantly decreased the number of newly born neurons in the dentate gyrus. Therefore, increasing the systemic level of CCL11 partially recapitulates the inhibitory effects observed with aging and heterochronic parabiosis. Similar results were obtained in a more detailed study of B2m [12]. Collectively, these data link molecular changes in the systemic milieu observed with aging to the age-related decline in adult neurogenesis, synaptic plasticity and cognitive functions.

Genome-wide microarray analysis of hippocampi from aged (18 months-old) isochronic and heterochronic parabiont mice revealed specific differences in gene expression profiles [13]. A gene ontology category most enriched in heterochronic parabionts was synaptic plasticity regulation. Likewise, in silico analysis using the Ingenuity Pathway Analysis software detected the plasticity-related signaling pathways, such as Creb, in the top-signaling network. Increased numbers of cells expressing the immediate early genes *Egr1* and *c-Fos* and an increase in phosphorylated Creb were detected in the dentate gyrus of heterochronic compared with isochronic parabionts. Also, the dendritic spine number on granule cell neurons in the dentate gyrus increased, and synaptic plasticity improved in heterochronic parabionts. At the cognitive level, repeated injections of young blood plasma into aged mice improved age-related cognitive impairments. Structural and cognitive enhancements elicited by exposure to young blood are mediated, in part, by activation of the Creb in the aged hippocampus.

Two distinct strategies for reversing aging brain phenotypes could be used. The first one is the introduction of "pro-youthful' factors from young blood to reverse age-related impairments in the brain. The second one is the abrogation of "pro-aging" factors in aged blood to counteract aging-associated impairments. Of course, these two possibilities are not mutually exclusive and could be combined to combat the effects of aging successfully.

Deterioration of blood vessels with a consequent reduction in blood flow in the neurogenic stem cell niche could be the leading cause of reduced neuroplasticity and impaired cognition in aged animals. Whether or not extrinsic signals from young blood can restore these age-related impairments was investigated in a mouse heterochronic parabiosis model [14]. Heterochronic parabiosis between aged (15 months-old) and young (2 months-old) mice for 5 weeks significantly increased the numbers of proliferative $Ki67^+$ cells, $Sox2^+$ stem cells, and $Olig2^+$ transit-amplifying progenitor cells in the subventricular zone of the aged parabiont mice compared with age-matched mice from isochronic parabiotic pairs. Notably, these cell populations were unaffected in the young parabiont mice from heterochronic parabiotic pairs, an apparent contradiction with the detrimental effects of old blood on hippocampal neurogenesis in young animals described above [8]. This discrepancy could reflect the differences between the subventricular zone and the hippocampus stem cell niches or just the fact that younger animals were used as aged parabionts in the current study. Indeed, in heterochronic parabiont pairs between 21- and 2-month-old mice, decreased numbers of proliferative $Ki67^+$ cells and $Sox2^+$ stem cells were observed in the young parabiont mice compared with age-matched mice from isochronic parabiont pairs. Thus, age-dependent accumulation of factors in the blood of older mice does interfere with neurogenesis in both the hippocampus and the subventricular zone. When cultured as neurospheres, neural stem cells from aged mice of heterochronic parabiont pairs showed increased ability to proliferate and differentiate into neurons compared with aged mice from isochronic parabiont pairs. Volumetric analysis of the 3D reconstructions of the blood vessels showed that aging causes a decrease in blood vessel volume, whereas heterochronic parabiosis reversed this decline, increasing blood vessel volume by

87% and blood vessel branching by 21%. Similar vascular remodeling in the aged heterochronic parabiont mice was observed in other neurogenic areas such as the hippocampus and non-neurogenic areas such as the neocortex. Notably, this remodeling of the aged cerebral vasculature in response to young systemic factors increased cerebral blood flow to levels seen in young animals. Thus, circulating factors have diverse positive effects in aging mice, including enhancing neurogenesis and improving the vasculature in the cortex and other parts of the brain. Collectively, the data described above show that the balance of positive and negative circulating factors changes with aging. Since the blood from 15 month-old mice does not have a detrimental effect on young mice, whereas older blood (21 months-old) decreases neural stem-cell activity in the young brain, there is an age at which "pro-aging" circulating factors accumulate to a significant level, and the "pro-youthful" factors are reduced.

Since the circulating factor GDF11 (a member of the BMP/TGF-β family) has been shown to reproduce many of the beneficial effects of heterochronic parabiosis on aging hypertrophic cardiac muscle [15] (to be discussed in detail lower), its possible effects on the age-related decline in neurogenesis has also been investigated [14]. The volume of blood vessels in 21- to 23 month-old mice treated with daily injections of recombinant GDF11 (0.1 mg/kg body weight) for 4 weeks increased by 50% and the population of $Sox2^+$ stem cells increased by 29% compared with the age-matched phosphate-buffered saline (PBS)-treated mice. Therefore, GDF11 also increases blood flow and neurogenesis in aged mice, although its effects are not as large as those of heterochronic parabiosis.

It has been shown that the age-dependent decrease in adult neurogenesis in mouse hippocampus and concomitant cognitive impairment could be caused by loss of Tet2 activity and reduced hm5C levels [16]. An increase of Tet2 has been found in older parabionts after exposure to young blood compared with age-matched isochronic parabionts exposed to old blood. Hypermethylation of promoter-associated CpG islands in genes encoding transcription factors is a prevailing feature of the aged brain methylome [17]. Thus, reactivation of Tet2 DNA demethylase appears to be relevant to brain rejuvenation.

2.4 Endocrine Pancreas

The replicative potential of pancreatic β-cells declines dramatically with age in both rodents and humans. In a heterochronic parabiosis study, the frequency of β-cell replication was found to be significantly increased in old heterochronic parabiont mice compared with age-matched isochronic and non-parabiont mice [18]. Recent studies have suggested that the age-related decline in β-cell replication results from increased expression of cell-cycle inhibitors, particularly p16/INK4A [19]. However, the mRNA levels of p16/INK4A have not been found to be changed on the exposure of old mice to a young circulation. Thus, the systemic factor regulating the decline in β-cell proliferation with age is unlikely to act through this pathway.

The potential contributions of insulin and insulin-like growth factor 1 (IGF-1) were also examined. Serum levels of these factors did not change in heterochronic parabiosis. Further studies are needed to identify circulating factors that regulate the proliferation of β-cells and which are altered with the aging process.

2.5 Heart

Among the diseases and disorders associated with advanced age, heart failure is one of the most prevalent. Cardiac hypertrophy is a prominent pathological feature of age-related diastolic heart failure. Cardiac aging in C57Bl/6 mice recapitulates human cardiac aging, including the development of age-related cardiac hypertrophy, and therefore was used as a model to study the role of systemic circulation factors in age-related heart failure in the heterochronic parabiosis paradigm [15]. Four weeks after joining the effects of the young (2 months-old) milieu on old (23 months-old) hearts were readily visible. Hearts of old mice were noticeably smaller than the hearts of identically aged isochronic parabiont (old–old) mice. A morphometric analysis of cardiac histological sections revealed a concomitant reduction in myocyte size. Thus, exposure to a young milieu reversed the hypertrophic cellular phenotype of aged hearts to the morphologic phenotype typical of a young adult mouse. Atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP) are molecular markers of cardiac myocyte hypertrophy. Both appeared to be significantly reduced at the transcript level in the hearts of old mice exposed to a young circulation, as compared to the isochronic age-matched controls. Therefore, young circulating factors modify discrete molecular pathways associated with cardiac myocyte hypertrophy. In an attempt to identify circulating molecules that might account for the regression of cardiac hypertrophy in old heterochronic parabiont mice, metabolomic profiling of amino acids, amines, and lipids was carried out. However, no significant differences were detected between the heterochronic and isochronic parabiont mice. A broad-scale proteomic analysis using aptamer-based technology further revealed 13 analytes that were present at significantly different levels between young and old mice, of which GDF11, a member of the activin/TGF-β super family, showed differential abundance in the blood plasma between isochronic-old and isochronic-young mice and a more youthful profile in old-heterochronic animals. GDF11 appeared to be widely expressed in a range of tissues and cell populations, with the spleen showing the highest levels of GDF11 mRNA. Significantly decreased levels of GDF11 mRNA and protein were detected in the spleens of old compared with young mice. Daily injection of old mice with recombinant GDF11 for 30 days led to lower heart weight, smaller cardiomyocytes, and reduced BNP and ANP compared with age-matched saline-injected controls. Thus, GDF11 could be involved in heterochronic parabiosis effects on age-related cardiac hypertrophy, but the participation of other factors cannot be excluded.

2.6 Bone

Bone repair and related processes, such as osseous integration of implants, occurs at a slower pace in older than in younger patients. Likewise, *in vitro* differentiation of bone marrow stromal cells (BMSCs) to osteoblasts is less effective in cultures from older compared to younger patients. Exposure to a young circulation in a heterochronic parabiosis paradigm was found to rejuvenate *in vivo* bone-fracture repair and *in vitro* osteoblast differentiation in old parabiont mice [20]. Heterochronic parabiosis led to the rejuvenation of both fracture repair phenotype and osteogenic potential in 20 month-old mice. Fracture calluses from old mice in heterochronic pairings contained twice the bone tissue and substantially less fibrotic tissue than fracture calluses from age-matched mice in isochronic parabiotic pairings. BMSC cultures from old mice in heterochronic pairs contained more osteoblastic colonies and produced higher levels of mineralization than their isochronic counterparts. Rescue of the aged phenotype was successful when osteoblasts were ablated from the young parabionts in heterochronic pairings but failed when old mice in these pairings were depleted of osteoblasts. Young mice in heterochronic pairings showed no significant changes in bone repair or *in vitro* osteogenic potential. These data indicate the existence of a circulating youth factor able to rejuvenate fracture repair and osteogenic potential in older mice. This rejuvenation is not dependent on osteoblasts residing in the parabiont partner, but rather arises from a circulating cell or molecule able to influence the endogenous, aged osteoblasts during this repair process. Engraftment of young bone marrow rescued fracture repair and osteogenic potential in aged animals to a similar degree, as seen in heterochronic parabiosis. Similar to the findings in parabiosis, the ablation of osteoblasts in host animals led to complete loss of fracture repair and osteogenic potential, while ablation of donor osteoblasts had no effect. Interestingly, 100% of the cells engrafted into the bone marrow space were $CD45^+$ hematopoietic cells. Media conditioned by young BMSC cultures were able to rescue the age-related decrease in osteogenic potential of old BMSC cultures. The transferred media did not contain cells or particulate materials, but centrifugation-based isolation confirmed the rejuvenation factor to have a molecular weight greater than 10 kDa. Heat denaturation of the media abolished rejuvenation. Hence, young BMSC cultures secrete a dissolved, transferable, heat-sensitive molecule that can rejuvenate the osteoblast differentiation capacity of old BMSC cultures. The flow cytometry identification showed that as much as 50% of cells in the BMSC cultures were $CD45^+$ hematopoietic cells. Thus, young $CD45^+$ cells are likely to be the source of the transferable factor that can rejuvenate aged osteoblastic differentiation. Mice in which the aged fracture repair was rejuvenated through heterochronic parabiosis showed a reduced level of total β-catenin, activated β-catenin and a β-catenin target gene *Axin2* transcript in the fracture calluses. In the rejuvenated old BMSC cultures, β-catenin levels were lower than in the control old cultures and similar to the young cultures. Together, these data show that fracture repair in aged mice can be rejuvenated through the modulation of β-catenin activity by exposure to a youthful circulation.

One cell type that might be responsible for the rejuvenation effect of young hematopoietic cells may be of the monocyte/macrophage lineage. After tissue injury, macrophages are recruited to areas of trauma, where they undergo phenotypic and functional changes coordinating tissue repair. During fracture, healing macrophages are found at the fracture site, and when depleted, fractures will not heal effectively. In addition, macrophage population and phenotype can change with aging.

3 Looking for a "Silver Bullet"

3.1 GDF11: A Controversial Hero

The specific rejuvenating role of GDF11 has been questioned in subsequent studies. In the skeletal muscle, the best-studied inhibitor of muscle growth is the closest homolog (90% identity) of GDF11, myostatin, also called GDF8. It inhibits muscle differentiation and causes differentiated myotubes to undergo atrophy [21]. GDF11 and myostatin function through the same receptor complex – type II activin receptor, which induces activation of type I receptors ALK4 or ALK5. Their activation induces the phosphorylation and activation of the transcription factors SMAD2 and SMAD3 that repress genes involved in muscle differentiation. The expression pattern of GDF11 is different from myostatin, however when added to muscle, GDF11 elicits identical signaling patterns to induce myotube atrophy and inhibit differentiation [21, 22]. In the serum and muscle of rats, the GDF11 level does not decrease but rather increases with age [22]. It was found that GDF11 detection methods used in previous studies [10, 15] did not distinguish between GDF11 and myostatin and that the much more abundant myostatin was actually measured [22, 23]. Concerning the ability of GDF11 to improve muscle regeneration, this was retested and the opposite results were obtained. At the doses that the prior reports used, no effects on skeletal muscle regeneration were seen, whereas at higher doses, GDF11 inhibited muscle regeneration [22]. These findings were independently supported by other authors [24]. On the other hand, the rejuvenation effects of GDF11 on the regenerative capacity and cognitive functions of the aged brain have not been contested [14]. When the aged (22 months-old) mice were subjected to daily intraperitoneal injections with recombinant GDF11 (1 mg/kg) for 1 week, the average value of blood GDF11 was about 400 pg/mL – equal to that in control young mice (saline-injected, 3–4 months-old), whereas the intrinsic circulating GDF11 could not be detected in saline-injected aged control mice [25]. Since the specificity of the enzyme-linked immunoadsorbent assay (ELISA) used for GDF11 measurements has been confirmed by using recombinant myostatin, which was not detected at any concentration, these results showed that intraperitoneal injection at 1 mg/kg in aged mice increased the blood GDF11 to a youthful level. After 1 week of daily injections, GDF11-treated old mice were significantly leaner than age-matched controls, with

an average reduction of 8% of their initial body weight. No further weight loss occurred after an additional 2 weeks of GDF11 treatment, and GDF11-treated aged mice remained as lean as young mice and maintained a statistically significant weight difference compared to aged control mice. Visceral (epididymal) white adipose tissue was significantly reduced in old mice after 3 weeks of GDF11 treatment, whereas the tibial muscle mass was not changed. Moreover, no morphological differences in muscle were observed between the two aged groups. In the brain subventricular zone of aged mice, increased neurogenic capacity was observed after GDF11 treatment, suggesting a simultaneous role for GDF11 in both brain rejuvenation and weight loss. Interestingly, these mice maintained the same significantly reduced weight 3 weeks after the GDF11 injections were stopped. Therefore, systemic GDF11 administration triggers changes in organismal physiology that have a long-lasting effect. Interestingly, calorie-restricted (CR) aged mice showed increased levels of circulating GDF11 compared with their *ad libitum* fed aged counterparts. Aged GDF11-treated mice that underwent fasting exhibited a significant decrease in insulin levels, whereas no significant change was observed in fed mice. Likewise, plasma IGF-1 levels were significantly decreased in old GDF11-treated compared to old control mice. Adiponectin, an adipose-secreted hormone, is known to induce appetite-independent weight loss, is inversely correlated with adipose mass, and increased by CR. Elevated levels of adiponectin were observed in aged GDF11-treated mice. These results demonstrate that systemic GDF11 administration in aged mice affects metabolic pathways by inducing sustainable hormonal changes similar to those activated in CR. The insulin/IGF-1 axis of aging is also affected in CR and IGF-1 levels are inversely correlated with obesity and aging. GDF11 treatment led to a 27% reduction in serum levels of IGF-1, similar to the effects of CR, together with a decrease in fasting insulin levels. Given that reduced levels of IGF-1 and insulin are tightly linked to increased longevity, it would be interesting to examine whether or not longer GDF11 treatment could increase longevity. Lastly, it is essential to note that the CR-like phenotype induced by GDF11 is correlated with a rejuvenation phenotype in the brain, suggesting that GDF11 treatment might reverse brain dysfunctions related to aging along with a pleiotropic effect on whole-body metabolism.

In humans, circulating GDF11 levels vary between 0.4 and 0.6 ng/mL and are not statistically different between variously aged (21–93 years-old) subjects of both genders, as measured by a GDF11-specific liquid chromatography tandem mass spectrometry (LC-MS/MS) assay [26]. Myostatin levels are highest in men in their twenties (about 6 ng/mL) and slightly decline throughout subsequent decades (to about 4 ng/mL). In females, myostatin levels are lower than in men (about 3.5 ng/mL) and do not change with age. Severe aortic stenosis is an age-associated condition and the most common form of valvular cardiovascular disease in developed countries. In an extensively characterized cohort of older adults undergoing surgical valve replacement for the treatment of severe aortic stenosis, plasma GDF11 levels ranged from 0.224 to 0.841 ng/mL, and plasma myostatin concentrations ranged from 0.64 to 6.27 ng/mL. When the participants were stratified into low, middle, and high GDF11 or MSTN tertiles, increased circulating GDF11 was associated with a

higher proportion of study participants who had diabetes and a history of previous cardiac conditions, including coronary artery bypass. Participants with the highest GDF11 levels at surgery had a significantly higher predicted risk of mortality. In contrast to GDF11, no statistically significant associations were found between myostatin and comorbid conditions or mortality risk. Individuals who were rehospitalized at least once post-operation had significantly higher GDF11 levels at baseline than non-rehospitalized counterparts. Moreover, participants that experienced multiple adverse health outcomes had higher baseline GDF11 levels than those with only one or no post-operative health complications. These data show that GDF11 could be a biomarker of impaired organismal resiliency to surgical stress but not a reliable indicator of chronological aging. Therefore, GDF11 is not likely to be a magic 'silver bullet' to combat aging or age-related diseases.

3.2 Other Contenders

About 70 circulatory proteins from young parabiont mice were found in muscle tissue of old parabiont mice [27]. Many of these proteins possess rejuvenating promyogenic properties and are expected to synergize when reaching the old muscle tissue simultaneously. LIF-1 has been shown to enhance the repair of the injured muscle. Cripto and cerberus1 act as antagonists of TGFβ1, which increases with age and inhibits regeneration of old muscle. GDF5 is a TGFβ family member that promotes muscle innervation known to decline with age. Follistatin might counteract the effect of myostatin known to inhibit muscle stem cell proliferation. Cerberus1 and DKK-1 antagonize the age-elevated Wnt pathway activity. These "young" proteins also have known beneficial effects on other tissues. Of particular note, leptin was identified as one of these "young" proteins, and it broadly regulates hormonal networks, including those controlling reproduction and metabolism, and it has numerous anti-aging effects. Leptin interacts with oxytocin in its positive effects on the health of muscle, bone, brain and in the reduction of obesity. Moreover, since both leptin and oxytocin activity decline with aging, the increase of the leptin/oxytocin axis might represent a key event in mammalian aging that is rescued by heterochronic parabiosis.

Heterochronic parabiosis studies exploiting young mice in which macrophages can be depleted, and fractionated bone marrow transplantation experiments have shown that young macrophages rejuvenate bone fracture repair, whereas old macrophage cells slow healing in young mice [28]. Proteomic analysis of the macrophage secretomes identified differential proteins between old and young macrophages, such as low-density lipoprotein receptor-related protein 1 (Lrp1) produced by young macrophages. Depleting Lrp1 in young mice abrogated the ability to rejuvenate fracture repair, while in old mice, recombinant Lrp1 improved fracture repair. Notably, when β-catenin was depleted at the fracture site in old mice, Lrp1treatment no longer affected fracture repair. Therefore, the rejuvenating effect of Lrp1 on bone repair appears to be mediated by modulation of the β-catenin pathway activity.

4 Conclusions

Heterochronic parabiosis studies have addressed some of the most fundamental questions about the systemic regulation of cell and tissue aging. Recent advances in omics techniques have opened new avenues of research in this area, of which the identification of "pro-aging" or "anti-aging" factors that are carried in the circulation are the most important. Effectors of the Wnt and TGF-β signaling pathways and cytokines with direct actions on stem cells are likely to be among these factors, but many others remain to be identified. Application of the heterochronic parabiosis paradigm to mouse strains with genetically altered signal pathways would allow for direct tests of their role in regulating cell and tissue aging. Heterochronic plasma and blood transfusion studies hopefully will help in the identification of circulating factors that regulate aging at the cell, tissue, and organismal levels. Studies of heterochronic parabiosis could provide an experimental means of understanding the epigenetic regulation of aging. Molecular changes that have been induced in parabiont animals are known to persist for some time when the treatment has stopped, which indicates an epigenetic reprogramming occurs *in vivo* in response to changes of systemic milieu. However, this does not involve the loss of cellular differentiation and is therefore distinct from the epigenetic reprogramming that occurs in induced pluripotent stem cells. The cells keep their identity as before parabiosis, but their regenerative performance becomes rejuvenated by the young blood milieu. Therefore, heterochronic parabiosis allows for the dissociation of "dedifferentiation" from "rejuvenation." Heterochronic parabiosis experiments have shown that, at the organismal level, aging can not only be slowed down but also reversed to a significant extent, at least in some organs. Therefore, further research is warranted to study the epigenetic profiles of cells exposed to a heterochronic parabiosis paradigm. As a powerful experimental system to study aging at various levels, heterochronic parabiosis shows considerable promise, especially when applied to the epigenetics of aging and rejuvenation. The ultimate aim is to discover new ways of reducing the effects of age-related diseases to help people live longer healthier lives.

References

1. Finerty JC (1952) Parabiosis in physiological studies. Physiol Rev 32(3):277–302
2. Eggel A, Wyss-Coray T (2014) A revival of parabiosis in biomedical research. Swiss Med Wkly 144:w13914. https://doi.org/10.4414/smw.2014.13914
3. Conboy MJ, Conboy IM, Rando TA (2013) Heterochronic parabiosis: historical perspective and methodological considerations for studies of aging and longevity. Aging Cell 12(3):525–530
4. Ludwig FC, Elashoff RM (1972) Mortality in syngeneic rat parabionts of different chronological age. Trans N Y Acad Sci 34(7):582–587
5. Conboy IM, Conboy MJ, Wagers AJ, Girma ER, Weissman IL, Rando TA (2005) Rejuvenation of aged progenitor cells by exposure to a young systemic environment. Nature 433(7027):760–764

6. Brack AS, Conboy MJ, Roy S, Lee M, Kuo CJ, Keller C et al (2007) Increased Wnt signaling during aging alters muscle stem cell fate and increases fibrosis. Science 317(5839):807–810
7. Carlson ME, Hsu M, Conboy IM (2008) Imbalance between pSmad3 and Notch induces CDK inhibitors in old muscle stem cells. Nature 454(7203):528–532
8. Villeda SA, Luo J, Mosher KI, Zou B, Britschgi M, Bieri G et al (2011) The ageing systemic milieu negatively regulates neurogenesis and cognitive function. Nature 477(7362):90–94
9. Sinha M, Jang YC, Oh J, Khong D, Wu EY, Manohar R et al (2014) Restoring systemic GDF11 levels reverses age-related dysfunction in mouse skeletal muscle. Science 344(6184):649–652
10. Sinha I, Sinha-Hikim AP, Wagers AJ, Sinha-Hikim I (2014) Testosterone is essential for skeletal muscle growth in aged mice in a heterochronic parabiosis model. Cell Tissue Res 357(3):815–821
11. Ruckh JM, Zhao JW, Shadrach JL, van Wijngaarden P, Rao TN, Wagers AJ et al (2012) Rejuvenation of regeneration in the aging central nervous system. Cell Stem Cell 10(1):96–103
12. Smith LK, He Y, Park JS, Bieri G, Snethlage CE, Lin K et al (2015) β2-microglobulin is a systemic pro-aging factor that impairs cognitive function and neurogenesis. Nat Med 21(8):932–937
13. Villeda SA, Plambeck KE, Middeldorp J, Castellano JM, Mosherm KI, Luo J et al (2014) Young blood reverses age-related impairments in cognitive function and synaptic plasticity in mice. Nat Med 20(6):659–663
14. Katsimpardi L, Litterman NK, Schein PA, Miller CM, Loffredo FS, Wojtkiewicz GR et al (2014) Vascular and neurogenic rejuvenation of the aging mouse brain by young systemic factors. Science 344(6184):630–634
15. Loffredo FS, Steinhauser ML, Jay SM, Gannon J, Pancoast JR, Yalamanchi P et al (2013) Growth differentiation factor 11 is a circulating factor that reverses age-related cardiac hypertrophy. Cell 153(4):828–839
16. Gontier G, Iyer M, Shea JM, Bieri G, Wheatley EG, Ramalho-Santos M et al (2018) Tet2 rescues age-related regenerative decline and enhances cognitive function in the adult mouse brain. Cell Rep 22(8):1974–1981
17. Hernandez DG, Nalls MA, Gibbs J, Arepalli S, van der Brug M, Chong S et al (2011) Distinct DNA methylation changes highly correlated with chronological age in the human brain. Hum Mol Genet 20(6):1164–1172
18. Salpeter SJ, Khalaileh A, Weinberg-Corem N, Ziv O, Glaser B, Dor Y (2013) Systemic regulation of the age-related decline of pancreatic β-cell replication. Diabetes 62(8):2843–2848
19. Chen H, Gu X, Su IH, Bottino R, Contreras JL, Tarakhovsky A et al (2009) Polycomb protein Ezh2 regulates pancreatic beta-cell Ink4a/Arf expression and regeneration in diabetes mellitus. Genes Dev 23(8):975–985
20. Baht GS, Silkstone D, Vi L, Nadesan P, Amani Y, Whetstone H et al (2015) Exposure to a youthful circulation rejuvenates bone repair through modulation of β-catenin. Nat Commun 6:7131. https://doi.org/10.1038/ncomms8131
21. Trendelenburg AU, Meyer A, Rohner D, Boyle J, Hatakeyama S, Glass DJ (2009) Myostatin reduces Akt/TORC1/p70S6K signaling, inhibiting myoblast differentiation and myotube size. Am J Physiol Cell Physiol 296(6):C1258–C1270
22. Egerman MA, Cadena SM, Gilbert JA, Meyer A, Nelson HN, Swalley SE et al (2015) GDF11 increases with age and inhibits skeletal muscle regeneration. Cell Metab 22(1):164–174
23. Rodgers BD, Eldridge JA (2015) Reduced circulating GDF11 is unlikely responsible for age-dependent changes in mouse heart, muscle, and brain. Endocrinology 156(11):3885–3888
24. Hinken AC, Powers JM, Luo G, Holt JA, Billin AN, Russell AJ (2016) Lack of evidence for GDF11 as a rejuvenator of aged skeletal muscle satellite cells. Aging Cell 15(3):582–584
25. Katsimpardi L, Kuperwasser N, Camus C, Moigneu C, Chiche A, Tolle V et al (2019) Systemic GDF11 stimulates the secretion of adiponectin and induces a calorie restriction-like phenotype in aged mice. Aging Cell 19:e13038. https://doi.org/10.1111/acel.13038

26. Schafer MJ, Atkinson EJ, Vanderboom PM, Kotajarvi B, White TA, Moore MM et al (2016) Quantification of GDF11 and myostatin in human aging and cardiovascular disease. Cell Metab 23(6):1207–1215
27. Liu Y, Conboy MJ, Mehdipour M, Liu Y, Tran TP, Blotnick A et al (2017) Application of bio-orthogonal proteome labeling to cell transplantation and heterochronic parabiosis. Nat Commun 8:643. https://doi.org/10.1038/s41467-017-00698-y
28. Vi L, Baht GS, Soderblom EJ, Whetstone H, Wei Q, Furman B (2018) Macrophage cells secrete factors including LRP1 that orchestrate the rejuvenation of bone repair in mice. Nat Commun 9(1):5191. https://doi.org/10.1038/s41467-018-07666-0

Chapter 6
Skeletal Muscle Aging Atrophy: Assessment and Exercise-Based Treatment

Gabriel Nasri Marzuca-Nassr, Yuri SanMartín-Calísto, Pablo Guerra-Vega, Macarena Artigas-Arias, Andrea Alegría, and Rui Curi

1 Introduction

In 2019, there were 703 million persons aged 65 years or over in the world. In 2050, the number of older persons will double to 1.5 billion. From 1990 to 2019, the number of people aged 65 years or over increased from 6% to 9%. This proportion will increase to 16%, meaning that one in six people in the world will be aged 65 years or over in 2050 [1].

Aging is associated with high cell damage occurrence. Aging leads to a decrease in physical and mental capacity and a growing risk of disease. These changes, however, are neither linear nor consistent, and they are only loosely associated with a

G. N. Marzuca-Nassr (✉)
Departamento de Medicina Interna, Facultad de Medicina, Universidad de La Frontera, Temuco, Chile

Magíster en Terapia Física con menciones, Facultad de Medicina, Universidad de La Frontera, Temuco, Chile
e-mail: gabriel.marzuca@ufrontera.cl

Y. SanMartín-Calísto · M. Artigas-Arias · A. Alegría
Magíster en Terapia Física con menciones, Facultad de Medicina, Universidad de La Frontera, Temuco, Chile

P. Guerra-Vega
Magíster en Terapia Física con menciones, Facultad de Medicina, Universidad de La Frontera, Temuco, Chile

Escuela de Kinesiología, Facultad de Ciencias de la Salud, Universidad San Sebastián, Puerto Montt, Chile

R. Curi
Interdisciplinary Post-Graduate Program in Health Sciences, Cruzeiro do Sul University, Sao Paulo, Brazil

P. C. Guest (ed.), *Reviews on New Drug Targets in Age-Related Disorders*,
Advances in Experimental Medicine and Biology 1260,
https://doi.org/10.1007/978-3-030-42667-5_6

person's age in years. While some 70 year-olds enjoy perfect health and functioning, other 70 year-olds are frail and require significant help from others [2].

With aging, there is a loss of skeletal muscle mass (skeletal muscle atrophy called sarcopenia) that decreases muscular strength and physical performance, that also influences cognitive status. Assessing these changes becomes vital in an older adult. In this line, there are several strategies which have been proposed to combat skeletal muscle atrophy due to aging, such as physical exercise, nutritional supplements, or drugs. Although some researchers showed the combination of these strategies, we will focus in this chapter only on the physical activity methods. This chapter aims to demonstrate the most used existing test/machines to evaluate the loss of skeletal muscle mass due to aging and, consequently, the decrease in muscle strength and physical performance. It will also propose physical exercise as an "effective drug" to counteract the effects produced by the loss of muscle mass and its consequences due to aging.

2 Skeletal Muscle Aging Atrophy

A young person has 48% muscle mass, 19% fat, and 33% non-muscle fat-free mass (FFM) at the age of around 22 years-old. On the other hand, a subject near the age of 78 years-old has a body composition of 25% muscle mass, 35% fat, and 40% of non-muscle FFM [3].

This decrease in global skeletal muscle mass (mainly reflected in the lower extremities) related by aging may be the product of a reduction in the synthesis pathways and through an increase in the degradation pathways of skeletal muscle proteins. In protein synthesis, the primary signaling networks that been investigate are the insulin-like growth factor 1 (IGF-1) axis and the protein kinase B/mammalian target of rapamycin/ribosomal S6 kinase (Akt/mTOR/S6) pathway. On the other hand, in protein degradation, there are several systems such as apoptosis, calpains, autophagy, ubiquitin-proteasome and oxidative stress. Also, there is interplay between sarcopenia and chronic inflammation [4–8].

At the level of muscle fibers, there are many cellular and molecular changes that contribute to muscle aging. For instance, reduced number of satellite cells, decreased number of muscle fibers (predominantly type II), reduced myosin protein content, reduced number of mitochondria, increased inter- and intra-muscular adipose tissue, disruption of excitation-contraction coupling, and others [9]. According to all of these changes in skeletal muscle due to aging, an older person shows a blunted muscle protein synthetic response to anabolic stimuli like amino acid administration and physical activity when compared with the effects seen in young persons. This condition is known as anabolic resistance [10]. Therefore, an older person will have to have a more significant anabolic stimulus to obtain beneficial responses at the muscular level.

The decrease in skeletal muscle mass loss due to aging is called sarcopenia. Irwin H. Rosenberg was the first to propose the sarcopenia term at a meeting in

1988. The sarcopenia Greek meaning is s*arx* for flesh and *penia* for loss [11]. Initially, the concept of sarcopenia was coined for the decrease of muscle mass and function [11], although most people associate it with only skeletal muscle mass loss.

Cruz-Jentoft and Sayer presented a reasonable timeline on the international definition of sarcopenia [12]. In 2010, the EWGSOP (European Working Group on Sarcopenia in Older People) defined sarcopenia using muscle mass, muscle strength, and physical performance (cut-offs not specified). In 2011, the International Working Group on Sarcopenia and Society of Sarcopenia, Cachexia, and Wasting Disorders (SSCWD) defined the disease using muscle mass and physical performance, with defined cut-offs. The SSCWD used the phrase sarcopenia with limited mobility. In 2014, the Asian Working Group on Sarcopenia gave the same definition as the EWGSOP and also defined cut-offs for Asia. In the same year, the Foundation for the National Institutes of Health described the disease using muscle mass and muscle strength, and also defined cut-offs, using physical performance as an outcome. The EWGSOP updated its definition in 2019 (EWGSOP2) with cut-offs determined, using physical performance to assess the severity of the condition [12].

3 Basic and Instrumental Activities of Daily Living Assessment

The geriatric assessment allows knowing the baseline situation of the subjects, evaluates the impact of diseases, and establishes specific treatments. The evaluation of the health condition of the older people includes assessment scales of basic Activities of Daily Living (ADL) and Instrumental Activities of Daily Living (IADL), physical and functional assessment scales, and instruments that assess the cognitive state (Table 6.1) [25].

3.1 Barthel Index

Mahoney and Barthel created this index in 1955 [13]. They measured the evolution of subjects with neuromuscular and skeletal muscle processes in a hospital for chronic patients in Maryland and published the results 10 years later. This was modified in 1979. The fundamental change occurs in the item of transfer by wheelchair to bed, which changes to the transfer of armchair to bed [13]. This index comprises 10 elements in their original version, with each item receiving a score of zero if the subject is unable to perform the task or a variable score between 5, 10, and 15 points, which reflect independence or intervals of relative autonomy for tasks. The values assigned to each activity are based on the time and amount of physical help required if the patient cannot perform that activity. Full credit is not granted for an activity if the subject needs help and/or minimal supervision [13].

Table 6.1 Valuation instruments in older people

Instrument	Parameters to evaluate	Interpretation
Barthel Index [13]	Basic Activities of Daily Living (ADL)	100 Independency ≥60 Slight dependency 40–55 Moderate dependency 20–35 Severe dependency <20 Total dependency
Katz Index [14]	Instrumental Activities of Daily Living (IADL)	6 = High (patient independent) 0 = Low (patient very dependent)
Lawton & Brody [15]	Instrumental Activities of Daily Living (IADL)	0 Dependent 8 Independent
Grip Strength [16]	Grip strength	In accordance with EWGSOP2∗, values <27 kg in men and <16 kg in women is associated with sarcopenia
Gait Speed [16]	Gait speed	In accordance with EWGSOP2∗, ≤0.8 m/s indicates low functional performance and association with sarcopenia
Timed Up and Go [17]	Dynamic balance	≤10 segments normal 11–19 segments slight risk of falls > 20 segments high risk of falls
Unipedal stance [18]	Dynamic balance	≥ 5 segments high risk of falls
Tinetti [19]	Dynamic balance	12 Independence
	Gait	16 Independence
Short Physical Performance Battery [20]	Static balance Dynamic balance Lower+ body strength	0–3 Severe limitations 4–6 Moderate limitations 7–9 Mild limitations 10–12 Minimal limitations The sum of the subtests has a maximum score of 12, indicates functional independence
Senior Fitness Test [21]	Lower body strength Upper body strength Aerobic capacity Superior train flexibility Superior train flexibility	Presents reference values by age range for each subtest
Mini Mental State Examination [22]	Cognitive state	30–35 Normal cognitive status 25–29 Slight deficit 20–24 Mild cognitive impairment 15–19 Moderate cognitive impairment 0–14 Severe cognitive impairment, dementia
Pfeffer Scale [23]	Cognitive state	0–2 errors: normal mental functioning 3–4 errors: mild cognitive impairment 5–7 errors: moderate cognitive impairment 8–10 errors: severe cognitive impairment
Geriatric Depression Scale [24]	Geriatric depression	0–5 Normal 6–9 Probable depression 10 or more established depression

aEWGSOP2: European Working Group on Sarcopenia in Older People 2

Environmental conditions can influence the index score since if the subject has special requirements to perform their ADL and the corresponding adaptations are not available, and therefore their score will be lower when they cannot be performed. Examples of this are the bars or handles in the bathroom, tub, and toilet. If needed and not available, the subject will not be able to perform the activity [13, 26].

Some authors have proposed reference scores to facilitate their interpretation. For example, it has been observed that an initial rating of more than 60 is related to a shorter duration of hospital stay and a higher probability of reintegrating into the community after discharge. This score seems to represent a limit [27–29].

The Barthel Index is a handy instrument in rehabilitation. Because of its validity and reliability, it is easy to apply and interpret. Its application is low cost and is useful for monitoring the evolution of subjects. It takes between 2 and 5 min, while the self-assessment is done in approximately 10 min. It can be applied by rehabilitation professionals or by other health professionals and by trained interviewers, who are trained in times requiring no longer than 1 h. It can also be self-administered, valued by third parties, or through a telephone interview [26].

3.2 Katz Index

The multidisciplinary team led by S. Katz created this index in 1958 at the Benjamin Rose Hospital in Ohio [30]. The purpose was to delimit dependence of subjects with hip fractures. It was published in 1959 under the name of the Index of Independence in Activities of Daily Living.

The purpose was to measure functionality in chronic patients and aging populations. It is a tool used to obtain important information about the prognosis and disability associated with aging [14]. In addition, it allows clinicians and researchers to assess the need for health care and determine treatment effectiveness, and it can be used as a therapeutic guide [25, 31].

The index considers performance in six essential activities: bathing, clothing, use of toilet, mobility, continence, and food consumption [30]. According to the assigned performance scores of A, B, C, D, E, F, or G, A is the most independent grade of the scale, and G is the most dependent grade. Through questions and/or observations, the evaluator forms a mental image of the patient's ADL status. The assessor determines if the patient performed his activities alone, with active personal assistance, directive assistance, or if he/she required supervision [31].

The Katz index describes a similarity between the patterns of loss and recovery of functions with the development of the infant, ordering dependence and the restoration of independence, and inverse processes, where the first capacity to recover is that of feeding and the latest are the ability to dress and bathe [30, 31].

3.3 Lawton and Brody Scale

The Lawton and Brody scale was first published in 1969 [15]. It was initially used at the Philadelphia Geriatric Center to evaluate physical autonomy and IADL in older people who may or may not be institutionalized. It is a widely used instrument internationally for IADL measurement, with the advantage of allowing analysis of each individual score used in the scale. It also allows evaluation of therapeutic plans used for older people, being sensitive in the detection of the first and most minimal signs of functional deterioration in this age group [15].

This index evaluates the functional capacity in 8 items: (i) use of the telephone, (ii) purchasing, (iii) ability to prepare food, (iv) home care, (v) doing laundry, (vi) use of means of transport, (vii) responsibility for medicines and (viii) management of economic affairs [15, 32]. The information is obtained by directly asking the subject or his/her usual caregiver. Each item is assigned a score of 0 if there is dependence and 1 point if there is independence for that activity. The total sum of points varies between 0 corresponding to maximum dependence and 8 points, which corresponds to complete independence for the operations [33].

4 Physical Performance Assessment

4.1 Grip Strength Test

The grip strength test is the simplest and recommended method for the evaluation of muscle strength in clinical practice due to its strong association with lower limb muscle strength [34]. This parameter is the maximum isometric contraction force generated around a dynamometer measured in kilograms, Newtons, pounds, or millimeters of mercury [34]. Older people commonly have difficulties with the operation of the hands and manual dexterity in tasks that require a delicate and precise grip, and the loss of the strength of the hands can affect simple everyday actions [35]. Impaired hand function occurs as a result of healthy aging and established disorders frequently found in older people, such as osteoporosis, osteoarthritis, and rheumatoid arthritis [35, 36].

For more details on this evaluation method, please see Sect. 6.2 in the hospital context."

4.2 Gait Speed Test

The gait is an intrinsic function in the human being, which is the reason why its deterioration determines the loss of dependence. The alteration in the gait speed in older people is also an indicator of increased risk of falls, fractures, and more significant morbidity and mortality [37]. Walking speed predicts the state of health and

risk of future functional decline, including hospitalization and institutionalization [38]. This parameter requires a comparison with reference values that vary between 0.6 and 1.70 m/s. There is no standardized assessment consensus to evaluate this parameter. However, there are several physical performance assessment batteries that include the parameter "Short Physical Performance Battery and Senior Fitness Test", which is described below.

4.3 Timed Up and Go Test

In the beginning, the Getup and Go test was used, which was designed as a screening instrument to detect balance changes in the population. The subject had to get up from a chair with armrests, walk 3 m, turn on himself, step back 3 m, and sit down again [39]. To make the measurement more objective, Podsiadlo and Richardson, in 1991, made a modified and timed version of the test, now called Timed Up and Go [17]. The time starts when the participant takes off from the straight back of the chair and ends when, after traveling, the circuit returns to the starting position. For its realization, the participant will use his usual or necessary technical help and will walk at a rapid pace, without running, walking at a light but safe speed [40].

Adults without neurological problems, who are independent in the natural balance and mobility tasks, perform the test in less than 10 s. Meanwhile, older people who need between 11 and 19 s to complete the activity present a slight risk of falls, and a high risk if they require more than 20 s to complete it [17].

4.4 Unipedal Stance Test

The Unipedal Stance test is used to assess static balance. For its realization, the older person must stand with his arms crossed on his chest, resting hands on shoulders, performing triple flexion of one leg at 90°, keeping in this position for as long as possible, to a maximum of 30 s. This activity is repeated 3 times, alternating the lower limb of support, considering the best time obtained [18]. An older person has a high risk of falls if he/she fails to maintain the position for a time shorter than or equal to 5 s [18, 41].

4.5 Tinetti Scale

The Tinetti scale was made in 1986 to assess the mobility of older people. Its main objective is to detect subjects with a high risk of falls, based on the two domains of gait and balance, composed of nine and seven items, respectively [42]. A zero score is assigned if the person does not achieve or maintain stability in position changes

or has an inappropriate gait pattern according to the parameters established in the scale (considered abnormal). A score of 1 indicates that the individual achieves changes in position or gait patterns with compensations (the adaptive condition). A score of 2 indicates that the subject does not present difficulties for the different activities of the scale (the average condition). The maximum balance score obtained is 16 points, and the maximum gait score corresponds to 12 points. The sum of both tests provides a maximum total score of 28, with which the risk of falls is determined. Between 19 and 28 points is considered to represent a minimum risk of falls, while a score lower than 19 is regarded as a high risk of falls [19]. This instrument is mainly used in older people who live in the community and institutionalized, to assess the risk of falls and their consequent relationship with the functional alterations of each subject [43].

4.6 Short Physical Performance Battery

The Short Physical Performance Battery (SPPB), developed by Guralnik in 1994, is an instrument to be used safely in older people with or without underlying acute pathologies [20]. The SPPB consists of three tests: balance, gait speed, and getting up and sitting in a chair five times. In the first balance test, the subject must maintain three positions: feet together, semi-tandem, and tandem, following a hierarchical order of difficulty, for at least 10 s to obtain the full score of the item [20, 44]. The gait speed test is carried out so that the subject walks at his usual speed for a distance of 4 m twice, recording the shortest time. Finally, for the test to get up from the chair, the subject is asked to cross his arms over his chest and get up. If successful, the time is recorded from the moment when the subject stands for the first time until he sits at the fifth time. Each test is awarded a score in favor of the time used for each activity, where 0 corresponds to the worst performance and 4 to the best performance. A global test battery score ranging from 0 to 12 points is obtained. The low scores on this instrument have a high predictive value for a considerable amount of health consequences, including disability in the AVD, loss of mobility, disability, hospitalization, length of hospital stay, admission to nursing facilities, and mortality [45].

4.7 Senior Fitness Test

In 2001, Rikli and Jones designed the Senior Fitness Test battery to address the need to create tools that allow assessment of the physical condition of older people residing in the community [21]. It applies to older people from 60 years-old and over with different levels of physical and functional abilities. It does not require equipment or sophisticated spaces to be realized [46]. The tests that make up the battery include various components of functional capacity, this being the particularity of

existing batteries that focus on only one component. It is composed of differentiated tests or evaluations: chair stand, arm curl, 6 min walk, 2 min step, chair-sit and reach, back scratch, foot up and go and body mass index [21, 47].

The chair stand test starts with the evaluation subject sitting in a chair with a straight back, feet flat on the floor and arms crossed on the chest. The subject should rise fully and return to the initial position as many times as possible during 30 s. It is essential for the examiner to consider the demonstration of the correct movement before starting the test and ask the subject to replicate it to ensure their understanding [21].

In the arm curl test, the participant starts sitting in a chair with a straight back, feet flat on the floor, with an extended elbow and then lifts a weight of 5 pounds in the case of women and 8 pounds in the case of men in the dominant hand, which is oriented towards the body. From that position, an elbow flexion and supination of the forearm is performed, and then the forearm returns to the initial position by extending the elbow and rotating the wrist. The score is assigned with the number of complete moves made in 30 s [21].

The 6 min walk test is used to evaluate aerobic endurance. This particular test requires ample space for its realization (30-m corridor). Ideally, it is done after finishing the other tests. The participant will walk as quickly as possible for 6 min in a marked circuit, and a score will be assigned to each return he/she makes. For each elapsed min, the subject will be informed of the remaining evaluation time associated with the pace of walking. The participant will stand sideways performing leg lift movements alternately at the end of the 6 min [21].

Before starting the 2 min step test, it is necessary to measure the height at which the participant should raise the knee. The average distance between the iliac crest and the upper part of the patella is calculated, thus marking the midpoint of the thigh corresponding to the height of the knee while walking. To visualize this mark, it must be transferred to the wall so that the participant has a reference. Start the test when the signal is given, and the subject begins to march in place as many times as possible for 2 min. Both knees must reach the indicated height. The number of times the right knee reaches the reference height is considered. If it does not reach the stated level, the speed should be reduced so that the test is valid without stopping the time. The final score will be awarded according to the number of steps taken and the right knee has reached the set height [21].

The chair-sit and reach test assesses the flexibility of the lower extremities, mainly the biceps femoris. The participant sits in the chair with the gluteal fold at the front edge of the chair. One leg will be with the knee in flexion and the foot resting on the ground, while the other leg will be extended forward as much as possible. With the arms extended and the hands together, the participant will flex the hip slowly to touch the tip of the foot with both middle fingers or beyond this point. If the extended leg begins to flex, the subject should return to the starting position. The score is assigned according to the remaining cm to reach the tip of the foot or the amount by which it is exceeded [21].

The back scratch test evaluates the flexibility of the upper body, mainly shoulders. The participant starts standing with his favorite hand on the same shoulder

with the palm facing down. In this position, he/she will bring the hand towards the middle of the back as far as possible while keeping the elbow towards the ceiling. The other arm is located on the back around the waist, trying to take it as far as possible, trying to touch both hands. The middle fingers should be oriented as close as possible, and the distance between them or, failing that, the number of cm that are exceeded is measured [21].

The foot up and go test assesses agility and dynamic balance. The participant will be placed in a chair with a straight back, hands on the thighs and feet resting on the floor with one more advanced. At the signal, the subject will rise from the chair without supporting their hands to push themselves and will quickly walk towards a cone located at an established distance and then return to sit down and position themselves in the initial position [21].

The body mass index of each subject is measured using the formula: body weight (kg) divided by height (m^2).

Finally, the scores obtained in each of the tests that make up the battery are added to a standardized record sheet and are compared with the existing reference values for each age group within the elderly population [47].

5 Cognitive Assessment

5.1 Mini Mental State Examination

The Mini Mental State Examination (MMSE) is an instrument created in 1975 to assess cognitive status systematically and thoroughly. It consists of 11 questions that analyze areas of cognitive functioning, including orientation, registration, attention, calculation, memory, and language. This tool requires 5 min of application, so it is considered practical in its administration in older people [22]. The performance of this evaluation has variables, which may or may not influence the final result. Among these variables, we can find the educational level of the participant and the sensory deficit, such as difficulty in hearing or sight [48]. The maximum score is 19 points, and a value less than or equal to 13 points is considered suggestive of cognitive deficit [49].

5.2 Pfeffer Scale

The Pfeffer scale has been used since 1982 and applies to the companion of the older person who obtained a score less than or equal to 13 in the MMSE. It is used to complement the assessment of cognitive status with information obtained from a relative or caregiver of the participant [22, 23]. This scale measures the ability to perform IADL, designed for studies in the community in individuals with good

health or with mild alterations. The instrument evaluates 11 functional activities scored on a scale of 0–33 points, with score of 0 being an ideal performance [50]. This instrument has a high correlation with the Lawton and Brody scale [15].

5.3 Geriatric Depression Scale

Brink and Yesavage created this instrument in 1983 to assess depression in older people with or without cognitive impairment and as a measure of symptom evolution [24]. It can be used in older people who have an optimal state of health, with medical illness, and in those with mild to moderate cognitive impairment. The scale is a self-report, brief, and dichotomous (yes/no). It has 30 questions, although there is an abbreviated version with 15 questions to avoid fatigue and loss of concentration that are sometimes associated with longer instruments. The score is the sum of all positive responses with a cut-off point of 11. A score from 0 to 11 is considered healthy, while a score between 21 and 30 is associated with moderate to severe depression [24, 51].

6 Skeletal Muscle Atrophy: Aging and Hospitalization Combined

The process of skeletal muscle atrophy in the context of hospitalization, a consequence of bed rest, disuse, and decreased physical activity, is a critical factor that relates to the deterioration of functionality in hospitalized patients [52, 53]. In this context, and as compared to their state of fragility before hospital admission, older patients have a high risk of loss of autonomy, worsening of their physical capacity, and a decrease in their primary and instrumental ADL [54]. Additionally, hospital stay leads to a reduction of muscle strength and aerobic capacity. In this sense, the presence of skeletal muscle atrophy is associated with an increase in the days of mechanical ventilation (MV), stay in intensive care units (ICUs), stay in hospital, and risk of mortality [55–57]. In the short-term, the hospitalization effects of older people have a more significant negative impact on the generation of strength and skeletal muscle mass loss compared to young adults. This fact suggests a higher vulnerability of the older age groups, especially in variables such as functional independence [58, 59]. This latter is an indicator of short-term disability and, consequently, a high risk in the deterioration of the quality of life of these patients after the hospitalization stage [60].

6.1 Sarcopenia and Costs Associated with Hospitalization

Due to the increased prevalence in the development of sarcopenia, its effects significantly affect older adults causing an increased risk of suffering a disability, more significant functional impairment, high risk of falls, a high incidence of hospitalization, and a high mortality rate compared to a healthy older adult. The above varies according to age from 4.6% between 70 and 74 years to 31.9% in people over 85 years [61, 62]. For these purposes, the costs associated with this condition in the home environment, outpatient care, and in-hospital services should be considered. In European countries, the total costs related to sarcopenia were estimated at € 1125.3 ± 1367.2 compared to € 561.4 ± 762.6 for non-sarcopenic older people. This has led us to consider sarcopenia as a public health problem [62, 63]. Also, this condition is associated with multiple comorbidities such as osteoporosis, obesity, and type 2 diabetes mellitus, so the economic impact is probably even higher than reported. Older adults with sarcopenia significantly increase their hospital stay compared to non-sarcopenic older adults (13.4 days versus 9.4 days, respectively) [64]. Along the same lines, the average costs per day of hospitalization reach € 68 for people with sarcopenia and € 40 for those who do not suffer from the syndrome, which equals total expenses during hospital stays of € 11,294 and € 6878, respectively [65]. Currently, in the health systems of the USA, data are reported showing a total hospitalization cost of USD 40.4 billion in individuals with sarcopenia, an average of USD 260 per person, and for those over 65 years old, the cost was USD 19.1 billion [66].

6.2 Decrease in Skeletal Muscle Mass and Strength: Clinical Evaluation in the Hospital Context

There is a series of factors to be considered when selecting an element for the evaluation of the decrease in skeletal muscle mass and strength in older people. The factors include: A) the purpose of the measurement, B) the clinical utility that represents decision making, C) the patient's ability to collaborate in the procedure, and D) the validity properties of each instrument [67]. Thus, researchers have made several recommendations based on the tools that provide accurate information regarding the evaluation in the hospital setting of the skeletal muscle mass loss in older patients. The tools to assess skeletal muscle mass and volume are: ultrasonography, computed tomography (CT) or magnetic resonance imaging (MRI). Assessment of muscle strength requires the Medical Research Council sum-score scale (MRC-SS), and grip strength through dynamometry [67–69].

The current changes in the definition of sarcopenia include the loss of muscle function associated with a skeletal muscle mass loss. The management of this condition needs to identify preventive interventions that may delay, improve, reverse, or eliminate the changes produced in muscle strength, muscle mass, and quality. This

will allow us to define and to standardize parameters that include a large percentage of the population [70]. In ICUs, the skeletal muscle mass loss occurs between 25% and 40% in adult patients who are under mechanical ventilation for periods longer than 48 h, with a percentage of skeletal muscle atrophy that reaches 21% during the first 10 days of hospitalization. Additionally, in a study conducted in Asia, the entire hospitalized older adult population showed a severe decrease in muscle strength, and all recorded values located below the cut-off score for the diagnosis of sarcopenia. Currently, the prevalence of sarcopenia in hospitalized older adults reaches between 22% and 26% [68, 69, 71, 72].

Regarding the evaluation methodology, the moment of the measurements should be considered [70]. Accordingly, a delayed measure could mean errors in the actual identification of skeletal muscle atrophy [73]. In this regard and considering the rate of skeletal, muscular atrophy in hospitalized patients, researchers have proposed serial evaluation during the first weeks of hospitalization. During days 1, 3, 5, 7, and 10, it has been proposed to collect quantitative and qualitative dates of the muscle state, standardize the measurements and be able to guide the decision-making regarding specific therapeutic behaviors [74, 75].

MRC-SS

The MRC-SS scale has become the main measuring instrument in hospitalized patients to assess muscle strength [76]. For the application of this scale, there must be an active patient collaboration, both to understand the therapist's instruction and to perform the requested movement. This degree of cooperation is measured by applying the Standardized 5 Questions (S5Q) scale, in which the patient must be able to answer 3 of 5 questions favorably to determine an adequate cognitive state [67–73, 77]. The MRC-SS scale has been used mainly in critically ill patients for the assessment and diagnosis of ICU-acquired weakness (ICUAW). The scores have a range between 0 (complete paralysis) and 60 (normal force) points and a cut-off score below 48 points sets the basis for diagnosis [78]. The scale has an excellent level of reliability in the total sum of scores at the time of assessing all muscle groups. However, it may vary depending on the context of the patient hospitalized in the ICU, or surgical medical services [79].

Regarding the correlation degree with functional scales, the MRC-SS scale obtains adequate correlation degrees with the Barthel index and the elderly mobility scale [80]. Besides, those patients that have a measurement below 48 points, the use of mechanical ventilation could be more prolonged, or the individual could have a more extended stay in the ICU and hospital, and even higher mortality after hospital discharge [79, 81–83].

The MRC-SS scale has predictive value and a higher score in the sum of muscle forces is associated with better physical performance [76]. Due to this, the latest reports include the MRC-SS scale within the tools for assessing muscle strength, which has allowed a therapeutic approach to the physical performance of the subject [84].

Grip Strength in the Hospital

The assessment of grip strength, using dynamometry, is aimed at assessing isometric grip performance in patients who can collaborate and who have a score > 3 on the MRC-SS scale [85]. It is a straightforward application tool with essential clinical utility. The cut-off values for weakness in adult patients hospitalized in ICU, with high levels of sensitivity and specificity, are 7 kg for women and 11 kg for men. In addition, grip strength shows a high level of correlation with the MRC scale [84]. Along the same lines, hospitalized adults with grip strength value less than 5 kg have a high percentage of mortality in the ICU. In addition, it is considered as an independent variable associated with more days of connection to MV, increased hospital stay, and death [83–86]. On the other hand, for patients hospitalized in medicine-surgery services, values lower than 11.52 kg for women and 13.89 kg for men during hospital stay is related to a longer hospitalization time and may be used to predict the degree of functional capacity impairment in older adults after discharge [87].

Regarding the ways to evaluate grip strength, there is a standardized form of evaluation which often uses the right hand, the dominant hand or both sides [49]. However, the ideal way to perform the test consists of having a seated patient, with the elbow at 90°, and the prehensile effort made must be maintained for at least 3 s and with a 30 s pause between each attempt. The best value of three tries is the final record [88–90]. Therefore, grip strength could help to identify potential candidate patients for intervention to mitigate the exposure risk. According to the above, the use of this evaluation technique has advantages over the evaluation of the global strength in limbs, since it uses less time, and it is not necessary to reposition the patient for the measurements and deliver a more objective numerical value [87].

In general, in the context of the older patients hospitalized in medical-surgical units, the assessment of grip strength has been used to determine variables such as mortality, survival, disability, hospital complications, and increased hospital stay [87–91]. In clinical practice, the assessment of grip strength is an easy and quick method to execute, which means that it is an excellent tool for the recognition and diagnosis of skeletal muscular atrophy. This makes it a potential gold standard for measurement for assessment of this medical condition [92].

Ultrasonography

Ultrasonography allows confident quantification of skeletal muscle atrophy. This exam is a validated tool to determine the changes in skeletal muscle mass in the hospital context [93]. This includes the measurement of muscle thickness as well as quality concerning the degree of echogenicity. The association between muscle thickness and strength has not demonstrated with an adequate correlation in this method [94, 95]. The ultrasound image does not have the necessary information regarding the neuromuscular properties. The ultrasound image underestimates the strength loss in critical patients [73]. Despite the above, the application of this

technique has an excellent level of reliability in variables such as echogenicity, independent of the level of experience of the evaluator. It gives added value in the quantification of skeletal muscle atrophy [96].

The measurement of the quadriceps muscle and its rectus femoris and vastus lateral portions, in addition to being performed in full extension position, can be executed in 10°, 50° and 115° knee flexion, in semi-Fowler position, seated position with knee and hip flexion at 90°, and even in a standing position. Before measurement, the subject should remain at rest for 30 min and preferably the same amount of time in the position where the test will be performed, and the muscle should be completely relaxed [97].

On the other hand, in older hospitalized patients in units of low complexity, the reality regarding skeletal muscular atrophy does not differ concerning the data obtained in the ICU. The incidence in the development of sarcopenia is higher in those who remain in the hospital for an average 5 days of bed rest. In hospitalized older adults, the use of ultrasound allows us to measure parameters such as muscle architecture and its association with functional capacity. This tool gives advantages when identifying patients at risk of disability and also to prescribe rehabilitation programs during hospitalization. Thus, it avoids the deterioration of physical capacity, the increase in falls, and even a decrease in the quality of life [98, 99]. The quantification of skeletal muscle atrophy through the use of ultrasonography also allows identification of stages of sarcopenia. Varying degrees of echogenicity and decreased quadriceps muscle thickness relate to physical performance, specifically with the decrease in gait speed and lower limb strength [80].

In summary, ultrasonography is a tool that allows clinicians and researchers to detect changes in skeletal muscle mass before and after intervention programs. It is easy to apply, will enable examinations next to the patient bed, and does not generate damage associated with the measurement technique. It has a high degree of validity and reliability when comparing the measurements with more specific tools such as CT and MRI, and even with the electrical bioimpedance technique (methods described in Sect. 7) [68, 69].

7 Techniques of Skeletal Muscle Mass Assessment

Changes in body composition occur as part of the normal aging process and are associated with important effects on health and function [100]. The decrease in age-related skeletal muscle mass is widely known as one of the main components for the diagnosis of sarcopenia [16]. As mentioned earlier, the main aspects of interest in body composition during the aging process are the content and distribution of body fat and FFM [100]. It is necessary to use valid, precise, and accurate methods to identify high-risk groups of age-related muscle loss and monitor the potential efficacy of health interventions. The methods for the analysis of the body composition are fundamental for an in-depth assessment of the body state [101].

7.1 Body Composition

Wang et al. developed a widely accepted five-level model of body composition research [101]. They divided the human body into different compartments using the following levels: atomic, molecular, cell, tissue system, and the whole body. This model provides a structural framework to explain the relationships between the main compartments of the body.

7.2 Multi-compartment Model

The atomic level is characterized by 11 main elements that comprise more than 99% of body mass including oxygen, carbon, hydrogen, nitrogen, calcium, phosphorus, potassium, sulfur, sodium, chlorine, and magnesium [101]. On the other hand, the molecular level is the most studied level in the field of body composition research. The classic two-component model consisting of fat and FFM is a molecular level model. The molecular level is one level above the atomic scale in body composition and, therefore, has close links with the elements of the nuclear level. Some units of the molecular level such as proteins, fats, bone minerals and water are, in turn, composed of elements of the atomic level such as nitrogen, calcium, carbon, and oxygen [102]. Also, the cellular level consists of three main components: cells, extracellular fluids, and extracellular solids. The extracellular solids component is mainly composed of bone minerals and, to a lesser extent, other solid components such as collagen. This level has been critical in physiological studies [103]. The multi-component model of the tissue system includes adipose tissue and its subcomponents. It also includes various organs such as the brain, heart, liver, kidneys, spleen, lung, and skeletal muscle [104].

7.3 Body Imaging Techniques

Multiple and varied technological evaluation methods have been developed to measure the quantity and quality of skeletal muscle mass, which have revolutionized the current understanding of abnormalities in body composition. Imaging technologies used to detect skeletal muscle mass loss include MRI, CT, and dual-energy x-ray absorptiometry (DXA). These methods differ in terms of costs, reliability, radiation exposure, and availability. Qualitative changes in muscle fibers can only be investigated by histochemical analysis and microscopy using invasive quantification techniques, such as skeletal muscle biopsy.

The following sections describe the main characteristics of the imaging techniques used to assess skeletal muscle mass loss.

MRI

MRI is an imaging technique that estimates the volume of body components. The main advantage of MRI over other technologies is that it does not involve exposure to ionizing radiation and is based on the interaction between hydrogen nuclei in the human body [105]. Data acquisition is based on the generation of a magnetic field that focuses on the alignment of hydrogen nuclei. Then, a radiofrequency pulse is applied, which leads to the absorption of energy by hydrogen protons, which release energy as the pulse goes off. Then, the protons return to the original position. A receiver detects the energy released in the form of a radio frequency signal used to create whole-body or regional images [106].

Using analysis software, the images generated in grayscale can be determined based on the voxel information (volume and pixels) and the area (calculated based on cm^2). Using specific configurations, the sizes of the whole body and/or regions can be calculated based on a three-dimensional formula that represents the area of the tissue, the thickness of the cut, the distance between consecutive images, and the number of images [106]. The tissue mass (kg) can be calculated based on the assumed constant density values for skeletal muscle (1.04 g/cm^3) and adipose tissue (0.92 g/cm^3) [105].

From the skeletal muscle point of view, MRI has been a powerful non-invasive technique with which the structure and function of the skeletal muscle has been evaluated *in vivo*. Beyond the evaluation of the anatomical characteristics of the tissue using conventional MRI techniques, biochemical and physiological properties of the tissue have been studied [107], such as the presence of intramuscular lipids [108], the presence of edema and changes in the mitochondrial metabolism [109]. This latter makes muscle MRI a potent tool in the diagnosis and follow-up of patients with muscle disorders/conditions such as aging [110].

CT

CT is based on an X-ray beam that crosses the body. The intensity of X-ray output transmission is controlled by a series of detectors, which results in the visual production of cross-sections of approximately 10 mm thick. The output transmission is used to calculate the average attenuation coefficient along the length of the X-ray beam. Attenuation coefficients occur in terms of Hounsfield units (HUs), in which bone and other dense materials are equal to +1000, water is equal to zero, and the air is equivalent to −1000 [111]. Visceral organs, bone, skeletal muscle, and adipose tissue have ranges of specific HUs, allowing their identification in cross-sectional images [106, 112].

CT has been used to measure the quality of several tissues, particularly skeletal muscle tissue. CT analysis of latter can distinguish between different types of tissues based on their attenuation characteristics, which in turn can be presented according to tissue density and chemical composition. A typical density for skeletal

muscle is defined as having attenuation values in a range of 40–100 HUs [113]. Low mean values of attenuation will have higher lipid infiltration into the muscle [114].

Typical anatomical locations for measurements of skeletal muscle mass with CT are the thigh, the proximal femur, and the trunk [115]. However, the abdominal area at the level of the third lumbar vertebra is commonly used in most studies, as it relates well to the skeletal muscle mass throughout the body [116]. Also, researchers have used a single-slice CT of the total transverse psoas muscle area to identify sarcopenia [105].

DXA

One of the popular techniques today for estimating body composition is DXA. The principle of using DXA to measure body composition at the molecular level is based on the notion that when an X-ray beam is passed through a complex material, the beam is attenuated in proportion to the composition of the material [117]. The DXA scanner emits two X-ray beams composed of photons at two different energy levels and, as a result of the interaction within the human body, the energy of the X-ray photon undergoes an attenuation that is directly related to the specific chemical compounds with which interacts. By knowing how many photons are transmitted with respect to the detected number, the amount of mineral can be determined, as well as soft tissues (fat mass and FFM) at the level of the whole body or body region (although it does not distinguish visceral and subcutaneous fat in the abdominal region) [118]. Skeletal muscle and adipose tissue mainly consist of water and organic compounds, which restrict the flow of X-rays less than bone [111], so DXA will reflect changes in hydration as a change in lean tissue [100].

The radiation dose varies by model and manufacturer, but is generally small, which makes DXA a safe option for repeated measurements of body composition [119]. In addition, DXA allows the measurement of three compartments of body composition and can provide regional estimates for each of them. This last property has been used to estimate the mass of the appendicular skeletal muscle (ASM) by measuring the amount of lean soft tissue in the upper and lower extremities, which is mainly skeletal muscle [118]. ASM is largely used in the study of sarcopenia and a low ASM is one of the parameters on which all available definitions of sarcopenia are based [16].

However, one should be aware that DXA does not measure skeletal muscle mass directly since some percentage of the mass identified as lean is not muscular and probably includes connective or fibrotic tissue, water and organic mass [120]. There are many ways to represent body composition, but a popular approach in recent years has been the use of indexes normalized by height of the subject. Such as the lean mass index (LMI: total lean mass/height^2), appendicular lean mass (ALM: arms lean mass + legs lean mass) and skeletal muscle mass index (SMI: ALM/height^2) [121, 122]. These have been proposed as parameters for the evaluation of a reduction in skeletal muscle mass which, in turn, is critical in the sarcopenia diagnosis.

Bioelectrical Impedance Analysis (BIA)

The use of BIA to measure the composition is based on the notion that tissues rich in water and electrolytes are less resistant to the passage of an electric current compared to adipose tissue, which is rich in lipids. The accuracy of the estimation of BIA muscle mass is specific to the device and the test population [123]. The best results are obtained when the equation is validated for both the BIA device and the population. The most used equation for the estimation of skeletal muscle was developed by Janssen et al. and is presented below [124]:

$$\text{Skeletal muscle mass}\left(\text{kg}\right) = \left[\begin{array}{l}\left(\text{height}^2 \;/\; \text{bioelectrical resistance} \times 0.401\right) + \\ \left(\text{gender} \times 3.825\right) + \left(\text{age} \times -0.071\right)\end{array}\right] + 5.102$$

The SMI (kg/m^2) is obtained by dividing the absolute muscle mass by the squared height [125], being used as a variable for the diagnosis of sarcopenia [126]. However, it has been shown that BIA results are confused by fluid retention. Hydrostatic abnormalities, peripheral edema, and the use of diuretic medications may affect the validity of BIA measurements in older people [127].

The main concern about this tool is that BIA does not measure any compartment of the body and is considered a doubly indirect method. BIA does not measure anything beyond impedance or its two components, resistance and reactance [128]. By using these variables, in combination with other covariates such as sex, weight and height, BIA can estimate several body compartments that are used as a substitute measure of skeletal muscle mass, according to the reference method used to develop the equations/algorithms [129].

Anthropometry

The imaging equipment mentioned above is not available in low-income clinical settings, such as primary health care centers, which represent the first point of access for the majority of older people with muscle disorders [130]. In such situations, the estimation of body composition and skeletal muscle mass through anthropometric measurements may allow a safe and effective initial evaluation [131]. Anthropometry is a technique that offers excellent portability, applicability, and economy in its use for various environments, being also a non-invasive tool with which the health professional can evaluate size, proportions, and body composition. In contrast to some body imaging techniques, it does not employ ionizing radiation [132].

The inherent errors overshadow the clear advantages of anthropometric techniques. These errors at the level of the evaluator, the instrument, and changes in the body composition of the tissue. The evaluator mistake can be minimized by proper training and by performing several measurements on the same subject. On the other hand, to reduce the error of the instrument, it is necessary to use high quality

measuring devices, developed for anthropometric purposes. Regarding changes in the composition or physical properties of tissues, alterations in muscle tone or fluctuations in hydration are factors that can alter the results [132]. Changes in body water lead to changing the proportion of muscle area, such as can occur in the arm [133]. These same effects are caused by the infiltration of fat or connective tissue in lean mass [132].

Among the anthropometric measurements that can be found to measure skeletal muscle mass are arm circumference and calf circumference (CC) [134]. CC has been recommended for several years [135, 136] as a more sensitive measure than other anthropometric measurements (e.g., arm circumference) to assess the global muscle mass loss in the elderly. The first studies that used the measurement of CC reported a correlation between the decrease in CC and the decrease in physical activity, as well as the fact that CC has a significant relationship with the FFM in the elderly [135].

The World Health Organization (WHO) published a report in 1995 developed by a committee of experts in which it describes the use and interpretation of anthropometry. These reinforce the idea of previous studies recommending CC as the most sensitive measure of skeletal muscle mass in older people, capable of indicating changes in FFM that occur with aging and by decreased physical activity [137].

At present, this measurement has been widely investigated around the world, obtaining a significant variability of values in terms of ethnicity and geographical distribution. A correlation between CC and the appendicular skeletal muscle mass index (ASMI) [138] and skeletal appendicular muscle mass [139] has been described. It is also used in the diagnosis of sarcopenia [138–141].

7.4 Invasive Evaluation of Skeletal Muscle Mass

Skeletal Muscle Biopsy

The percutaneous biopsy technique is used to obtain skeletal muscle samples, this being a minimally invasive and relatively safe procedure. Most subjects undergoing skeletal muscle biopsy report few changes in their ability to perform their daily living activities [142]. Duchenne was the first to build a needle with a trocar to obtain a skeletal muscle sample from living subjects through a percutaneous biopsy [143]. In the 1960s, Bergström introduced a percutaneous biopsy needle similar to that described by Duchenne [144, 145]. This technique has encouraged the diagnosis of myopathies and the understanding of the structure and function of skeletal muscle. Molecular and cellular studies in skeletal muscle require samples obtained primarily from the vastus lateralis muscle. Classically, a muscle biopsy is described as an open procedure in which the skin is cut so that a needle connected to a vacuum pump can be inserted to aspirate skeletal muscle tissue [144]. The procedure takes 15–20 min, most of which to prepare the incision. In studies based on interventions such as physical exercise, muscle samples are often taken before and after the

activity, with one or two samples collected during recovery [146]. Alternatively, when shorter periods of physical activity are investigated, an incision can be made before exercise, covered with a sterile bandage and secured with surgical tape, thus allowing the biopsy sample to be taken quickly after completing a series of exercises [147]. The relative speed of the procedure allows the researcher to capture cellular and molecular events before, during, and after an intervention.

The collected muscle samples can be used to observe a large number of variables, such as the determination of the type and proportion of muscle fibers [148], muscle damage quantification [148, 149], capillary density of muscle tissue [150], enzymatic and oxidative activity [151], protein synthesis [152, 153], inflammatory response markers [154] and oxidative stress [155], among others.

8 Physical Exercise as a Drug to Combat Skeletal Muscle Atrophy in the Older Population

During aging, there is a reduction in physical activity levels, which contributes to the loss of functionality [156]. However, the regular practice of exercise can minimize the harmful effects of a sedentary lifestyle, increase active and independent life expectancy and control the development or progression of chronic diseases, which are characteristic of the aged population [157].

For such effects, the combination of aerobic and muscular strength activities seems to be more effective than any form of training alone, to counteract the detrimental effects on health, general well-being, and the functioning of the cardiovascular and skeletal muscle systems. In addition, it is recommended to include modalities of flexibility and balance to the prescription of exercises for older people to improve problems of joint range and stability. Therefore, an ideal physical intervention plan is one that comprehensively addresses the components that make up physical fitness (Table 6.2) [158].

8.1 Modalities of Physical Exercise in Older People

Resistance Exercise Training (RET) or Muscle Strength

RET is an excellent intervention tool to combat skeletal muscle disuse, sarcopenia, frailty and consequently improve the functional capacity of older people [167], by increasing strength, FFM) and cross-sectional areas of muscle and muscle fiber [168]. RET is defined according to the type of exercise, characterized by repeated muscle contractions against an external load [169]. The contractions can be static (isometric), producing strength without joint movement or changes in muscle length. This is useful in older people when joint movement is restricted due to pain

Table 6.2 Recommendations to practice physical exercise in older people

Exercise type	Recommendations	Doses	Examples
Resistance exercise training (RET) or muscle strength [158–160]	The development of muscle strength and endurance is progressive over time. This means that gradual increases in the amount of weight and the days per week of exercise should be planned so that sessions are not held on consecutive days	*Frequency:* 2–3 days/week *Workload:* Progressive training, low (40% 1RM), moderate (60% 1RM) and high (80% 1RM) load and power exercises (20% –40% 1RM) *Repetitions:* 2–3 sets of 8–12 repetitions that address major muscle groups (it is suggested to include stabilizing spine and core muscles) A specific amount of time for muscle strengthening is not recommended	Exercises using exercise bands, weight machines, hand weights or calisthenic exercises (body weight provides resistance to movement)
Aerobic exercise training (AET) or endurance [159, 170, 173]	Continuous exercise Prefer aerobic activity or endurance that do not impose excessive joint stress The increases should be gradual of cardiorespiratory resistance and preferably distribute the exercise to non-consecutive days, depending on the intensity of the training	*Frequency:* 3–5 days/week *Intensity:* Start with moderate load (50% –60% VO_{2max}) to progress to high load (70% –80% VO_{2max}) *Training time:* 30 min (moderate) or 20 min (vigorous), or divided into 3 series of 10 min Older adults should do at least 150 min a week of moderate intensity, according to the effort perception scale (PSE) 5 or 6/10, or 75 min/week of vigorous intensity (PSE) 7 or 8/10 to obtain substantial benefits for aerobic health.	Walking, dancing, swimming, water aerobics, jogging, aerobic exercise classes, bicycle riding (stationary or on a path)
Stretching or flexibility training [159, 164, 165]	Defined as any activity that maintains or increases flexibility using sustained stretches for each major muscle group Static mode is preferred over ballistic stretching This type of exercise is recommended to maintain the normal range of motion for daily activities, and is usually combined with warm-up or calm-down activities	*Frequency:* ≥ 2–3 days/week They generally complement aerobic or strength training sessions *Intensity:* stretch to the point of tightness or slight discomfort *Repetitions:* 2–4 for each stretch, maintaining the technique for 30–60 s	Static and dynamic elongations are the most used in the older population Achieving an improvement in ROM, regardless of the type of stretch chosen The use of ballistic stretching is not promoted, due to its complexity and associated risks

(continued)

Table 6.2 (continued)

Exercise type	Recommendations	Doses	Examples
Balance training [159, 166]	Older adults at risk of falls should do balance training ≥3 days/week and do standardized exercises of a strengthening program shown to reduce falls Progressively difficult postures that gradually reduce the support base, with dynamic movements that disturb the center of gravity and stress the postural muscle groups	Frequency: ≥2–3 days/week. Training with proprioceptive characteristics, agility, walking With progressive complexity, around postures, disturbance exercises, reduction of sensory input (eyes closed)	Walking backwards, sideways, on heels, on toes, and standing from a sitting position Exercises can increase in difficulty by progressing from holding to a stable support (such as furniture) while performing the exercises to doing so without support Tai Chi and yoga are alternatives that can help prevent falls

or injury. There are also dynamic contractions, which can be divided in concentric or eccentric [169].

Another form of RET is high-speed resistance training, or also known as power. This involves the use of rapid contractions with low external resistances at approximately 40% of the 1 repetition maximum (1RM). In older adults, it may be relevant to practice this modality because the disproportionate reduction of type II muscle fibers, translates into a rapid and progressive loss of muscle power [163, 170]. Other improvements attributed to the RET act on the muscular quality especially during the first phases of the training, increasing the rates of recruitment and/or discharge of the motor units. The benefits of this are observed in a similar way between older and younger people [170].

Regarding the prescription of this type of physical exercise, some reports show favorable changes on muscular endurance, the FFM, and body fat [171]. However, for increases in lean body mass in older people, training volume and age are vital determinants of therapeutic effectiveness, suggesting that higher doses result in a more significant adaptive response. Given this context, the current recommendations, which recommend a sequential increase in the load, should be modified towards the control of the total dose. This means the series performed, as well as the repetitions and weight lifted, to generate significant improvements in the physical condition [161].

In summary, the intervention of RET should be progressive in the total volume load, that is from 60% to 70% of 1RM towards high intensity (80% of 1RM), controlling the number of repetitions and series, with a physical work applied to the whole body, 2 or 3 times a week [159].

Most of the research applied in older people is carried out using strength training machines, such as leg press, chest press, knee extension, and lat pulldown devices.

All of these are chosen because they represent the totality of muscles associated with functionality. It should be noted how essential the incorporation of the exercises for the lower extremities is because the decrease in strength and skeletal muscle atrophy during aging is superior in this region when compared to the upper extremities. For these reasons, the intervention of the lower extremities provides an excellent means of improving the capacity of locomotion and reduction of the risk of falling [160].

Aerobic Exercise Training (AET) or Endurance

Aerobic training involves the participation of large muscle groups, which move rhythmically and steadily for prolonged periods [170]. The aerobic capacity measured using the maximum oxygen consumption (VO_{2max}) shows a constant decrease with age of up to 10% per decade after 25 years. This decrease is mainly due to the reduction in cardiac output caused by an increase in peripheral circulatory resistance [172]. Therefore, it is important to incorporate aerobic exercise in older adults to mediate age-related circulatory system detriments, such as elastic arterial stiffness and endothelial vascular dysfunction. Favorable adaptations occur including lower heart rate at rest or at any submaximal workload, decrease in systolic, diastolic and mean blood pressure during exercise or improvements in vasodilation and oxygen absorption capacity in trained muscle groups [162].

Similarly, AET programs contribute to protective metabolic cardiovascular effects. These effects include reductions in atherogenic levels (reduced triglycerides and higher concentrations of HDL), increased transport of glucose in skeletal muscle, and improved insulin action throughout the body, which ultimately leads to the reduction of cardiovascular risk [173]. This type of exercise also improves body composition, such that at moderate intensity (≥60% of VO_{2max}), it is useful for the loss of total body fat and fat of the intra-abdominal region (>10%), both in a young or older population who are overweight. Also, it has effects on FFM by stimulating protein synthesis in healthy older individuals, so that it can lead to skeletal muscle growth, mainly of the slow myofibers [174]. Despite this, the statistical power of the effect of muscular hypertrophy is significantly higher for resistance training, which also benefits the population suffering from chronic diseases such as diabetes, obesity, and heart failure [175, 176].

Supervised programs that seek to improve aerobic capacity should be executed at a sufficient intensity (≥60% VO_{2max}), frequency (≥3 days/week), and adequate duration (≥16 weeks), both in healthy middle-aged and older adults [159].

Stretching or Flexibility Training

Flexibility corresponds to the ability to move a joint through a full range of motion (ROM) and is mainly dependent on tendons, bones, and muscle length [170, 177].

During aging the ROM changes, triggering a loss that varies in each individual. From the age of 71, flexibility decreases on average by 20–30% in the hip and spine and 30–40% in the ankle, especially in women [164]. These effects result in a limited range of movement in the joints, which can lead to an increased risk of skeletal muscle injuries, falls, and less efficiency in the gait due to the reduction in stride length, speed, and balance, typical of the elderly [178].

Given this context, stretching can maintain and/or improve musculoskeletal flexibility and increase the quality of body movement [179, 180]. There are different modalities, such as treating this component of physical fitness, among which static stretching (SS) exercises stand out. SS is defined as the most effective alternative to improve joint range and prevent damage to muscles and tendons. Its application is recommended after an aerobic training of resistance or muscular power. The contractile capacity of the musculoskeletal tissue is not altered, unlike the dynamic stretching (DS) that can also be used as an alternative to warming or in preparation to the movement and is recommended before the main training phase [181].

Despite the link between functionality and this type of training in older people, there is little research dedicated to examining dosage, types of elongations, timing of application, and regarding the impact on flexibility around health outcomes in general [165, 182].

Concerning dosage in older people, there are more significant gains in the ROM with longer durations of stretching (30–60 s). The repetition of each elongation exercise is most effective when done 2–4 times. Improvements after 3–12 weeks of training are observed if performed at a frequency of at least 2–3 times weekly, with more significant progress if done daily. The main objective is to reach 60 s of total stretching time per flexibility exercise, resting between stretches for approximately 30–60 s [159].

Balance Training

The balance gives the possibility of maintaining the center of mass of the body within the limits of the support base. For this purpose, the postural control synchronizes several systems including sensory (i.e., vestibular, visual, somatosensory), cognitive (central nervous system), and skeletal muscle ones [183].

Balance disorders increase in the geriatric population, as a result of multifactorial causes, presenting weakness in the core stabilizing muscles, alteration in muscle activation patterns, loss of proprioception and the ability to control central processing and normal muscular effectors, which contributes to deficiencies in stability and balance. This exposes older people to the risk of falls in situations that demand balance [184]. Consequently, many older adults are at risk of falling during their ADL. In most cases, falls and associated injuries impair the quality of life and cause physical limitations, anxiety, loss of confidence, and fear of movement [185]. For these reasons, adapted physical activity programs have been suggested to improve the balance control of older participants. Among the components of this type of training, of the main challenge is to integrate the sensory and neuromuscular

systems in accordance with the information and adaptation to the needs of the environment. This must consider changes in the direction of travel, orientation in space, speed or height of the center of mass and thus allow the overcoming of challenges such as double or multiple tasks improving the stability and speed of walking [186, 187].

Following the beneficial effects of the inclusion of balance exercises to a training program in the older people, the combination of movements that include balance and coordination are recommended, such as tai chi and yoga, that incorporate motor skills, agility, and proprioceptive training [188–191]. For balance exercises, it is recommended to perform 2–3 sessions per week, for periods of at least 8 weeks, as a tool to improve part of physical fitness, agility, quality of life, and reduce the risk of falling [159, 161, 170, 171].

Final Considerations

Aerobic and resistance exercises should be applied to restore or maintain independence in ADLs, where mainly activities that involve the muscle strength component prevent, delay or modulate frailty, along with regulating the disproportionate increase in sarcopenia. Increasing muscle protein synthesis, skeletal muscle mass, improvement of neural recruitment, and muscle strength ensure more positive effects. The combination of these components of physical fitness (cardiorespiratory capacity and muscular strength) [158, 165, 171] has produced beneficial effects on body composition [161, 168, 174, 175] and functionality, as well as in general well-being among elderly users [159, 166].

It should be kept in mind that older people have a high risk of falling, due to difficulties in their motor capacity and locomotion, resulting from reduced flexibility, balance, or coordination. Therefore, among the variety of modalities of physical exercise are the tools to improve the detriments of older people. It is essential, also consider each component of the physical condition decreased or altered, in the development of a training program for older adults along with adequate planning around dosing and periodization, to achieve improvements in physical performance and quality of life.

9 Conclusions

The best way to evaluate an older person is through their muscular strength, skeletal muscle mass and physical performance. There are several ways to assess these parameters, which will depend on the clinician or researcher on which one to choose according to the context in which it is found and the available economic resources. Among the strategies we have to combat skeletal muscle atrophy due to aging (or sarcopenia) is physical exercise, which has shown greater beneficial effects compared to other strategies such as nutritional or pharmacological ones. Therefore,

different training modalities have been carried out to counteract the problems associated with decreased muscle strength, skeletal muscle mass loss, and decreased physical performance. Among these, aerobic and resistance training have been shown to have more significant benefits over those of balance and flexibility, with resistance training being the most effective due to its ability to increase skeletal muscle mass and muscle strength in older people and, consequently, improve physical performance. Funding Supported by FONDECYT - Chile (Grant Number 11180949) and Dirección de Investigación (DIUFRO) of Universidad de La Frontera (Grant Number DI18-0068). FAPESP, CNPq, and CAPES support the Rui Curi Research team.

References

1. United Nations, Department of Economic and Social Affairs PD. World Population Ageing 2019: Highlights https://www.un.org/development/desa/family/wp-content/uploads/sites/23/2018/05/BACKGROUND-PAPER.SDGs1611.FINAL_.pdf
2. World Health Organization (2018) Ageing and health. https://www.who.int/news-room/fact-sheets/detail/ageing-and-health
3. Short KR, Nair KS (2000) The effect of age on protein metabolism. Curr Opin Clin Nutr Metab Care 3(1):39–44
4. Giovannini S, Marzetti E, Borst SE, Leeuwenburgh C (2008) Modulation of GH/IGF-1 axis: potential strategies to counteract sarcopenia in older adults. Mech Ageing Dev 129(10):593–601
5. Jackman RW, Kandarian SC (2004) The molecular basis of skeletal muscle atrophy. Am J Physiol Cell Physiol 287(4):C834–C343
6. Kudryavtseva AV, Krasnov GS, Dmitriev AA, Alekseev BY, Kardymon OL, Sadritdinova AF et al (2016) Mitochondrial dysfunction and oxidative stress in aging and cancer. Oncotarget 7(29):44879–44905
7. Fan J, Kou X, Jia S, Yang X, Yang Y, Chen N (2016) Autophagy as a potential target for sarcopenia. J Cell Physiol 231(7):1450–1459
8. Dalle S, Rossmeislova L, Koppo K (2017) The role of inflammation in age-related sarcopenia. Front Physiol 8:1045. https://doi.org/10.3389/fphys.2017.01045
9. Miljkovic N, Lim JY, Miljkovic I, Frontera WR (2015) Aging of skeletal muscle fibers. Ann Rehabil Med 39(2):155–162
10. Burd NA, Gorissen SH, Van Loon LJC (2013) Anabolic resistance of muscle protein synthesis with aging. Exerc Sport Sci Rev 41(3):169–173
11. Rosenberg IH (1997) Sarcopenia: origins and clinical relevance. J Nutr 127(5):990S–991S
12. Cruz-Jentoft AJ, Sayer AA (2019) Sarcopenia. Lancet 393(10191):2636–2646
13. Mahoney FI, Barthel DW (1965) Functional evaluation: the Barthel index. Md State Med J 14:61–65
14. Katz S, Heiple KG, Downs TD, Ford AB, Scott CP (1967) Long term course of 147 patients with fracture of the hip. Surg Gynecol Obstet 124(6):1219–1230
15. Lawton MP, Brody EM (1969) Assessment of older people: self-maintaining and instrumental activities of daily living. Gerontologist 9(3):179–186
16. Cruz-Jentoft AJ, Bahat G, Bauer J, Boirie Y, Bruyère O, Cederholm T et al (2019) Sarcopenia: revised European consensus on definition and diagnosis. Age Ageing 48(1):16–31
17. Podsiadlo DRS (1991) The timed “up & go”: a test of basic functional mobility for frail elderly persons. J Am Geriatr Soc 39(2):142–148

18. Eladio Mancilla S, José Valenzuela H, Máximo Escobar C (2015) Timed up and go right and left unipodal stance results in Chilean older people with different degrees of disability. Rev Med Chil 143(1):39–46
19. Tinetti ME, Franklin Williams T, Mayewski R (1986) Fall risk index for elderly patients based on number of chronic disabilities. Am J Med 80(3):429–434
20. Guralnik JM, Ferrucci L, Simonsick EM, Salive ME, Wallace RB (1995) Lower-extremity function in persons over the age of 70 years as a predictor of subsequent disability. N Engl J Med 332(9):556–561
21. Rikli RE, Jones CJ (2000) Senior fitness test manual. Human Kinetics Publishers, Champaign. ISBN-10: 9780736033565
22. Folstein MF, Folstein SE, McHugh PR (1975) "Mini-mental state". A practical method for grading the cognitive state of patients for the clinician. J Psychiatr Res 12(3):189–198
23. Pfeffer RI, Kurosaki TT, Harrah CH, Chance JM, Filos S (1982) Measurement of functional activities in older adults in the community. J Gerontol 37(3):323–329
24. Yesavage JA, Brink TL, Rose TL, Lum O, Huang V, Adey M et al (1982) Development and validation of a geriatric depression screening scale: a preliminary report. J Psychiatr Res 17(1):37–49
25. Stuck AE, Siu AL, Wieland GD, Rubenstein LZ, Adams J (1993) Comprehensive geriatric assessment: a meta-analysis of controlled trials. Lancet 342(8878):1032–1036
26. González N, Bilbao A, Forjaz MJ, Ayala A, Orive M, Garcia-Gutierrez S et al (2018) Psychometric characteristics of the Spanish version of the Barthel index. Aging Clin Exp Res 30(5):489–497
27. Granger CV, Hamilton BB, Gresham GE (1988) The stroke rehabilitation outcome study--part I: general description. Arch Phys Med Rehabil 69(7):506–509
28. Granger CV, Hamilton BB, Gresham GE, Kramer AA (1989) The stroke rehabilitation outcome study: part II. Relative merits of the total Barthel index score and a four-item subscore in predicting patient outcomes. Arch Phys Med Rehabil 70(2):100–103
29. Mahoney FI, Wood OH, Barthel DW (1958) Rehabilitation of chronically ill patients: the influence of complications on the final goal. South Med J 51(5):605–609
30. Katz S, Ford AB, Moskowitz RW, Jackson BA, Jaffe MW (1963) Studies of illness in the aged: the index of ADL: a standardized measure of biological and psychosocial function. JAMA 185(12):914–919
31. Katz S, Downs TD, Cash HR, Grotz RC (1970) Progress in development of the index of ADL. Gerontologist 10(1):20–30
32. Jiménez-Caballero PE, López-Espuela F, Portilla-Cuenca JC, Pedrera-Zamorano JD, Jiménez-Gracia MA, Lavado-García JM et al (2019) Evaluation of the instrumental activities of daily living following a stroke by means of the Lawton and Brody scale. Rev Neurol 55(6):337–242
33. Graf C (2008) The Lawton instrumental activities of daily living scale. Medsurg Nurs 17(5):343–344
34. Massy-Westropp NM, Gill TK, Taylor AW, Bohannon RW, Hill CL (2011) Hand grip strength: age and gender stratified normative data in a population-based study. BMC Res Notes 4:127. https://doi.org/10.1186/1756-0500-4-127
35. Carmeli E, Patish H, Coleman R (2003) The aging hand. J Gerontol A Biol Sci Med Sci 58(2):146–152
36. Kong YK, Lee JH, Shin JM, Shim HH, Kim JK, Cho MU et al (2019) Evaluation of subjective perceived rating for grip strength depending on handedness for various target force levels. Work 62(1):21–26
37. Sanders JB, Bremmer MA, Comijs HC, van de Ven PM, Deeg DJH, Beekman ATF (2017) Gait speed and processing speed as clinical markers for geriatric health outcomes. Am J Geriatr Psychiatry 25(4):374–385
38. Hackett RA, Davies-Kershaw H, Cadar D, Orrell M, Steptoe A (2018) Walking speed, cognitive function, and dementia risk in the English longitudinal study of ageing. J Am Geriatr Soc 66(9):1670–1675

39. Hofheinz M, Mibs M (2016) The prognostic validity of the timed up and go test with a dual task for predicting the risk of falls in the elderly. Gerontol Geriatr Med 2:2333721416637798. https://doi.org/10.1177/2333721416637798
40. Barry E, Galvin R, Keogh C, Horgan F, Fahey T (2014) Is the timed up and go test a useful predictor of risk of falls in community dwelling older adults: a systematic review and meta-analysis. BMC Geriatr 14:14. https://doi.org/10.1186/1471-2318-14-14
41. Ortega A (2016) Prevention of falls in the elderly: a review of new concepts based on the evidence. Eur J Investig Heal Psychol Educ 6(2):71–82
42. Guevara CR, Lugo Validez LH (2012) Validity and reliability of Tinetti Scale for Colombian people. Revista Colombiana de Reumatología 19:213–233
43. Curcio F, Basile C, Liguori I, Della-Morte D, Gargiulo G, Galizia G et al (2016) Tinetti mobility test is related to muscle mass and strength in non-institutionalized elderly people. Age (Omaha) 38(5–6):525–533
44. Stookey AD, Katzel LI, Steinbrenner G, Shaughnessy M, Ivey FM (2014) The short physical performance battery as a predictor of functional capacity after stroke. J Stroke Cerebrovasc Dis 23(1):130–135
45. Volpato S, Cavalieri M, Sioulis F, Guerra G, Maraldi C, Zuliani G et al (2011) Predictive value of the short physical performance battery following hospitalization in older patients. J Gerontol Ser A Biol Sci Med Sci 66(1):89–96
46. Langhammer B, Stanghelle JK (2015) The senior fitness test. J Physiother 61(3):163. https://doi.org/10.1016/j.jphys.2015.04.001
47. Rikli RE, Jones CJ (2013) Development and validation of criterion-referenced clinically relevant fitness standards for maintaining physical independence in later years. Gerontologist 53(2):255–267
48. Creavin ST, Wisniewski S, Noel-Storr AH, Trevelyan CM, Hampton T, Rayment D et al (2016) Mini-mental state examination (MMSE) for the detection of dementia in clinically unevaluated people aged 65 and over in community and primary care populations. Cochrane Database Syst Rev 1:CD011145. https://doi.org/10.1002/14651858.CD011145.pub2
49. Muñoz Silva CA, Rojas Orellana PA, Marzuca-Nassr GN (2015) Criterios de valoración geriátrica integral en adultos mayores con dependencia moderada y severa en centros de atención primaria en chile. Rev Med Chil 143(5):612–618
50. Pfeffer RI, Kurosaki TT, Chance JM, Filos S, Bates D (1984) Use of the mental function index in older adults: reliability, validity, and measurement of change over time. Am J Epidemiol 120(6):922–935
51. Brink TL (1989) Proper scoring of the geriatric depression scale. J Am Geriatr Soc 37(8):819–819
52. Covinsky KE, Palmer RM, Fortinsky RH, Counsell SR, Stewart AL, Kresevic D et al (2003) Loss of independence in activities of daily living in older adults hospitalized with medical illnesses: increased vulnerability with age. J Am Geriatr Soc 51(4):451–458
53. Kortebein P, Symons TB, Ferrando A, Paddon-Jones D, Ronsen O, Protas E et al (2008) Functional impact of 10 days of bed rest in healthy older adults. J Gerontol A Biol Sci Med Sci 63(10):1076–1081
54. Bodilsen AC, Pedersen MM, Petersen J, Beyer N, Andersen O, Smith LL et al (2013) Acute hospitalization of the older patient: changes in muscle strength and functional performance during hospitalization and 30 days after discharge. Am J Phys Med Rehabil 92(9):789–796
55. Kramer CL (2017) Intensive care unit–acquired weakness. Neurol Clin 35(4):723–736
56. Schreiber A, Bertoni M, Goligher EC (2018) Avoiding respiratory and peripheral muscle injury during mechanical ventilation: diaphragm-protective ventilation and early mobilization. Crit Care Clin 34(3):357–381
57. Ferrante LE, Pisani MA, Murphy TE, Gahbauer EA, Leo-Summers LS, Gill TM (2016) Factors associated with functional recovery among older intensive care unit survivors. Am J Respir Crit Care Med 194(3):299–307
58. Dos Santos C, Hussain SNA, Mathur S, Picard M, Herridge M, Correa J et al (2016) Mechanisms of chronic muscle wasting and dysfunction after an intensive care unit stay: a pilot study. Am J Respir Crit Care Med 194(7):821–830

59. Rossi AP, Rubele S, Pelizzari L, Fantin F, Morgante S, Marchi O et al (2017) Hospitalization effects on physical performance and muscle strength in hospitalized elderly subjects. J Gerontol Geriatr Res 06(02). https://doi.org/10.4172/2167-7182.1000401
60. Guidet B, Vallet H, Boddaert J, de Lange DW, Morandi A, Leblanc G et al (2018) Caring for the critically ill patients over 80: a narrative review. Ann Intensive Care 8(1):114. https://doi.org/10.1186/s13613-018-0458-7
61. Beaudart C, Zaaria M, Pasleau F, Reginster JY, Bruyère O (2017) Health outcomes of sarcopenia: a systematic review and meta-analysis. PLoS One 12(1):e0169548. https://doi.org/10.1371/journal.pone.0169548
62. Steffl M, Sima J, Shiells K, Holmerova I (2017) The increase in health care costs associated with muscle weakness in older people without long-term illnesses in the Czech Republic: results from the survey of health, ageing and retirement in Europe (SHARE). Clin Interv Aging 12:2003–2007
63. Janssen I, Shepard DS, Katzmarzyk PT, Roubenoff R (2004) The healthcare costs of sarcopenia in the United States. J Am Geriatr Soc 52(1):80–85
64. Beaudart C, Rizzoli R, Bruyère O, Reginster J-Y, Biver E (2014) Sarcopenia: burden and challenges for public health. Arch Public Health 72(1):45. https://doi.org/10.1186/2049-3258-72-45
65. Van Vugt JLA, Buettner S, Levolger S, Coebergh Van Den Braak RRJ, Suker M, Gaspersz MP et al (2017) Low skeletal muscle mass is associated with increased hospital expenditure in patients undergoing cancer surgery of the alimentary tract. PLoS One 12(10):e0186547. https://doi.org/10.1371/journal.pone.0186547
66. Goates S, Du K, Arensberg MB, Gaillard T, Guralnik J, Pereira SL (2019) Economic impact of hospitalizations in US adults with sarcopenia. J Frailty Aging 8(2):93–99
67. Parry SM, Huang M, Needham DM (2019) Evaluating physical functioning in critical care: considerations for clinical practice and research. Crit Care 21(1):249. https://doi.org/10.1186/s13054-017-1827-6
68. Looijaard WGPM, Molinger J, Weijs PJM (2018) Measuring and monitoring lean body mass in critical illness. Curr Opin Crit Care 24(4):241–247
69. Mijnarends DM, Meijers JMM, Halfens RJG, ter Borg S, Luiking YC, Verlaan S et al (2013) Validity and reliability of tools to measure muscle mass, strength, and physical performance in community-dwelling older people: a systematic review. J Am Med Dir Assoc 14(3):170–178
70. Gruther W, Benesch T, Zorn C, Paternostro-Sluga T, Quittan M, Fialka-Moser V et al (2008) Muscle wasting in intensive care patients: ultrasound observation of the M. quadriceps femoris muscle layer. J Rehabil Med 40(3):185–189
71. Yamanouchi A, Yoshimura Y, Matsumoto Y, Jeong S (2016) Severely decreased muscle mass among older patients hospitalized in a long-term care ward in Japan. J Nutr Sci Vitaminol (Tokyo) 62(4):229–234
72. Welch C, Hassan-Smith ZK, Greig CA, Lord JM, Jackson TA (2018) Acute sarcopenia secondary to hospitalisation – an emerging condition affecting older adults. Aging Dis 9(1):151–164
73. Parry SM, Granger CL, Berney S, Jones J, Beach L, El-Ansary D et al (2015) Assessment of impairment and activity limitations in the critically ill: a systematic review of measurement instruments and their clinimetric properties. Intensive Care Med 41(5):744–762
74. Parry SM, El-Ansary D, Cartwright MS, Sarwal A, Berney S, Koopman R et al (2015) Ultrasonography in the intensive care setting can be used to detect changes in the quality and quantity of muscle and is related to muscle strength and function. J Crit Care 30(5):1151.e9–1151.e14
75. Puthucheary ZA, Phadke R, Rawal J, McPhail MJW, Sidhu PS, Rowlerson A et al (2015) Qualitative ultrasound in acute critical illness muscle wasting. Crit Care Med 43(8):1603–1611
76. Connolly B, Thompson A, Moxham J, Hart N (2012) Relationship of Medical Research Council sum-score with physical function in patients post critical illness. Am J Respir Crit Care Med 185:A3075–A3075

77. Parry SM, Berney S, Granger CL, Dunlop DL, Murphy L, El-Ansary D et al (2015) A new two-tier strength assessment approach to the diagnosis of weakness in intensive care: an observational study. Crit Care 19(1):52. https://doi.org/10.1186/s13054-015-0780-5
78. Stevens RD, Marshall SA, Cornblath DR, Hoke A, Needham DM, De Jonghe B et al (2009) A framework for diagnosing and classifying intensive care unit-acquired weakness. Crit Care Med 37(10 Suppl):S299–S308
79. Brunello AG, Haenggi M, Wigger O, Porta F, Takala J, Jakob SM (2010) Usefulness of a clinical diagnosis of ICU-acquired paresis to predict outcome in patients with SIRS and acute respiratory failure. Intensive Care Med 36(1):66–74
80. Yamada M, Kimura Y, Ishiyama D, Nishio N, Abe Y, Kakehi T et al (2017) Differential characteristics of skeletal muscle in community-dwelling older adults. J Am Med Dir Assoc 18(9):807.e9–807.e16
81. Sharshar T, Bastuji-Garin S, Stevens RD, Durand MC, Malissin I, Rodriguez P et al (2009) Presence and severity of intensive care unit-acquired paresis at time of awakening are associated with increased intensive care unit and hospital mortality. Crit Care Med 37(12):3047–3053
82. Ali NA, O'Brien JM, Hoffmann SP, Phillips G, Garland A, Finley JCW et al (2008) Acquired weakness, handgrip strength, and mortality in critically III patients. Am J Respir Crit Care Med 178(3):261–268
83. Lee JJ, Waak K, Grosse-Sundrup M, Xue F, Lee J, Chipman D et al (2012) Global muscle strength but not grip strength predicts mortality and length of stay in a general population in a surgical intensive care unit. Phys Ther 92(12):1546–1555
84. Vanpee G, Hermans G, Segers J, Gosselink R (2014) Assessment of limb muscle strength in critically ill patients: a systematic review. Crit Care Med 42(3):701–711
85. García-Peña C, García-Fabela LC, Gutiérrez-Robledo LM, García-González JJ, Arango-Lopera VE, Pérez-Zepeda MU (2013) Handgrip strength predicts functional decline at discharge in hospitalized male elderly: a hospital cohort study. PLoS One 8(7):e69849. https://doi.org/10.1371/journal.pone.0069849
86. Roberson AR, Starkweather A, Grossman C, Acevedo E, Salyer J (2018) Influence of muscle strength on early mobility in critically ill adult patients: systematic literature review. Hear Lung J Acute Crit Care 47(1):1–9
87. Bohannon RW (2008) Hand-grip dynamometry predicts future outcomes in aging adults. J Geriatr Phys Ther 31(1):3–10
88. Mohamed-Hussein AAR, Makhlouf HA, Selim ZI, Gamaleldin Saleh W (2018) Association between hand grip strength with weaning and intensive care outcomes in COPD patients: a pilot study. Clin Respir J 12(10):2475–2479
89. Cottereau G, Dres M, Avenel A, Fichet J, Jacobs FM, Prat D et al (2015) Handgrip strength predicts difficult weaning but not extubation failure in mechanically ventilated subjects. Respir Care 60(8):1097–1104
90. Syddall H, Cooper C, Martin F, Briggs R, Sayer AA (2003) Is grip strength a useful single marker of frailty? Age Ageing 32(6):650–656
91. Schmidt D, Coelho AC, Vieira FN, Torres VF, Savi A, Vieira SRR (2019) Critical illness polyneuromyopathy in septic patients: is it possible to diagnose it in a bedside clinical examination? Arq Neuropsiquiatr 77(1):33–38
92. Bragança RD, Ravetti CG, Barreto L, Ataíde TBLS, Carneiro RM, Teixeira AL et al (2019) Use of handgrip dynamometry for diagnosis and prognosis assessment of intensive care unit acquired weakness: a prospective study. Hear Lung 48(6):532–537
93. Reid CL, Campbell IT, Little RA (2014) Muscle wasting and energy balance in critical illness. Clin Nutr 23(2):273–280
94. Baldwin CE, Bersten AD (2014) Alterations in respiratory and limb muscle strength and size in patients with sepsis who are mechanically ventilated. Phys Ther 94(1):68–82

95. Grimm A, Teschner U, Porzelius C, Ludewig K, Zielske J, Witte OW et al (2013) Muscle ultrasound for early assessment of critical illness neuromyopathy in severe sepsis. Crit Care 17(5):R227. https://doi.org/10.1186/cc13050
96. Sarwal A, Parry SM, Berry MJ, Hsu FC, Lewis MT, Justus NW et al (2015) Interobserver reliability of quantitative muscle sonographic analysis in the critically ill population. J Ultrasound Med 34(7):1191–1200
97. Perkisas S, Baudry S, Bauer J, Beckwée D, De Cock AM, Hobbelen H et al (2018) Application of ultrasound for muscle assessment in sarcopenia: towards standardized measurements. Eur Geriatr Med 9(6):739–757
98. Aubertin-Leheudre M, Martel D, Narici M, Bonnefoy M (2019) The usefulness of muscle architecture assessed with ultrasound to identify hospitalized older adults with physical decline. Exp Gerontol 125:110678. https://doi.org/10.1016/j.exger.2019.110678
99. Martone AM, Bianchi L, Abete P, Bellelli G, Bo M, Cherubini A et al (2017) The incidence of sarcopenia among hospitalized older patients: results from the Glisten study. J Cachexia Sarcopenia Muscle 8(6):907–914
100. Woodrow G (2009) Body composition analysis techniques in the aged adult: indications and limitations. Curr Opin Clin Nutr Metab Care 12(1):8–14
101. Wang ZM, Pierson RN, Heymsfield SB (1992) The five-level model: a new approach to organizing body-composition research. Am J Clin Nutr 56(1):19–28
102. Wang ZM, Heshka S, Pierson RN, Heymsfield SB (1995) Systematic organization of body-composition methodology: an overview with emphasis on component-based methods. Am J Clin Nutr 61(3):457–465
103. Pietrobelli A, Heymsfield S, Wang Z, Gallagher D (2019) Multi-component body composition models: recent advances and future directions. Eur J Clin Nutr 55(2):69–75
104. Gallagher D, Belmonte D, Deurenberg P, Wang Z, Krasnow N, Pi-Sunyer FX et al (1998) Organ-tissue mass measurement allows modeling of REE and metabolically active tissue mass. Am J Phys 275(2):E249–E258
105. Prado CMM, Heymsfield SB (2014) Lean tissue imaging: a new era for nutritional assessment and intervention. J Parenter Enter Nutr 38(8):940–953
106. Ross R, Janssen I (2005) Human body composition. 2nd ed. Heymsfield SB, Lohman T, Wang Z GS (eds) Human kinetics (ADVANTAGE) (Consignment), Leeds, United Kingdom, 2nd revised edition, pp 89–108. ISBN-10: 0736046550
107. Chang G, Wang L, Cárdenas-Blanco A, Schweitzer ME, Recht MP, Regatte RR (2010) Biochemical and physiological MR imaging of skeletal muscle at 7 Tesla and above. Semin Musculoskelet Radiol 14(2):269–278
108. Alizai H, Chang G, Regatte RR (2015) MRI of the musculoskeletal system: advanced applications using high and ultrahigh field MRI. Semin Musculoskelet Radiol 19(4):363–374
109. Parida GK, Roy SG, Kumar R (2017) FDG-PET/CT in skeletal muscle: pitfalls and pathologies. Semin Nucl Med 47(4):362–372
110. Juras V, Mlynarik V, Szomolanyi P, Valkovič L, Trattnig S (2019) Magnetic resonance imaging of the musculoskeletal system at 7T: morphological imaging and beyond. Top Magn Reson Imaging 28(3):125–135
111. Lustgarten MS, Fielding RA (2011) Assessment of analytical methods used to measure changes in body composition in the elderly and recommendations for their use in phase II clinical trials. J Nutr Heal Aging 15(5):368–375
112. Mattsson S, Thomas BJ (2006) Development of methods for body composition studies. Phys Med Biol 51(13):R203–R228
113. Kelley DE, Slasky BS, Janosky J (1991) Skeletal muscle density: effects of obesity and non-insulin-dependent diabetes mellitus. Am J Clin Nutr 54(3):509–515
114. Ross R (2003) Advances in the application of imaging methods in applied and clinical physiology. Acta Diabetol 40(Suppl 1):S45–S50
115. Engelke K, Museyko O, Wang L, Laredo JD (2018) Quantitative analysis of skeletal muscle by computed tomography imaging – state of the art. J Orthop Translat 15:91–103

116. Shen W, Punyanitya M, Wang ZM, Gallagher D, St. Onge MP, Albu J et al (2004) Total body skeletal muscle and adipose tissue volumes: estimation from a single abdominal cross-sectional image. J Appl Physiol 97(6):2333–2338
117. Lukaski HC (1993) Soft tissue composition and bone mineral status: evaluation by dual-energy X-ray absorptiometry. J Nutr 123(2 suppl):438–443
118. Heymsfield SB, Adamek M, Gonzalez MC, Jia G, Thomas DM (2014) Assessing skeletal muscle mass: historical overview and state of the art. J Cachexia Sarcopenia Muscle 5(1):9–18
119. Damilakis J, Adams JE, Guglielmi G, Link TM (2010) Radiation exposure in X-ray-based imaging techniques used in osteoporosis. Eur Radiol 20(11):2707–2714
120. Cawthon PM (2015) Assessment of lean mass and physical performance in sarcopenia. J Clin Densitom 18(4):467–471
121. Shepherd JA, Ng BK, Sommer MJ, Heymsfield SB (2017) Body composition by DXA. Bone 104:101–105
122. Guglielmi G, Ponti F, Agostini M, Amadori M, Battista G, Bazzocchi A (2016) The role of DXA in sarcopenia. Aging Clin Exp Res 28(6):1047–1060
123. Offord NJ, Witham MD (2017) The emergence of sarcopenia as an important entity in older people. Clin Med (Lond) 17(4):363–366
124. 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
125. Janssen I, Baumgartner RN, Ross R, Rosenberg IH, Roubenoff R (2004) Skeletal muscle cutpoints associated with elevated physical disability risk in older men and women. Am J Epidemiol 159(4):413–421
126. Cruz-Jentoft AJ, Baeyens JP, Bauer JM, Boirie Y, Cederholm T, Landi F et al (2010) Sarcopenia: European consensus on definition and diagnosis. Age Ageing 39(4):412–423
127. Haapala I, Hirvonen A, Niskanen L, Uusitupa M, Kröger H, Alhava E et al (2002) Anthropometry, bioelectrical impedance and dual-energy X-ray absorptiometry in the assessment of body composition in elderly Finnish women. Clin Physiol Funct Imaging 22(6):383–391
128. Heymsfield SB, Gonzalez MC, Lu J, Jia G, Zheng J (2015) Skeletal muscle mass and quality: evolution of modern measurement concepts in the context of sarcopenia. Proc Nutr Soc 74(4):355–366
129. Sergi G, De Rui M, Veronese N, Bolzetta F, Berton L, Carraro S et al (2015) Assessing appendicular skeletal muscle mass with bioelectrical impedance analysis in free-living Caucasian older adults. Clin Nutr 34(4):667–673
130. Landi F, Martone AM, Calvani R, Marzetti E (2014) Sarcopenia risk screening tool: a new strategy for clinical practice. J Am Med Dir Assoc 15(9):613–614
131. Landi F, Onder G, Russo A, Liperoti R, Tosato M, Martone AM et al (2014) Calf circumference, frailty and physical performance among older adults living in the community. Clin Nutr 33(3):539–544
132. Heymsfield SB, Casper K (1987) Anthropometric assessment of the adult hospitalized patient. J Parenter Enter Nutr 11(5 suppl):36S–41S
133. Heymsfield SB, Stevens V, Noel R, McManus C, Smith J, Nixon D (1982) Biochemical composition of muscle in normal and semistarved human subjects: relevance to anthropometric measurements. Am J Clin Nutr 36(1):131–142
134. Tosato M, Marzetti E, Cesari M, Savera G, Miller RR, Bernabei R et al (2017) Measurement of muscle mass in sarcopenia: from imaging to biochemical markers. Aging Clin Exp Res 29(1):19–27
135. Patrick JM, Bassey EJ, Fentem PH (1982) Changes in body fat and muscle in manual workers at and after retirement. Eur J Appl Physiol Occup Physiol 49(2):187–196
136. Pearson MB, Bassey EJ, Bendall MJ (1985) The effects of age on muscle strength and anthropometric indices within a group of elderly men and women. Age Ageing 14(4):230–234
137. http://www.who.int/childgrowth/publications/physical_status/en/

138. Ukegbu PO, Kruger HS, Meyer JD, Nienaber-Rousseau C, Botha-Ravyse C, Moss SJ et al (2018) The association between calf circumference and appendicular skeletal muscle mass index of black urban women in Tlokwe City. J Endocrinol Metab Diabetes 23(3):86–90
139. Kim M, Won CW (2019) Prevalence of sarcopenia in community-dwelling older adults using the definition of the European Working Group on Sarcopenia in Older People 2: findings from the Korean Frailty and Aging Cohort Study. Age Ageing 48(6):910–916
140. Kusaka S, Takahashi T, Hiyama Y, Kusumoto Y, Tsuchiya J, Umeda M (2017) Large calf circumference indicates non-sarcopenia despite body mass. J Phys Ther Sci 29(11):1925–1928
141. Pagotto V, Santos KFD, Malaquias SG, Bachion MM, Silveira EA (2018) Calf circumference: clinical validation for evaluation of muscle mass in the elderly. Rev Bras 71(2):322–328
142. Andrew Shanely R, Zwetsloot KA, Travis Triplett N, Meaney MP, Farris GE, Nieman DC (2014) Human skeletal muscle biopsy procedures using the modified Bergström technique. J Vis Exp 91:51812. https://doi.org/10.3791/51812
143. Duchenne GB (1868) Recherches sur la paralysie musculaire pseudo-hypertrophique ou paralysie myoo-sclerosique. Archs Gen Med 6(5)
144. Bergström J (1962) Muscle electrolytes in man determined by neutron activation analysis on needle biopsy specimens. Scand J Clin Lab Invest 14(Supp 68):7–110
145. Bergström J, Hultman E (1967) A study of the glycogen metabolism during exercise in man. Scand J Clin Lab Invest 19(3):218–228
146. Coyle EF, Coggan AR, Hemmert MK, Ivy JL (1986) Muscle glycogen utilization during prolonged strenous exercise when fed carbohydrate. J Appl Physiol 61(1):165–172
147. Utter AC, Kang J, Nieman DC, Dumke CL, McAnulty SR, Vinci DM et al (2004) Carbohydrate supplementation and perceived exertion during prolonged running. Med Sci Sports Exerc 36(6):1036–1041
148. Horstman AMH, Backx EMP, Smeets JSJ, Marzuca-Nassr GN, Van Kranenburg J, De Boer D et al (2019) Nandrolone decanoate administration does not attenuate muscle atrophy during a short period of disuse. PLoS One 14(1):e0210823. https://doi.org/10.1371/journal.pone.0210823
149. Langer HT, Senden JMG, Gijsen AP, Kempa S, van Loon LJC, Spuler S (2018) Muscle atrophy due to nerve damage is accompanied by elevated myofibrillar protein synthesis rates. Front Physiol 9:1220. https://doi.org/10.3389/fphys.2018.01220
150. Holloway TM, Snijders T, Van Kranenburg J, Van Loon LJC, Verdijk LB (2018) Temporal response of angiogenesis and hypertrophy to resistance training in young men. Med Sci Sports Exerc 50(1):36–45
151. Niemeijer VM, Snijders T, Verdijk LB, Van Kranenburg J, Groen BBL, Holwerda AM et al (2018) Skeletal muscle fiber characteristics in patients with chronic heart failure: impact of disease severity and relation with muscle oxygenation during exercise. J Appl Physiol 125(4):1266–1276
152. Kilroe SP, Fulford J, Holwerda AM, Jackman SR, Lee BP, Gijsen AP et al (2019) Short-term muscle disuse induces a rapid and sustained decline in daily myofibrillar protein synthesis rates. Am J Physiol Endocrinol Metab 19. https://doi.org/10.1152/ajpendo.00360.2019. [Epub ahead of print]
153. Dirks ML, Smeets JSJ, Holwerda AM, Kouw IWK, Marzuca-Nassr GN, Gijsen AP et al (2019) Dietary feeding pattern does not modulate the loss of muscle mass or the decline in metabolic health during short-term bed rest. Am J Physiol Endocrinol Metab 316(3):E536–E545
154. Tsintzas K, Stephens FB, Snijders T, Wall BT, Cooper S, Mallinson J et al (2017) Intramyocellular lipid content and lipogenic gene expression responses following a single bout of resistance type exercise differ between young and older men. Exp Gerontol 93:36–45
155. Whitfield J, Ludzki A, Heigenhauser GJF, Senden JMG, Verdijk LB, van Loon LJC et al (2016) Beetroot juice supplementation reduces whole body oxygen consumption but does not improve indices of mitochondrial efficiency in human skeletal muscle. J Physiol 594(2):421–435

156. Wullems JA, Verschueren SMP, Degens H, Morse CI, Onambélé GL (2016) A review of the assessment and prevalence of sedentarism in older adults, its physiology/health impact and non-exercise mobility counter-measures. Biogerontology 17(3):547–565
157. Watson KB, Carlson SA, Gunn JP, Galuska DA, O'Connor A, Greenlund KJ et al (2014) Physical inactivity among adults aged 50 years and older – United States, 2014. MMWR Morb Mortal Wkly Rep 65(36):954–958
158. Mora JC, Valencia WM (2018) Exercise and older adults. Clin Geriatr Med 34(1):145–162
159. Chodzko-Zajko WJ, Proctor DN, Fiatarone Singh MA, Minson CT, Nigg CR, American College of Sports Medicine et al (2009) American College of Sports Medicine position stand. Exercise and physical activity for older adults. Med Sci Sports Exerc 41(7):1510–1530. https://doi.org/10.1249/MSS.0b013e3181a0c95c
160. 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
161. 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
162. Seals DR, DeSouza CA, Donato AJ, Tanaka H (2008) Habitual exercise and arterial aging. J Appl Physiol (1985) 105(4):1323–1332
163. Sayers SP, Gibson K (2010) A comparison of high-speed power training and traditional slow-speed resistance training in older men and women. J Strength Cond Res 24(12):3369–3380
164. Stathokostas L, McDonald MW, Little RMD, Paterson DH (2013) Flexibility of older adults aged 55–86 years and the influence of physical activity. J Aging Res 2013:743843. https://doi.org/10.1155/2013/743843
165. Montero-Fernández N, Serra-Rexach JA (2013) Role of exercise on sarcopenia in the elderly. Eur J Phys Rehabil Med 49(1):131–143
166. Dunsky A (2019) The effect of balance and coordination exercises on quality of life in older adults : a mini-review. Front Aging Neurosci 11:318. https://doi.org/10.3389/fnagi.2019.00318
167. Fragala MS, Cadore EL, Dorgo S, Izquierdo M, Kraemer WJ, Peterson MD et al (2019) Resistance training for older adults: position statement from the national strength and conditioning association. J Strength Cond Res 33(8):2019–2052
168. Binder EF, Yarasheski KE, Steger-May K, Sinacore DR, Brown M, Schechtman KB et al (2011) Effects of progressive resistance training on body composition in frail older adults: results of a randomized, controlled trial. J Gerontol Ser A Biol Sci Med Sci 60(11):1425–1431
169. Kraemer WJ, Ratamess NA, Flanagan SD, Shurley JP, Todd JS, Todd TC (2017) Understanding the science of resistance training: an evolutionary perspective. Sport Med 47(12):2415–2435
170. Galloza J, Castillo B, Micheo W (2017) Benefits of exercise in the older population. Phys Med Rehabil Clin N Am 28(4):659–669
171. Phu S, Boersma D, Duque G (2015) Exercise and Sarcopenia. J Clin Densitom 18(4):488–492
172. Seals DR, Walker AE, Pierce GL, Lesniewski LA (2009) Habitual exercise and vascular ageing. J Physiol 587(Pt 23):5541–5549
173. Rivera-Brown AM, Frontera WR (2012) Principles of exercise physiology: responses to acute exercise and long-term adaptations to training. PM R 4(11):797–804
174. Harber MP, Konopka AR, Douglass MD, Minchev K, Kaminsky LA, Trappe TA et al (2009) Aerobic exercise training improves whole muscle and single myofiber size and function in older women. Am J Physiol Regul Integr Comp Physiol 297(5):R1452–R1459
175. Sipilä S, Elorinne M, Alen M, Suominen H, Kovanen V (1997) Effects of strength and endurance training on muscle fibre characteristics in elderly women. Clin Physiol 17(5):459–474
176. Liu Y, Ye W, Chen Q, Zhang Y, Kuo CH, Korivi M (2019) Resistance exercise intensity is correlated with attenuation of HbA1c and insulin in patients with type 2 diabetes: a systematic review and meta-analysis. Int J Environ Res Public Health 16(1). pii: E140. https://doi.org/10.3390/ijerph16010140
177. Micheo W, Baerga L, Miranda G (2012) Basic principles regarding strength, flexibility, and stability exercises. PM R 4(11):805–811

178. Seco J, Abecia LC, Echevarría E, Barbero I, Torres-Unda J, Rodriguez V et al (2013) A long-term physical activity training program increases strength and flexibility, and improves balance in older adults. Rehabil Nurs 38(1):37–47
179. Behm DG, Blazevich AJ, Kay AD, McHugh M (2015) Acute effects of muscle stretching on physical performance, range of motion, and injury incidence in healthy active individuals: a systematic review. Appl Physiol Nutr Metab 41(1):1–11
180. Zhou WS, Lin JH, Chen SC, Chien KY (2019) Effects of dynamic stretching with different loads on hip joint range of motion in the elderly. J Sport Sci Med 18(1):52–57
181. Reid JC, Greene R, Young JD, Hodgson DD, Blazevich AJ, Behm DG (2018) The effects of different durations of static stretching within a comprehensive warm-up on voluntary and evoked contractile properties. Eur J Appl Physiol 118(7):1427–1445
182. Stathokostas L, Little RMD, Vandervoort AA, Paterson DH (2012) Flexibility training and functional ability in older adults: a systematic review. J Aging Res 2012:306818. https://doi.org/10.1155/2012/306818
183. Horak FB (2006) Postural orientation and equilibrium: what do we need to know about neural control of balance to prevent falls? Age Ageing 35(Suppl 2):ii7–ii11
184. Dunsky A, Zeev A, Netz Y (2017) Balance performance is task specific in older adults. Biomed Res Int 2017:6987017. https://doi.org/10.1155/2017/6987017
185. Kojima G (2015) Frailty as a predictor of future falls among community-dwelling older people: a systematic review and meta-analysis. J Am Med Dir Assoc 16(12):1027–1033
186. Silsupadol P, Shumway-Cook A, Lugade V, van Donkelaar P, Chou LS, Mayr U et al (2009) Effects of single-task versus dual-task training on balance performance in older adults: a double-blind, randomized controlled trial. Arch Phys Med Rehabil 90(3):381–387
187. Azadian E, Torbati HRT, Kakhki ARS, Farahpour N (2016) The effect of dual task and executive training on pattern of gait in older adults with balance impairment: a randomized controlled trial. Arch Gerontol Geriatr 62:83–89
188. Zhong D, Xiao Q, He M, Li Y, Ye J, Zheng H et al (2019) Tai Chi for improving balance and reducing falls: a protocol of systematic review and meta-analysis. Medicine (Baltimore) 98(17):e15225. https://doi.org/10.1097/MD.0000000000015225
189. Huang ZG, Feng YH, Li YH, Lv CS (2017) BMJ open systematic review and meta-analysis: Tai Chi for preventing falls in older adults. BMJ Open 7(2):e013661. https://doi.org/10.1136/bmjopen-2016-013661
190. Saravanakumar P, Higgins IJ, Van Der Riet PJ, Marquez J, Sibbritt D (2014) The influence of tai chi and yoga on balance and falls in a residential care setting: a randomised controlled trial. Contemp Nurse 48(1):76–87
191. Sivaramakrishnan D, Fitzsimons C, Kelly P, Ludwig K, Mutrie N, Saunders DH et al (2019) The effects of yoga compared to active and inactive controls on physical function and health related quality of life in older adults- systematic review and meta-analysis of randomised controlled trials. Int J Behav Nutr Phys Act 16(1):33. https://doi.org/10.1186/s12966-019-0789-2

Chapter 7
Polyphenols as an Effective Therapeutic Intervention Against Cognitive Decline During Normal and Pathological Brain Aging

S. Asha Devi and Anudita Chamoli

1 Introduction

Globally, an alarming increase in the elderly population has had profound implications, not only on the individuals' health but also for society and the economy. A prediction based on statistics by the World Health Organisation has indicated an enormous increase of the global population over 60 years of age to 22% by 2050 [1]. However, as attempts to improve the longevity of the population are increasing, the burden of the increasing incidences of age-related neurodegenerative disorders, such as Alzheimer's and Parkinson's disease, is on the rise. Alongside this rise are the crucial and fundamental questions that need to be resolved, i.e. at what age do these brain diseases occur and what are the age-related factors that predispose patients to neurodegenerative diseases?

In this review, we focus specifically on middle-age as an important risk factor for cognitive decline in normal aging subjects and how this decline is further impacted by neurons in specific regions of the brain leading to neurodegenerative diseases in subjects over 80 years of age. However, vigorous efforts towards any preventive measure against the onset of various brain disorders should also consider prioritising mechanisms related to normal aging such as inflammatory processes and impaired redox balance as essential tissue factors responsible for initiating the loss of neurons in sub-fields of the brain that are specific for cognitive functions. The literature on intervention studies has described in mechanistic terms polyphenols' effects through interactions with cellular signal transduction pathways. In addition, polyphenol-rich foods, such as fruit and vegetables, have been shown to either protect or slow down the progression of cerebrovascular diseases, such as strokes, and

S. A. Devi (✉) · A. Chamoli
Laboratory of Gerontology, Department of Zoology, Bangalore University, Bangalore, India
e-mail: sambeashadevi@bub.ernet.in

P. C. Guest (ed.), *Reviews on New Drug Targets in Age-Related Disorders*,
Advances in Experimental Medicine and Biology 1260,
https://doi.org/10.1007/978-3-030-42667-5_7

many neurological disorders, including dementia [2–5] and cognitive impairment in elderly populations. Polyphenol consumption in middle-age is also related to better cognitive function much later in life [6].

2 Polyphenols

Polyphenols are secondary metabolites in plants. The main components of polyphenols are phenolic acids, anthocyanins, flavonoids and simple and complex flavonoids as well. Flavonoids are the largest group of polyphenols that can be further classified into four main classes: flavonoids, phenolic acids, stilbenes, and lignans. A detailed classification of polyphenols has been reviewed by Archivo and his coscientists [7]. Of particular interest are the flavonoid anthocyanins, which impart red and blue colours to berries, grapes, and red wine. The beneficial effects of grape seeds on human health lie in the fact that they have highest concentrations of antioxidant activities in comparison with many other polyphenolic extracts from plants [8] and this is largely related to its flavan-3-ols and condensed tannins [9]. The flavonoids include gallic acid, the monomeric flavan-3-ols catechin, epicatechin, gallocatechin, epigallocatechin, and epicatechin 3-O-gallate. In addition, they contain procyanidin dimers, trimers, and more highly polymerised procyanidins. Of these, the simplest are dimeric proanthocyanidins, possessing ten to eight linked monomers [10–12]. Using liquid chromatography-tandem mass spectrometry (LC-MS/MS) technique, we have shown the bioavailability of tannins, (+)-catechin, and (−)-epicatechin in the hippocampus [13] and prefrontal cortex [14] of grape seed proanthocyanidin extract (GSPE)-supplemented young and middle-aged male Wistar rats.

Polyphenols supplied by the diet as functional foods are providing several benefits, especially for the elderly populations across the globe. In fact, some studies have demonstrated an interest of the consumers in such foods enriched with antioxidants, and these are now referred to as ‘nutraceuticals’ [15–17]. There is active intestinal absorption of the polyphenols following ingestion of polyphenol-rich foods [18, 19]. Polyphenols possess distinctive physiologically-supportive properties that are described as anti-diabetic, anti-inflammatory, anti-thrombotic, anti-hypertensive, and more importantly, anti-oxidant [20, 21]. In fact, experimental evidence has described polyphenols as micronutrients with anti-aging properties. Polyphenols are often perceived as pleiotropic, exerting their antioxidant and anti-inflammatory potential against several disease-relevant biological pathways [16]. Studies have shown that polyphenols and their metabolites in mammals can pass across the blood brain barrier (BBB) into the brain and bolster neurological functions [22–26]. Furthermore, the bioavailable concentrations of certain polyphenols such as anthocyanins have been identified in the hippocampus and cortex of rats supplemented with blueberry for 8 weeks [27] and 4 weeks in pigs [28]. In addition, the study also showed that the extent of deposition of anthocyanin in the brain is not in proportion to that of the plasma levels when measured immediately after consumption of the berry, thus concluding that uptake of polyphenols in the brain can also happen by

mechanisms other than passive diffusion. However, it is uncertain whether polyphenols and its metabolites primarily enter via simple or facilitated diffusion [29, 30]. Interestingly, uptake of the monomer constituents of GSPE, (+)-catechin and (−)-epicatechin, is through an isomer-selective transport in endothelial cells of the BBB [31]. In addition, Liang and co-workers [32] have demonstrated the accumulation of a product of catechin metabolism, 3-*O*-Me-catechin-5-*O*-glucuronide, in the rat brain. However, the limited bioavailability of polyphenols in the brain has been related to the selective permeability of the BBB, weak absorption, and rapid elimination from circulation [25].

3 Polyphenols and the Normal Aging Brain

Brain aging is associated with loss in volume and dendritic atrophy in the hippocampus (HC) and medial prefrontal cortex (mPFC) in rats [33] and humans [34–36]. Middle-aged rats experience reductions in neuronal number, volume, and density in the anterior cingulate cortex (ACC) and prelimbic cortex (Prl) of the dorsomedial prefrontal cortex (dmPFC) [14]. Studies have shown that young rats of 4–6 months of age have longer dendritic trees, elevated levels of synaptic markers, and better cognition compared to older rats 22–24 months-old, which have shorter dendrites and lower levels of synaptic markers [37, 38]. These age-related morphological changes represent an imbalance between generation and degeneration of dendrites in the old and their role in pathological neurodegeneration [39].

The brain is characterised by high levels of polyunsaturated fatty acids and oxidative stress (OS) is highly prevalent in normal aging. Some areas related to cognition, such as the PFC and HC, become dysfunctional as a result of increased oxidative injury by macromolecules that are essential for neuronal functions. As a result, several cytotoxic free radicals (FRs) contribute to the formation of lipid peroxides within the neurons [40]. Thus, neurons of aging brains suffer from a loss of intracellular concentrations of micronutrients and ions which leads to weak synaptic plasticity. Oxidative stress is highly related to cognitive impairments in aging humans and is largely a result of an imbalance between reactive oxygen and nitrogen species (RONS) and the antioxidant defence system. The heightened OS occurring in the aging brain is concomitantly accompanied by reductions in redox-active iron [41] with significant lipofuscin accumulation [14, 42].

Among the flavonoid polyphenols, proanthocyanidins are excellent scavengers of superoxide radicals and hydroxyl radicals [43]. Inhibition of oxidative DNA damage in the neural tissue has been reported in rats that were supplemented with GSE (100 mg/kg b.wt.) for 30 days [44] along with a decreased incidence of FR-induced lipid peroxidation (LPO) in the central nervous system of aged rats [45]. Better cognitive performance with reduced acetylcholine esterase (AChE) activity has been reported for adult mice following intra-peritoneal (i.p.) supplementation for 7 days with the polyphenol-rich blueberry extract [46] and in adult and middle-aged rats orally supplemented for 8 weeks with proanthocyanidin-rich GSE at 400 mg/kg body weight [47].

Normal aging of the brain is largely confined to the frontal and temporal lobes compared to the parietal and occipital lobes [48] with a progressive decline in cognition due to disturbances in the hippocampal circuit, including the dentate gyrus (DG) and the PFC [49]. It is known that the functional changes in the pre-existing synaptic connections and the synthesis of new proteins and more importantly, their capacity for establishing new connections, are critical for short-term and long term memory storage. It is made possible due to their potential to interact with the molecular components in the brain sites for memory. Alterations in cognition with age are manifested by a significant decline in spatial and working memory as evidenced by a delayed retrieval of a learned task. Polyphenols, when supplemented daily, can reverse age-related declines in memory because of their potential to interact with the molecules in cognitive sites and modify the pathways within neurons and synapses, as well as facilitate *de novo* protein synthesis, and in turn, are effective in improving the process of memory [50].

Animal studies on cocoa and tea flavanol supplementation have also demonstrated that dietary polyphenols are beneficial in reversing the course of neuronal and behavioural aging [51]. For instance, human studies have shown that cocoa flavanol consumption improved working memory and attention [52].

The anti-aging effects of GSE are attributable to the polyphenolics in reversing the neurobehavioral aging. Animal studies have shown that polyphenol extracts and individual polyphenols can benefit older and impaired rats that suffer cognitive deficits as a result of age, brain insults, or induced pathologies [53, 54]. The possible mechanisms that can be attributed to polyphenolic protection involve neurogenesis in the DG [55–57].

Polyphenolic activity in scavenging FRs can protect the brain tissue from oxidative injury. The evidence for this comes from behavioural studies in 19–21 month-old rats that consumed 10% grape juice wherein improvements were detected in the release of dopamine from striatal slices and improved cognitive performance in the Morris water maze [58], and from studies where 12 month-old rats were on a daily oral dose of GSPE at 75 mg/kg body weight for 30 days and had better cognition and memory as seen in a T-maze test [42]. Grape seed proanthocyanidin extract can neutralise FRs [59], protect against oxidative damage [60], and reduce the occurrence of diseases. Ample evidence through human and experimental studies on polyphenols and their beneficial effects for improving cognitive ability, more so, in normal aging and those with neurodegenerative disorders [47, 61–65] has led to the new term, neuro-nutraceutical.

4 Polyphenols and Neurodegenerative Diseases

As scientists are trying to achieve longevity in the lifespan, the incidence of several disorders, including neurodegenerative diseases, especially in ages above 70 years, is on the rise. Therefore, attempts in increasing the retention of cognitive functions have also been equally important. It is relevant to emphasise the significance of

sirtuin 1 (SIRT1) which is notably expressed in brain neurons with a role not only in neuronal plasticity but in protection against neuronal disorders [66, 67]. Numerous studies have proven a role of SIRTs in DNA repair, antioxidant defence, and anti-inflammatory mechanisms. Resveratrol has neuroprotective action through alleviating oxidative stress and inflammation, by enhancing vascular function and activating longevity genes and SIRTs [63].

Alzheimer's disease has been seen often, the incidence being about 15–20% in the world population [68]. Among Alzheimer's disease patients, 7% are of familial genetic patterns while environment and epigenetics have a role in the sporadic onset of the disease. Oxidative stress initiates the accumulation of amyloid plaques, a product of the membrane amyloid precursor protein (APP) being fragmented into β-amyloid (Aβ), with 39–43 amino acids being the pathological hallmark in the neocortex of AD patients [69]. As the disease advances, tau-laden tangles, referred to as neurofibrillary tangles (NFT), enlarge with a loss of neurons and synapses in the cerebral cortex and subcortical regions [70–72] followed by cognitive decline and memory loss [73]. The situation is further aggravated through the activation of microglia and astrocytes [74, 75]. The AChE inhibitory activities of grape skin anthocyanin (GSA) extract and the oligomerisation of Aβ by GSPE may be important considerations for designing therapeutic drugs against Alzheimer's disease [76], thus preventing the onset and progression of cognitive deterioration in Alzheimer's disease.

Parkinson's disease is now recognised as the second most prevalent neurodegenerative disease in elderly subjects with a similar economic and social impact as that of Alzheimer's disease. Individuals over the age of 85 years have at least a 5% risk of developing Parkinson's disease [77–79]. The symptoms of Parkinson's disease appear as a result of cell loss in the substantia nigra (SN) that is necessary for motor function, the dopaminergic neurons of the pars compacta are lost. It is also notable hat normal aging is accompanied by pathological changes in other regions of the brain which is exacerbated further in Parkinson's disease [80, 81]. Advanced age promotes a loss of neurons and a loss key mitochondrial proteins and mitochondrial potential, and fragmentation of mitochondrial network. All of these effects lead to loss of neurons with aging. Importantly, in these neurons is a summation effect of reactive oxygen species (ROS) within the mitochondria and OS due to the metabolism of dopamine within them [82]. Reeve and his co-scientists [83] have reviewed extensively on dopaminergic neurons of the pars compacta and advanced age as an important risk factor for the aetiology and pathophysiology of Parkinson's disease in humans.

Despite the fact that Alzheimer's and Parkinson's disease have different clinical symptoms, they have similar pathological mechanisms. In Alzheimer's disease, protein aggregation and accumulation of plaques of Aβ peptide and intracellular NFT of tau protein occurs and Parkinson's disease is marked by appearance of Lewy bodies and Lewy neuritis of intracellular α-synuclein (αS) inclusions. In contrast to these diseases that have minor genetic factors but larger environmental stressors during one's lifetime, amyotrophic lateral sclerosis (ALS) and Huntington's disease (HD) are neurodegenerative disorders which have stronger genetic predispositions [84].

Table 7.1 lists a few representative studies on flavonoid and non-flavonoid polyphenols as enhancers of cognitive ability in animal and human studies.

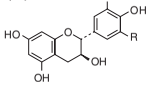
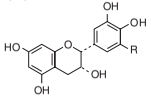
5 Polyphenols and Exercise for Aging Brain

Pure (−)-epicatechin (500 μg/g of food) has been observed to enhance the retention of spatial memory, especially when combined with exercise, in 8–10-week old C57BL/6 mice due to angiogenesis and increased spine density in the DG of the HC [85]. Further, our studies on male Wistar rats have demonstrated that GSPE intervention singly at a dose of 400 mg/kg body weight/day over a period of 16 weeks, in combination with swimming training, was beneficial in protecting the dmPFC [14] and HC [13] by alleviating mitochondrial FRs, and lipid and protein oxidations, as well as ameliorating the cytosolic antioxidant defences. The combined interventions imply a possible synergism between the two especially in middle-aged rats that are vulnerable to OS-induced mitochondrial functions (Fig. 7.1).

6 Conclusions

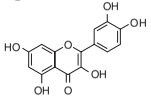
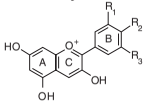
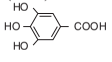
The normal age-related decline in the cognitive abilities in terms of learning and memory is largely traceable to a sizeable number of changes in the biochemical and molecular pathways at specific sites in the brain (HC, PFC, and amygdala). Such modifications are confirmed by several animal and human studies, wherein rigorous approaches have been attempted to delay the further progression towards pathological aging. Some are through dietary interventions related to natural products. Among these, the polyphenolic compounds have been found to have positive effects on brain health and cognitive function. Studies from our laboratory have revealed improved acquisition and retrieval of a learned task with aging by alterations at the biochemical, molecular, and anatomical levels through flavonoid-containing grape seed extract. The emerging evidence is that polyphenols have potential as a natural therapeutic product for treating neurodegenerative diseases. A flavonoid such as GSPE could be an appropriate ingredient for the manufacture of functional and neuro-nutraceutical food products for the elderly. However, these findings underline the physiological complexity that must be examined in designing therapeutic interventions to evoke similar responses in clinical situations.

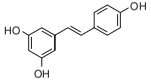
Table 7.1 Representative studies on dietary polyphenols with possible therapeutic use in age-related neurodegenerative diseases

Polyphenols Flavanoids Major constituents	Dietary sources	Effects	Study model	Type of study	References
(+)-Catechin 	Green tea Red wine	Prevent the formation of the endogenous neurotoxin 5-S-cysteinyldopamine in PD similar to in vivo conditions	Cysteinyl-dopamine adducts were formed by reaction of dopamine (100 lM) and L-cysteine (400 lM) in the presence of mushroom tyrosinase (250 U/ml) and catechin	*In vitro*	[86]
	Blue berry (BB)	Better performance in Morris water-maze. 14-unit T-maze. Kainic acid-treated rats impaired learning reduces with BB diet	Young male Fischer-344 rats	*In vivo*	[87]
(−)-Epicatechin (+)-Catechin  (−)-Epicatechin	Grape seed (GSE)	Prevents Aβ deposition in AD	Mice	*In vivo*	[88, 89]
		A noncovalent interaction of polyphenols with proline residues in the proline-rich domain of tau, with Pin1 sites at P213 and P232 Disintegration of PHF	Human brain	Frontal lobe temporal lobe, and parietal lobe of AD patients	[90]
		Improves climbing in *Drosophila*	Drosophila PD model	*In vivo*	[91]
	Grape seed and skin extract (GSSE)	GSSE acts at multiple levels to protect dopamine neurons from degeneration in a 6-OHDA-induced model of PD.	Mice	In vivo and in vitro Mesencephalic primary cell culture	[92]

(continued)

Table 7.1 (continued)

Polyphenols Flavanoids Major constituents	Dietary sources	Effects	Study model	Type of study	References
Quercetin 	Onion Buckwheat Tea Red wine	Brain-targeted polyphenol metabolites, quercetin-3-*O*-glucuronide,reduced the generation of Aβ peptides	Mice	Primary neuron cultures generated from the Tg2576 AD mouse model *In vitro*	[93]
		Reduces Aβ accumulation and protect against neurotoxicity and OS	Rat	Hippocampal neurons	[94]
Anthocyanidins  Anthocyanin	Red wine Berry fruits Cherry juice	Aged rats had lower levels of NF-κB than control animals	Aged Fischer-344 rats	*In vivo* Brain regions	[95]
		Improved short-and long-term memory	Humans	Randomized	[96]
Non-Flavanoids					
Gallicacid (GA) 	Red fruits Black radish Onions Tea leaves	Reduces HC neural damage Reduces FRs Inhibits oligomerization of Aβ	Adult *Wistar* rats	*In vivo* 1 μg/μL $A\beta_{1-42}$-induced AD	[97]
		Reduced ChEs and BACE-1 activity, ROS and MDA levels	Transgenic *D. melanogaster* expressing human amyloid precursor protein and β-secretase (BACE 1) genes as AD flies.	In vivo	[98]

Resveratrol 	Grapes Red wine Berries Pistachios peanuts	RESV and pharmacological activation of AMPK have therapeutic potential against Alzheimer disease.	Non-neuronal and neuronal cells, mouse primary neurons	*In vitro*	[99, 100]
		Anti-amyloidogenic activity of RESV	HEK293 cells stably transfected with human APP695	*In vitro*	[101]
		RESV diminished plaque formation	RESV fed for 45 days to transgenic	*In vitro*	[102]
		RESV negatively controls microglial inflammation triggered by Aβ	Mice	*In vitro* and *In vivo*	[103]
		RESV reduces amyloid protein enhances proteolytic cleavage due to its allosteric activity on the sirtuins	Murine microglial cell line BV-2	*In vitro*	[104]
		RES lowers motor and cognitive deficits	A53T α-synuclein mouse model of PD	*In vitro*	[105]
		Carbidopa / levodopa with + trans RESV improved cognition and movement	75 year old female with PD	*In vitro*	[106]
		RESV with an adjunct (dasatinib) against mitochondrial dysfunction and CDK5 dysregulation in neurodegeneration	Rotenone induced SH-SY5Y cell line models of Parkinson's disease.	*In vitro*	[107]
		RESV to rotenone exposed cells lowered cellular ROS, apoptosis, and increased survival rates	SH-SY5Y cell line models of PD	*In vitro*	[108]

Aβ amyloid beta, *AD* Alzheimer's disease, *AMPK* AMP-activated protein kinase, *APP* amyloid precursor protein, *BACE-1* β-secretase, *ChEs* Choline esterases, *LTP* long-term potentiation, *MDA* malondialdehyde, *NF-κB* (nuclear factor kappa-light-chain-enhancer of activated B cells, *OS* oxidative stress, *6-OHDA* 6-hydroxydopamine, *PD* Parkinsons' disease, *PHF* paired helical filaments, *RESV* resveratrol, *ROS* reactive oxygen species

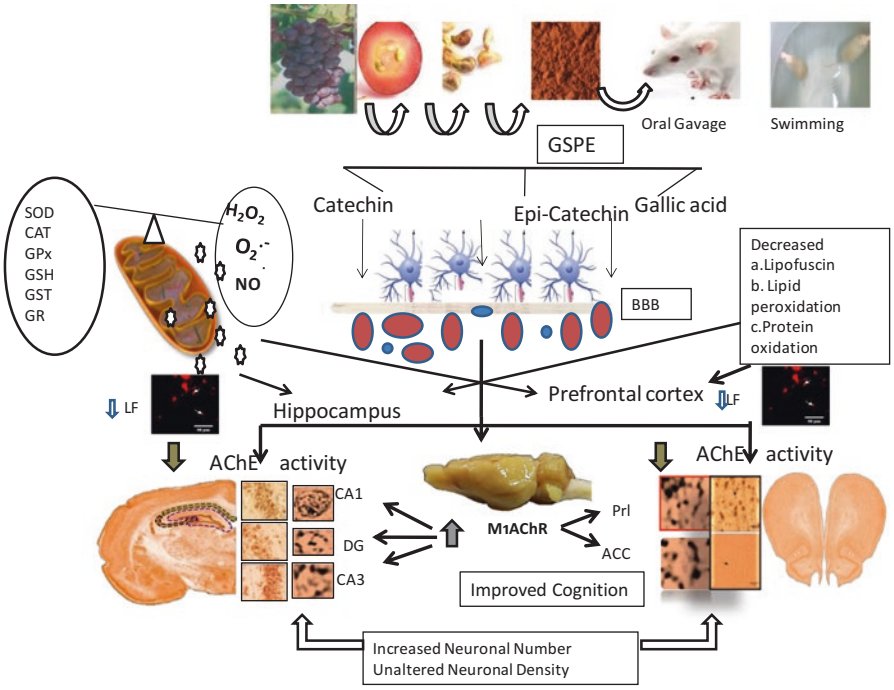


Fig. 7.1 Neuroprotection from grape seed proanthocyanidin extract and swimming training in middle-aged male Wistar rat. AChE, acetylcholine esterase; ACC, anterior cingulated cortex; BBB, blood brain barrier; CAT, catalase; CA1, cornus ammonis 1; CA3, cornus ammonis 3; DG, dentate gyrus; GSH, glutathione; GPx, glutathione peroxidase; GR, glutathione reductase; GSPE, grape seed proanthocyanidin extract; GST, glutathione-S-transferase; H2O2, hydrogen peroxide; LF, lipofuscin; M1AChR, muscarinic acetylcholine receptor NO•, nitric oxide;; O2•-, superoxide; Prl, prelimbic cortex; SOD, superoxide dismutase

Acknowledgements We thank all the lab members for their support in reading the manuscript. We apologize to research groups for not mentioning their studies due to space constraints. Financial support for our studies cited in this article has been through research grants from the Indian Council of Medical Research (ICMR, Referral No.54/9/CFP/GER/2011/NCD-II, dt.30.04.2012), New Delhi and the Promotion of University Research and Scientific Excellence (PURSE)-Department of Science and Technology (DST, Referral No. SR/59/Z-23/2010/38) dt.27.06.2011), New Delhi, India.

References

1. https://www.un.org/en/development/desa/population/publications/pdf/ageing/WorldPopulationAgeing2019-Highlights.pdf
2. Dai Q, Borenstein AR, Wu YG, Jackson JC, Larson EB (2006) Fruit and vegetable juices and Alzheimer's disease: the Kame Project. Am J Med 119:751–759

3. Devore EE, Kang JH, Breteler M, Grodstein F (2012) Dietary intakes of berries and flavonoids in relation to cognitive decline. Ann Neurol 72:135–143
4. Hollman PCH, Geelen A, Kromhout D (2010) Dietary flavonol intake may lower stroke risk in men and women. J Nutr 140:600–604
5. Letenneur L, Proust-Lima C, Le Gouge A, Dartigues JF, Barberger-Gateau P (2007) Flavonoid intake and cognitive decline over a 10-year period. Am J Epidemiol 165:1364–1371
6. Kesse-Guyot E, Fezeu L, Andreeva VA, Touvier M, Scalbert A, Hercberg S et al (2012) Total and specific polyphenol intakes in midlife are associated with cognitive function measured 13 years later. J Nutr 142:76–83
7. D'Archivio M, Filesi C, Di Benedetto R, Gargiulo R, Giovannini C, Masella R (2007) Polyphenols, dietary sources and bioavailability. Ann Ist Super Sanita 43:348–361
8. Peschel W, Sa'nchez-Rabaneda F, Diekmann W, Plescher A, Gartzı I, Jime'nez D et al (2006) An industrial approach in the search of natural antioxidants from vegetable and fruit wastes. Food Chem 97:137–150
9. Makris DP, Boskou G, Andrikopoulos NK (2007) Recovery of antioxidant phenolics from white vinification solid by-products employing water/ethanol mixtures. Bioresour Technol 98:2963–2967
10. Hernandez-Jimenez A, Gomez-Plaza E, Martinez-Cutillas A, Kennedy JA (2009) Grape skin and seed proanthocyanidins from Monastrell x Syrah grapes. J Agric Food Chem 57:10798–10803
11. Pastrana-Bonilla E, Akoh CC, Sellappan S, Krewer G (2003) Phenolic content and antioxidant capacity of muscadine grapes. J Agric Food Chem 51:5497–4503
12. Yilmaz Y, Toledo RT (2004) Major flavonoids in grape seeds and skins: antioxidant capacity of catechin, epicatechin, and gallic acid. J Agric Food Chem 52:255–260
13. Abhijit S, Sunil JT, Bhagya BS, Shankaranarayana Rao BS, Subramanyam MV, Asha Devi S (2018) Antioxidant action of grape seed polyphenols and aerobic exercise in improving neuronal number in the hippocampus is associated with decrease in lipid peroxidation and hydrogen peroxide in adult and middle-aged rats. Exp Gerontol 101:101–112
14. Abhijit S, Sunil JT, Shankaranarayana Rao BS, Asha Devi S (2019) Grape seed proanthocyanidin extract and swimming training enhances neuronal number in dorso-medial prefrontal cortex in middle-aged male rats by alleviating oxidative stress. J Funct Foods 60:103693. https://doi.org/10.1016/j.jff.2019.103693
15. Varzakas T, Zakynthinos G, Verpoort F (2016) Plant food residues as a source of nutraceuticals and functional foods. Foods 5(4). pii: E88. https://doi.org/10.3390/foods5040088
16. Kumar GP, Khanum F (2012) Neuroprotective potential of phytochemicals. Pharmacogn Rev 6:81–90
17. Weseler AR, Bast A (2017) Masquelier's grape seed extract: from basic flavonoid research to a well-characterized food supplement with health benefits. Nutr J 16:5. https://doi.org/10.1186/s12937-016-0218-1
18. Borges G, Lean MEJ, Roberts SA, Crozier A (2013) Bioavailability of dietary (poly) phenols: a study with ileostomists to discriminate between absorption in small and large intestine. Food Funct 4:754–762
19. Pimpão RC, Ventura MR, Ferreira RB, Williamson G, Santos CN (2015) Phenolic sulfates as new and highly abundant metabolites in human plasma after ingestion of a mixed berry fruit purée. Brit J Nutr 113:454–463
20. Hanhineva K, Torronen R, Bondia Pons I, Pekkinen J, Kolehmainen M, Mykkanen H (2010) Impact of dietary polyphenols on carbohydrate metabolism. Int J Mol Sci 11:1365–1402
21. Khurana S, Venkataraman K, Hollingsworth A, Piche M, Tai TC (2013) Polyphenols: benefits to the cardiovascular system in health and in aging. Nutrients 5:3779–3827
22. Gasperotti M, Passamonti S, Tramer F, Masuero D, Guella G, Mattivi F et al (2015) Fate of microbial metabolites of dietary polyphenols in rats: is the brain their target destination? ACS Chem Neurosci 6:1341–1352

23. Ho L, Ferruzzi MG, Janle EM, Wang J, Gong B, Chen TY et al (2013) Identification of brain-targeted bioactive dietary quercetin-3-O-glucuronide as a novel intervention for Alzheimer's disease. FASEB J 27:769–781
24. Youdim KA, Qaiser MS, Begley DJ, Rice-Evans CS, Abbott NJ (2004) Flavonoid permeability across an in situ model of the blood-brain barrier. Free Radic Biol Med 36:592–604
25. Chen TY, Kritchevsky J, Hargett K, Feller K, Klobusnik R, Song BJ et al (2015) Plasma bioavailability and regional brain distribution of polyphenols from apple/grape seed and bilberry extracts in a young swine model. Mol Nutr Food Res 59:2432–2447
26. Youdim KA, Dobbie MS, Kuhnle G, Proteggente AR, Abbott NJ, Rice-Evans C (2003) Interaction between flavonoids and the blood–brain barrier: in vitro studies. J Neurochem 85:180–192
27. Shukitt-Hale B, Lau FC, Joseph JA (2008) Berry fruit supplementation and the aging brain. J Agric Food Chem 56:636–641
28. Kalt W, Blumberg JB, McDonald JE, Vinqvist-Tymchuk MR, Fillmore SA, Graf BA et al (2008) Identification of anthocyanins in the liver, eye and brain of blueberry-fed pigs. J Agric Food Chem 56:705–725
29. Abbott NJ, Ronnback L, Hansson E (2006) Astrocyte-endothelial interactions at the blood-brain barrier. Nat Rev Neurosci 7:41–53
30. Cardoso FL, Brites D, Brito MA (2010) Looking at the blood-brain barrier: molecular anatomy and possible investigation approaches. Brain Res Rev 64:328–363
31. Faria A, Pestana D, Teixeira D, Couraud PO, Romero I, Weksler B et al (2011) Insights into the putative catechin and epicatechin transport across blood-brain barrier. Food Funct 2:39–44
32. Liang J, Xu F, Zhang YZ, Zang XY, Wang D, Shang MY et al (2013) The profiling and identification of the metabolites of (+)-catechin and study on their distribution in rats by HPLCDAD-ESI-IT-TOF-MSn technique. Biomed Chromatogr 28:401–411
33. Driscoll I, Davatzikos C, An Y, Wu X, Shen D, Kraut M et al (2009) Longitudinal pattern of regional brain volume change differentiates normal aging from MCI. Neurology 72:1906–1913
34. Driscoll I, Hamilton DA, Petropoulos H, Yeo RA, Brooks WM, Baumgartner RN et al (2003) The aging hippocampus: cognitive, biochemical and structural findings. Cereb Cortex 13:1344–1351
35. Freeman SH, Kandel R, Cruz L, Rozkalne A, Newell K, Frosch MP et al (2008) Preservation of neuronal number despite age-related cortical brain atrophy in elderly subjects without Alzheimer disease. J Neuropathol Exp Neurol 67:1205–1212
36. Raz N, Ghisletta P, Rodrigue KM, Kennedy KM, Lindenberger U (2010) Trajectories of brain aging in middle-aged and older adults: regional and individual differences. NeuroImage 51:501–511
37. Dickstein DL, Weaver CM, Luebke JI, Hof PR (2013) Dendritic spine changes associated with normal aging. Neuroscience 251:21–32
38. Mota C, Taipa R, Pereira das Neves S, Monteiro-Martins S, Monteiro S, Palha AJ et al (2019) Structural and molecular correlates of cognitive aging in the rat. Sci Rep 9:2005
39. Querfurth HW, LaFerla FM (2010) Alzheimer's disease. N Engl J Med 362:329–344
40. Asha Devi S, Jolitha AB, Ishii N (2006) Grape seed proanthocyanidin extract (GSPE) and antioxidant defense in the brain of adult rats. Med Sci Monit 12:BR124–BR129
41. Savory J, Rao JK, Huang Y (1999) Age-related hippocampal changes in Bcl-2:bax ratio, oxidative stress, redox-active iron and apoptosis associated with aluminum-induced neurodegeneration: increased susceptibility with aging. Neurotoxicology 20:805–817
42. Asha Devi S, Manjula KR, Sagar Chandrasekhar BK, Ishii N (2011) Grape seed proanthocyanidin lowers brain oxidative stress in the adult and middle-aged rats. Exp Gerontol 46:958–964
43. El-Beshbishy HA, Mohamadin AM, Abdel-Naim AB (2009) In vitro evaluation of the antioxidant activities of grape seed (vitis vinifere) exyract, blackseed (Nigella sativa) extract and curcumin. J Taibah Univ Med Sci 4:23–35

44. Balu M, Sangeetha P, Murali G, Panneerselvam C (2006) Modulatory role of grape seed extract on age-related oxidative DNA damage in central nervous system of rats. Brain Res Bull 68:469–473
45. 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 383:295–300
46. Papandreou MA, Dimakopoulou A, Linardaki ZI, Cordopatis P, Klimis-Zacas D, Margarity M et al (2009) Effect of a polyphenol-rich wild blueberry extract on cognitive performance of mice, brain antioxidant markers and acetylcholinesterase activity. Behav Brain Res 17:352–358
47. Abhijit S, Subramanyam MVV, Asha Devi S (2017) Grape seed proanthocyanidin and swimming exercise protects against cognitive decline: a study on M1 acetylcholine receptors in aging male rat brain. Neurochem Res 42:3573–3586
48. Bentourkia M, Bol A, Ivanoiu A, Labar D, Sibomana M, Coppens A et al (2000) Comparison of regional cerebral blood flow and glucose metabolism in the normal brain: effect of aging. J Neurol Sci 181:19–28
49. Morrison JH, Baxter MG (2012) The ageing cortical synapse: hallmarks and implications for cognitive decline. Nat Rev Neurosci 13:240–250
50. Manach C, Scalbert A, Morand C, Rémési C, Jiménez L (2004) Polyphenols: food sources and bioavailability. Am J Clin Nutr 79:727–747
51. Chan YC, Hosoda K, Tsai CJ, Yamamoto S, Wang MF (2006) Favorable effects of tea on reducing the cognitive deficits and brain morphological changes in senescence accelerated mice. J Nutr Sci Vitaminol 52:266–273
52. Field DT, Williams CM, Butler LT (2011) Consumption of cocoa flavanols results in an acute improvement in visual and cognitive functions. Physiol Behav 103:255–260
53. Miller MG, Shukitt-Hale B (2012) Berry fruit enhances beneficial signaling in the brain. J Agric Food Chem 60:5709–5715
54. Smith JM, Stouffer EM (2014) Concord grape juice reverses the age-related impairment in latent learning in rats. Nutr Neurosci 17:81–87
55. Burke S, Barnes C (2006) Neural plasticity in the ageing brain. Nat Rev Neurosci 7:30–40
56. Rendeiro C, Vauzour D, Kean RJ, Butler LT, Rattray M, Spencer JP et al (2012) Blueberry supplementation induces spatial memory improvements and region-specific regulation of hippocampal BDNF mRNA expression in young rats. Psychopharmacology (Berl) 223:319–330
57. Rendeiro C, Vauzour D, Rattray M, Waffo-Téguo P, Mérillon JM, Butler LT et al (2013) Dietary levels of pure flavonoids improve spatial memory performance and increase hippocampal brain-derived neurotrophic factor. PLoS One 8:e63535. https://doi.org/10.1371/journal.pone.0063535
58. Shukitt-Hale B, Carey A, Simon L, Mark DA, Joseph JA (2006) Effects of concord grape juice on cognitive and motor deficits in aging. Nutrition 22:295–302
59. Feng G, Chen LQ (2003) Determination of procyanidin in grape seed extracts. China Food Addit 6:103–105
60. Spranger I, Sun B, Mateus AM, Freitas V, Ricardo-da-Silva JM (2008) Chemical characterization and antioxidant activities of oligomeric and polymeric procyanidin fractions from grape seeds. Food Chem 108:519–532
61. Shukitt-Hale B, Bielinski DF, Lau FC, Willis LM, Carey AN, Joseph JA (2015) The beneficial effects of berries on cognition, motor behaviour and neuronal function in ageing. Br J Nutr 114:1542–1549
62. Choi MR, Lee MY, Hong JE, Kim JE, Lee JY, Kim TH et al (2014) Rubus coreanus miquel ameliorates scopolamine-induced memory impairments in ICR mice. J Med Food 17:1049–1056
63. Witte AV, Kerti L, Margulies DS, Floel A (2014) Effects of resveratrol on memory performance, hippocampal functional connectivity, and glucose metabolism in healthy older adults. J Neurosci 34:7862–7870

64. Cimrová B, Budáč S, Melicherová U, Jergelová M, Jagla F (2011) Electrophysiological evidence of the effect of natural polyphenols upon the human higher brain functions. Neuro Endocrinol Lett 32:464–468
65. Krikorian R, Boespflug EL, Fleck DE, Stein AL, Wightman JD, Shidler MD et al (2012) Concord grape juice supplementation and neurocognitive function in human aging. J Agric Food Chem 60:5736–5742
66. Herskovits AZ, Guarente L (2014) SIRT1 in neurodevelopment and brain senescence. Neuron 81:471–483
67. Ng F, Tang BL (2013) When is Sirt1 activity bad for dying neurons? Front Cell Neurosci 7:186. https://doi.org/10.3389/fncel.2013.00186
68. Sun AY, Wang Q, Simonyi A, Sun GY (2008) Botanical phenolics and brain health. NeuroMolecular Med 10:259–274
69. Wahlster L, Arimon M, Nasser-Ghodsi N, Post KL, Serrano-Pozo A, Uemura K et al (2013) Presenilin-1 adopts pathogenic conformation in normal aging and in sporadic Alzheimer's disease. Acta Neuropathol 125:187–199
70. Hajieva P (2017) The effect of polyphenols on protein degradation pathways: implications for neuroprotection. Molecules 22(1). pii: E159. https://doi.org/10.3390/molecules22010159
71. Heredia L, Lin R, Vigo FS, Kedikian G, Busciglio J, Lorenzo A (2004) Deposition of amyloid fibrils promotes cell-surface accumulation of amyloid β precursor protein. Neurobiol Dis 16:617–629
72. Utsuki T, Yu QS, Davidson D, Chen D, Holloway HW, Brossi A et al (2006) Identification of novel small molecule inhibitors of amyloid precursor protein synthesis as a route to lower Alzheimer's disease amyloid-β peptide. J Pharmacol Exp Ther 318:855–862
73. Scheltens P, Blennow K, Breteler MM, De Strooper B, Frisoni GB, Salloway S et al (2016) Alzheimer's disease. Lancet 388:505–517
74. Sadigh-Eteghad S, Majdi A, Mahmoudi J, Golzari SE, Talebi M (2016) Astrocytic and microglial nicotinic acetylcholine receptors: an overlooked issue in Alzheimer's disease. J Neural Transm 123:1359–1367
75. Von Bernhardi R (2007) Glial cell dysregulation: a new perspective on Alzheimer disease. Neurotox Res 12:215–232
76. Pervin M, Hasnat A, Lee Y, Kim D, Jo J, Lim B (2014) Antioxidant activity and acetylcholinesterase inhibition of grape skin anthocyanin (GSA). Molecules 19:9403–9418
77. de Lau LM, Breteler MM (2006) Epidemiology of Parkinson's disease. Lancet Neurol 5:525–535
78. Nussbaum RL, Ellis CE (2003) Alzheimer's disease and Parkinson's disease. N Engl J Med 348:1356–1364
79. Wood-Kaczmar A, Gandhi S, Wood NW (2006) Understanding the molecular causes of Parkinson's disease. Trends Mol Med 12:521–528
80. Bender A, Krishnan KJ, Morris CM, Taylor GA, Reeve AK, Perry RH et al (2006) High levels of mitochondrial DNA deletions in substantia nigra neurons in aging and Parkinson disease. Nat Genet 38:515–517
81. Kraytsberg Y, Kudryavtseva E, McKee AC, Geula C, Kowall NW, Khrapko K (2006) Mitochondrial DNA deletions are abundant and cause functional impairment in aged human substantia nigra neurons. Nat Genet 38:518–520
82. Sulzer D (2007) Multiple hit hypotheses for dopamine neuron loss in Parkinson's disease. Trends Neurosci 30:244–250
83. Reevea A, Eve Simcoxa E, Turnbulla D (2014) Ageing and Parkinson's disease: why is advancing age the biggest risk factor? Ageing Res Rev 14:19–30
84. Al-Chalabi A, van den Berg LH, Veldink J (2016) Gene discovery in amyotrophic lateral sclerosis: implications for clinical management. Nat Rev Neurol 13:96–104
85. van Praag H, Lucero MJ, Yeo GW, Stecker K, Heivand N, Zhao C et al (2007) Plant-derived flavanol (−)epicatechin enhances angiogenesis and retention of spatial memory in mice. J Neurosci 27:5869–5878

86. Vauzour D, Vafeiadou K, Spencer JPE (2007) Inhibition of the formation of the neurotoxin 5-S-cysteinyl-dopamine by polyphenols. Biochem Biophys Res Commun 262:340–346
87. Duffy KB, Spangler EL, Devan BD, Guo Z, Bowker JL, Janas AM et al (2008) A blueberry-enriched diet provides cellular protection against oxidative stress and reduces a kainite induced learning impairment in rats. Neurobiol Aging 29:1680–1697
88. Wang J, Ho L, Zhao W, Ono K, Rosensweig C, Chen L et al (2008) Grape-derived polyphenolics prevent A beta oligomerization and attenuate cognitive deterioration in a mouse model of Alzheimer's disease. J Neurosci 28:6388–6392
89. Wang YJ, Thomas P, Zhong JH, Bi FF, Kosaraju S, Pollard A et al (2009) Consumption of grape seed extract prevents amyloid-β deposition and attenuates inflammation in brain of an Alzheimer's disease mouse. Neurotox Res 15:3–14
90. Ksiezak-Redinga H, Hoa L, Santa-Mariaa I, Diaz-Ruiza C, Wanga J, Pasinettia GM (2012) Ultrastructural alterations of Alzheimer's disease paired helical filaments by grape seed-derived polyphenols. Neurobiol Aging 33:427–1439
91. Long J, Gao H, Sun L, Liu J, Zhao-Wilson X (2009) Grape extract protects mitochondria from oxidative damage and improves locomotor dysfunction and extends lifespan in a Drosophila Parkinson's disease model. Rejuvenation Res 12:321–331
92. Ben Youssef S, Brisson G, Doucet-Beaupré H, Castonguay AM, Gora C, Amri M et al (2019) Neuroprotective benefits of grape seed and skin extract in a mouse model of Parkinson's disease. Nutr Neurosci 25:1–15
93. Ho L, Ferruzzi MG, Elsa M, Wang J, Gong B, Chen TY et al (2013) Identification of brain-targeted bioactive dietary quercetin-3-O-glucuronide as a novel intervention for Alzheimer's disease. FASEB J 27:769–781
94. Bastianetto S, Zheng WH, Quirion R (2000) Neuroprotective abilities of resveratrol and other red wine constituents against nitric oxide-related toxicity in cultured hippocampal neurons. Br J Pharmacol 13:711–720
95. Goyarzu P, Malin DH, Lau FC, Taglialatela G, Moon WD, Jennings R et al (2004) Blueberry supplemented diet: effects on object recognition memory and nuclear factor-kappa B levels in aged rats. Nutr Neurosci 7:75–83
96. Kent K, Charlton K, Roodenrys S, Batterham M, Potter J, Traynor V et al (2017) Consumption of anthocyanin-rich cherry juice for 12 weeks improves memory and cognition in older adults with mild-to-moderate dementia. Eur J Nutr 56:333–341
97. Hajipour S, Sarkaki A, Farbood Y, Eidi A, Mortazavi P, Valizadeh Z (2016) Effect of gallic acid on dementia type of Alzheimer disease in rats: electrophysiological and histological studies. Basic Clin Neurosci 7:97–106
98. Ogunsuyi OB, Oboh G, Oluokun OO, Ademiluy AO, Ogunruku OO (2019) Gallic acid protects against neurochemical alterations in transgenic Drosophila model of Alzheimer's disease. Orient Pharm Exp Med. https://doi.org/10.1007/s13596-019-00393-x
99. Vingtdeux V, Dreses-Werringloer U, Zhao H, Davies P, Marambaud P (2008) Therapeutic potential of resveratrol in Alzheimer's disease. BMC Neurosci 9(Suppl. 2):S6. https://doi.org/10.1186/1471-2202-9-S2-S6
100. Vingtdeux V, Giliberto L, Zhao H, Chandakkar P, Wu Q, Simon JE et al (2010) AMP-activate.d protein kinase signaling activation by resveratrol modulates amyloid-beta peptide metabolism. J Biol Chem 285:9100–9113
101. Marambaud P, Zhao H, Davies P (2005) Resveratrol promotes clearance of Alzheimer's disease amyloid- peptides. J Biol Chem 280:37377–37382
102. Karuppagounder SS, Pinto JT, Xu H, Chen HL, Beal MF, Gibson GE (2009) Dietary supplementation with resveratrol reduces plaque pathology in a transgenic model of Alzheimer's disease. Neurochem Int 54:111–118
103. Capiralla H, Vingtdeux V, Zhao H, Sankowski R, Al-Abed Y, Davies P et al (2012) Resveratrol mitigates lipopolysaccharide- and Abeta-mediated microglial inflammation by inhibiting the TLR4/NF-kappaB/STAT sig-naling cascade. J Neurochem 120:461–472

104. Essa MM, Vijayan RK, Castellano-Gonzalez G, Memon MA, Braidy N, Guillemin GJ (2012) Neuroprotective effect of natural products against Alzheimer's disease. Neurochem Res 37:1829–1842
105. Zhang L, Yu X, Ji M, Liu S, Wu X, Wang Y et al (2018) Resveratrol alleviates motor and cognitive deficits and neuropathology in the A53T α-synuclein mouse model of Parkinson's disease. Food Funct 9:6414–6426
106. Barber EK (2017) The benefits of resveratrol with polyphenols in Parkinson's disease with Alzheimer's disease. Alzheimer's and Dementia 13(7 Supplement):P262. https://doi.org/10.1016/j.jalz.2017.06.132
107. Nair AT, Vadivelan R, Ramachandran, Ahamed HN (2019) Resveratrol with an adjunct for improved maintenance of mitochondrial homeostasis and dopamine neuronal rescue in neurodegeneration. J Pharm Sci Res 11:1210–1215
108. Lin KL, Lin KJ, Wang PW, Chuang JH, Lin HY, Chen SD et al (2018) Resveratrol provides neuroprotective effects through modulation of mitochondrial dynamics and ERK1/2 regulated autophagy. Free Radic Res 52:1371–1386

Chapter 8
Early Diagnosis and Targeted Treatment Strategy for Improved Therapeutic Outcomes in Alzheimer's Disease

Francesca L. Guest, Hassan Rahmoune, and Paul C. Guest

1 Introduction

As the average life expectancy continues to rise, the number of people afflicted with Alzheimer's disease worldwide has been projected to increase to 115 million by the year 2050, equivalent to 1.2% of the forecasted population [1, 2]. At the global level, Alzheimer's disease is a major cause of disability for the affected individuals, their families and societies in general. In 2016, the Global Burden of Diseases, Injuries, and Risk Factors (GBD) Study determined that dementia was the fifth leading cause of death and the twenty-third largest cause of disability adjusted life years (DALYs) [3]. The financial costs to society worldwide are also staggering with estimates of 818 million United States Dollars (USDs) in 2015 and projections suggest that this will increase to 2 trillion USD by the year 2030 [4]. It is now critical that new and better treatments are identified to delay the onset or slow the progression of this growing public health crisis.

Alzheimer's disease is characterized as a chronic degeneration of cortical neurons, leading to impaired memory and cognition, with a loss of executive functions [5]. As the disease advances, amyloid plaques and neurofibrillary tangles accumulate in specific brain regions, and this leads to disruption of neuronal signalling and loss of neurons and brain tissue [6, 7]. This results in a global decrease in synaptic plasticity in the hippocampus, the main brain region involved in co-ordination of

F. L. Guest
Musgrove Park Hospital, Taunton, UK

H. Rahmoune
Department of Chemical Engineering & Biotechnology, University of Cambridge, Cambridge, UK

P. C. Guest (✉)
Laboratory of Neuroproteomics, Department of Biochemistry and Tissue Biology, Institute of Biology, University of Campinas (UNICAMP), Campinas, Brazil

P. C. Guest (ed.), *Reviews on New Drug Targets in Age-Related Disorders*,
Advances in Experimental Medicine and Biology 1260,
https://doi.org/10.1007/978-3-030-42667-5_8

leaning, cognition and memory [8, 9]. Ultimately, these combined deficits lead to frailty and fatality, with an average survival time of approximately 5 years after diagnosis [10].

Although it is now recognized as a pathological continuum, three main stages of Alzheimer's disease have been recognized based on symptom type and severity [11]. The first is called the early stage with biomarker abnormalities but little or no cognitive impairment or memory difficulties. The second is the middle stage, characterized by abnormal pathophysiological biomarkers with episodic cognitive and memory impairments. The late stage is marked by pathological biomarker abnormalities and profound cognitive, memory and functional impairments. As with many chronic diseases, the progression through the various stages of Alzheimer's disease has been linked to advancing age, genetic risk factors, vascular disease, infections, immune system function, dietary factors, mitochondrial function and exposure to metals [12]. Furthermore, individuals with other disorders such as major depression, anxiety, metabolic disorders and cardiovascular disease have a higher risk of developing Alzheimer's disease [13].

Currently, treatment of Alzheimer's disease occurs once symptoms are already present. Thus, treatment is most likely initiated when it is too late and irreversible damage to neurons, synaptic connections and brain tissue has already occurred. With the ongoing research to identify new biomarkers and develop new treatments, earlier diagnosis of Alzheimer's disease could help to improve clinical outcomes [14]. Diagnosis is typically carried out via interviews with subject using standardized tests, considering parameters such as memory, higher thinking and other abilities. The patients may also be subjected to brain scans using magnetic resonance imaging (MRI) or computerised tomography (CT) to aid in assessing the degree of atrophy of the hippocampus or other brain regions.

No new drugs to treat Alzheimer's disease have been approved by the Food and Drug Administration (FDA) since the year 2003 [15]. Currently there are five FDA-approved medications which are either cholinesterase inhibiters (tacrine, donepezil, rivastigmine, galantamine) or an N-methyl-D-aspartate (NMDA) receptor antagonist (memantine) [15]. The cholinesterase inhibitors are usually prescribed to treat patients in the early to middle disease stages to improve memory and concentration. Memantine may be given in the middle to late stages for the same reasons and to reduce neuronal damage caused by over-stimulation of NMDA receptors [16]. The anticholinesterase medications can have potentially serious effects and require constant titration and monitoring [17]. Memantine has relatively few side effects but the benefits are mild and tend to diminish over a few months [17]. In addition, amyloid immunotherapies such as aducanumab have been tested but this showed only mild efficacy in the recent phase 3 EMERGE and ENGAGE trials, which may be due to low brain penetrance and poor selectivity between the various forms of the amyloid-beta (Aβ) peptide in the plaque formation cascade [18].

To address these challenges, a strategy is presented which advocates development of more successful therapies for treatment of Alzheimer's disease based on earlier diagnosis before neurological changes have occurred and targeting key precipitation points in the disease cascade pathways based on precision medicine approaches.

2 Alzheimer's Disease Pathophysiology and Biomarkers

Alzheimer's disease is characterized by progressive and irreversible loss of memory and cognitive function. The biggest risk factors are age and the presence of other diseases such as cardiovascular disorders, type 2 diabetes mellitus, obesity, cholesterolemia, kidney disease and psychiatric conditions [19–21]. Although most of the cases of Alzheimer's disease are sporadic, mutations in the amyloid precursor (APP), and presenilin 1 and 2 (PSEN-1 and -2) proteins [22–25], and the apolipoprotein E allele type 4 (APOEe4) have been associated with increased risk [26, 27]. In addition, other genes have been associated with subpopulations of Alzheimer's disease patients including those encoding the ATP binding cassette subfamily A member 7 (*ABCA7*), ADAM metallopeptidase domain 10 (*ADAM10*), A-kinase anchoring protein 9 (*AKAP9*), box dependant MYC interacting protein 1 (*BIN1*), sialic acid binding Ig-like lectin 3 (*CD33*), clusterin (*CLU*), complement C3b/C4b receptor 1 (*CR1*), death-associated protein kinase 1 (*DAPK1*), phospholipase D3 (*PLD3*), sortilin related receptor 1 (*SORL1*), triggering receptor expressed on myeloid cells 2 (*TREM2*) and netrin receptor UNC5C (*UNC5C*) [28, 29]. The main pathological characteristics of Alzheimer's disease are Aβ deposits and neurofibrillary tangles, as well as decreased metabolism, neuronal loss, atrophy of specific brain regions, mitochondrial dysfunction and increased oxidative stress [30]. The Aβ deposits are toxic oligomers which eventually amass to form the characteristic plaques and precipitation of neurofibrillary tangles occurs when the tau protein becomes hyperphosphorylated at threonine 181. Both of these processes are associated with neuroinflammation which leads to the characteristic neuropathology.

2.1 Aβ Plaques

The Aβ peptide is normally produced by proteolytic conversion of APP (Fig. 8.1). However, abnormalities in processing can lead to generation of two different versions of the peptide, comprised of either 40 ($A\beta_{40}$) or 42 ($A\beta 1_{42}$) amino acids. The "sticky" nature of the $A\beta_{42}$ form leads to generation of toxic insoluble plaques in specific brain regions [31]. In turn, this causes disruptions of the cytoskeleton and neuronal functions, which ultimately lead to cell death.

2.2 Neurofibrillary Tangles

The staging of tau pathology has been linked to disease progression with appearance first in the transentorhinal region of the brain (stages 1 and 2), followed by spreading to the limbic region (stages 3 and 4) and finally to the neocortex (stages 5 and 6) [32, 33]. Several reports have demonstrated that tau lesions occurred before

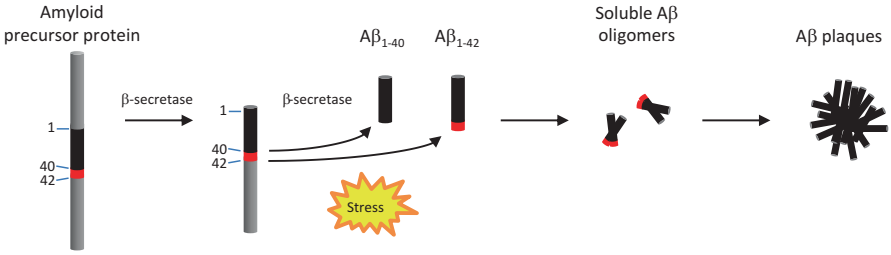


Fig. 8.1 The amyloid precursor protein is proteolytically-cleaved by the β- and γ-secretase enzymes to generate the $A\beta_{40}$ and $A\beta_{42}$ peptides. Under stress-related conditions, increased proportions of the extended $A\beta_{42}$ peptide are produced. These can form soluble toxic oligomers which seed formation of insoluble oligomers, leading to amyloid plaques

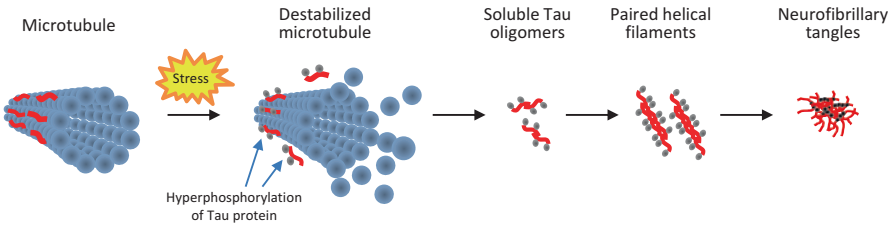


Fig. 8.2 Schematic representation showing hyperphosphorylation of Tau inside neurons leading to microtubule destabilization, formation of toxic soluble tau oligomers, paired helical fragments and neurofibrillary tangles

Aβ deposition and recent positron emission tomography (PET) imaging studies have shown that the patterns of tau patholgy are closely linked to those of the neurodegeneration and cognitive dysfunction in Alzheimer's disease patients. In addition, a positive correlation has been found between staging and tau distribution in both Alzheimer's disease and primary age-related taupathy [34]. Truncation or hyperphosphorylation of tau causes the protein to lose affinity for microtubules and initiates self-assembly of paired helical and straight filaments (Fig. 8.2) [35]. Tau oligomers have been associated with neurodegeneration and memory impairment even in the absence of Aβ plaques. The oligomers appear to consist of β-sheet structures which can promote conversion of fibrils to filaments in a dose-dependent manner.

2.3 Neuroinflammation

It has long been known that microglia help to maintain a healthy brain environment by clearing debris, including misfolded Aβ, tau and other molecules such as α-synuclein. Over-stimulation of microglia has now emerged as a major feature in Alzheimer's disease and other dementias as this drives inflammation, neuronal death and disease progression through the NOD-like receptor protein 3-apoptosis-associated speck-like protein containing a C-terminal caspase recruitment domain

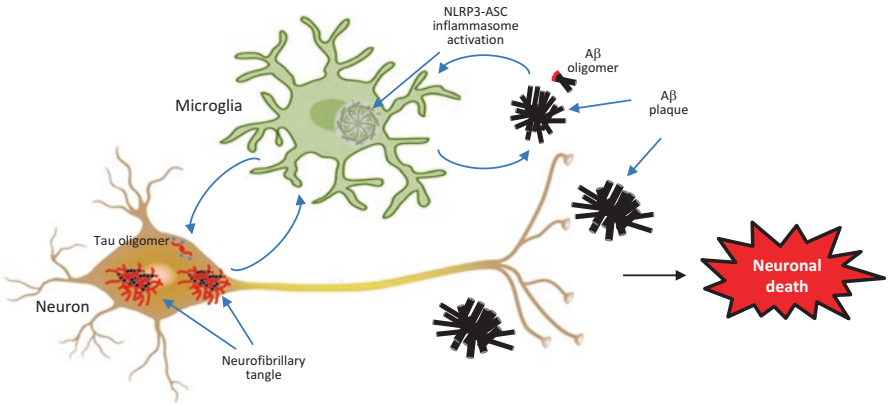


Fig. 8.3 Tau and amyloid pathology stimulates the NLRP3-ASC inflammosome complex in microglia, leading to exacerbation of the pathology and neuronal death

(NLRP3-ASC) pathway [36]. Microglia are the key immune regulators of the brain which detect misfolded proteins such as Aβ and tau, and they respond by initiating inflammation through secretion of cytokines like IL-1β [37]. In this way, ASC specks released by microglia bind rapidly to Aβ, leading to increased formation of oligomers and aggregates (Fig. 8.3) [38].

3 What Comes First – Aβ Plaques or Neurofibrillary Tangles?

The majority of Alzheimer's disease cases manifest as a late onset sporadic form with a small proportion of cases occurring as a genetic familial form with an earlier onset. Most of our understanding of the disease has come from studies of the familial form, which appear to arise from mutations in the *APP*, *PSEN1* or *PSEN2* genes, although a specific allele of the *APOE*gene appears to be a significant risk factor for the sporadic form of Alzheimer's disease. Despite this heterogeneity, the predominating theory has been that all cases of the disease arise from Aβ deposition in the brain. However, several studies have now led to the idea that the primary biomarker changes may arise in different temporal sequences depending on the form of the disease.

3.1 Age-Related Alzheimer's Disease

Research of age-related sporadic Alzheimer's disease subjects showed that tau inclusions appeared before Aβ plaque deposition [39]. One study which used a probabilistic generative model of cerebrospinal fluid (CSF) biomarkers in a hetero-

geneous sporadic Alzheimer's disease set showed that Aβ- or APOE-positive subjects had initial changes in $A\beta_{42}$, followed by changes in the levels of phosphorylated and total tau protein [40]. However, analysis of a broader population found that phosphorylated and total tau changes occurred earlier in the CSF than the $A\beta_{42}$ biomarker. In addition, one study of cognitively-normal individuals found that the longitudinal changes in CSF phosphorylated tau and hippocampal volume correlated with those of global cognitive decline [41].

3.2 Familial Alzheimer's Disease

In familial Alzheimer's disease, there are three subtypes based on the underlying genetic lesion (*APP*, *PSEN1*, *PSEN2*), all of which lead to increased Aβ plaque deposition. For example, most individuals with Down's syndrome produce excess APP with increased Aβ deposition and neuropathology. A meta-study of Down's syndrome subjects found that increased $A\beta_{40}$ and decreased $A\beta_{42}$ /$A\beta_{40}$ ratios may be promising biomarkers for predicting dementia [42]. Likewise, a comparative biomarker study of Columbian familial *PSEN1* carriers and non-carriers showed significant divergences in CSF levels of $A\beta_{42}$ at age 29 years, Aβ plaque deposition at age 31 years, CSF total tau at age 33 years, CSF phosphorylated tau at age 35 years and hippocampal volume reduction at age 42 years [43].

4 New Targets to Delay or Prevent Onset of Alzheimer's Disease

As indicated earlier, the poor to low efficacy achieved in clinical studies which have targeted Aβ may be related to the fact that these reagents do not selectively or effectively target soluble Aβ oligomers [18]. Also, these trials may not have used appropriately-stratified patient populations via a biomarker-based approach. Currently, Alzheimer's disease is diagnosed only after presentation of symptoms. This means that it is in the middle to late stages of the disease by this time and irreversible damage is already likely to have occurred.

4.1 The Potential Benefits of Early Detection

Like most diseases, earlier detection of Alzheimer's disease paves the way for earlier treatment which, in turn, allows for better patient outcomes (Fig. 8.4). With this in mind, studies of specific populations at higher risk of developing Alzheimer's disease has led to important insights regarding disease onset and progression. For example, most Down's syndrome patients with an extra copy of chromosome 21 show the pathological signs of Alzheimer's disease by the time they reach 40 years

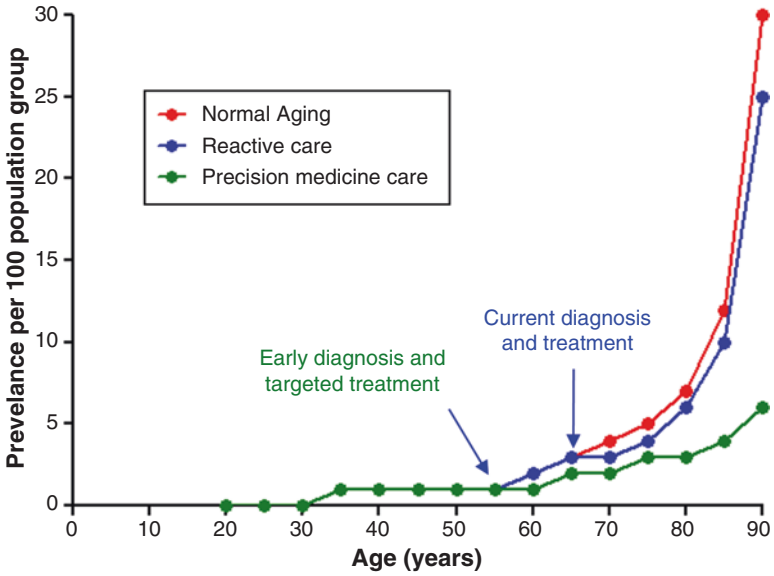


Fig. 8.4 Strategy showing early detection and precision medicine for slowing the progression of Alzheimer's disease

of age [44]. This is likely to be due to the over-expression of the *APP* gene, located on this chromosome. In addition, genes such as the dual specificity tyrosine-phosphorylation-regulated kinase 1A (*DYRK1A*) and the regulator of calcineurin 1 (*RCAN1*) which are thought to contribute to hyper-phosphorylation of tau are located on the same chromosome [45]. As mentioned above, a set of Colombian families with an early-onset autosomal-dominant form of Alzheimer's disease have been identified with a *PSEN1* mutation that leads to excessive production of the $A\beta_{42}$ peptide [46]. The individuals with this mutation have median ages of onset of Aβ deposition, with mild cognitive impairment (MCI) and dementia at the ages of 28, 44 and 49 years, respectively. Time-course studies of such patient populations with an ultra-high risk of developing Alzheimer's disease have a high potential of providing new information leading to the discovery of novel biomarkers and drug targets associated with the earliest stages of the disease. This would enable precision medicine approaches.

4.2 Targeting Soluble Aβ Oligomers

The challenge of generating new therapeutic approaches for Alzheimer's disease is to develop strategies that specifically target only the toxic misfolded forms of $A\beta_{42}$ and tau to maximize efficacy and decrease the chances of unwanted side effects. In addition, patients should be correctly identified as being at risk for developing Alzheimer's disease either by the use of biomarkers. A collaborative

study is currently investigating whether or not an Aβ antibody, crenezumab, can delay or prevent the clinical onset of Alzheimer's disease in cognitively-unimpaired individuals who carry the *PSEN1* mutation described above, compared to placebo [46]. In addition, 100 non-carriers from the same family will also receive placebo. Crenezumab is a humanized IgG4 antibody designed to neutralize Aβ oligomers by blocking the interaction of oligomers with neurons, and promoting the phacocytic removal of oligomers by microglia [47]. The primary outcome measure is a significant change in the Composite Cognitive Test Score from initiation of the study to week 260. The secondary outcomes are the time to progression to MCI or dementia, differences in dementia severity, memory and cognitive functioning, changes in Aβ PET imaging, MRI volumes, and CSF levels of Aβ, total tau and phosphorylated tau. In addition, safety and tolerability will be assessed. There are also plans to measure tau burden using PET in a subset of this study group. This represents the first large-scale clinical trial of a preclinical treatment for Alzheimer's disease.

4.3 Targeting Soluble Tau Oligomers

Tau oligomers are present in dementias such as Alzheimer's disease and may be the precipitating factor in the formation of intracellular tau protein aggregates and are more closely associated with neurodegeneration and memory impairment compared to Aβ plaques. The oligomers appear to consist of β-sheet structures which can promote fibril formation once a size of 20 nm is a attained. This occurs through formation of granular tau protofilaments comprised of approximately 40 tau molecules which can convert to filaments in a dose-dependent manner. Thus, oligomerization is a critical step for tau aggregation and abnormal filament formation. Studies have shown that the tau aggregation process is accompanied by conformational changes resulting from the hyper-phosphorylation at the amino-terminal end and truncations at the carboxy-terminal end of the protein, promoting elongation of the aggregates to form filaments.

Tau isoforms in the human brain can be classified into two groups known as 3-repeat (3R) or 4-repeat (4R), reflecting the number of microtubule binding domains [48]. In Alzheimer's disease both 3R and 4R forms are present which combine in a cross-β/β-helix configuration. These findings afford a means of assessing disease specificity since the presence of 3R and 4R forms may differ and the filaments can fold differently in other disorders such as progressive nuclear palsy, corticobasal degeneration and Pick's disease. Thus, the composition of the 3R and 4R forms can be used to determine the type of taupathy. For this, PET imaging can be used to measure the aggregated insoluble form of Aβ and the fibrillar deposited form of tau to aid early diagnosis [49].

4.4 Targeting Neuroinflammation

Current studies are targeting microglia and the NLRP3-ASC pathway as a means of reducing disease-associated neuroinflammation in dementias (Fig. 8.4). One study showed that NLRP3 inhibition significantly attenuated memory and cognition deficits and decreased microglia activation in hippocampus and cerebral cortex in an APP/PSEN1 mouse model [50]. Furthermore, a study showed that application of an ASC antibody reduced the number of ASC specks and the amyloid pathology in a similar mouse model [38].

A number of studies have provided evidence that immunity, inflammation and reductive-oxidative (redox) processes are also disrupted in Alzheimer's disease [51]. In addition, pro-inflammatory activation of microglia has been reported [52]. This has led to the testing of several anti-inflammatory drugs as potential means of improving symptoms and the pathology [53]. However, typical anti-inflammatory compounds such as cyclooxygenase inhibitors and glucocorticoids showed little or no efficacy and some adverse effects were found [54]. In contrast, a case study showed some improvement of cognitive symptoms using the anti-tumor necrosis factor drug etanercept [55] and this is now being tested in clinical studies [56]. Also, the natural anti-inflammatory/anti-oxidant curcumin has been shown to have neuroprotective effects through prevention of Aβ and tau aggregation [57, 58]. Further preclinical and clinical studies should be conducted to investigate the potential efficacy of this compound as a novel treatment for Alzheimer's disease since the limited numbers of human studies conducted thus far have met with mixed results [59]. The inconsistencies are likely to be due to differences in methodology or heterogeneity of the included study populations.

5 Biomarkers and the Importance of Patient Stratification

Currently, clinical diagnosis of Alzheimer's disease applies criteria outlined in the National Institute of Neurological and Communicative Disorders and Stroke, the Alzheimer's Disease and Related Disorders Association or the National Institute on Aging and Alzheimer's Association [60, 61]. However, imaging studies have shown that not all individuals diagnosed using these criteria have evidence of Alzheimer's disease-related biomarkers [62, 63]. Thus, if patients were to be recruited for a clinical study using these criteria alone without a confirmatory biomarker, it is likely that the test group will be comprised of a mixed population and demonstration of changes in symptoms by disease-modifying agents will be masked. For this reason, biomarkers are urgently needed to help diagnose and stratify patients correctly prior to initiating clinical studies (Fig. 8.4).

For example, MCI is a nonspecific syndrome that can be comprised of several conditions such as Alzheimer's disease, early forms of other dementias, frontal temporal dementia and depression. This has been demonstrated in studies which showed

progression of MCI to Alzheimer's disease or other dementias, as well as continuation of the original conditions or a return to normal cognition [64]. Furthermore, a PET imaging study showed that approximately one-third of amnestic MCI patients did not have evidence of brain Aβ, suggesting that Alzheimer's disease was not the primary pathological condition [65]. Another study found that 29% of MCI patients who developed dementia were given a non-Alzheimer's disease diagnosis at autopsy [66].

Individuals with preclinical Alzheimer's disease do not show cognitive abnormalities although they may show a marginal decrease from previous levels. It is likely that such individuals can only be identified using biomarkers. A meta-study involving PET imaging analysis of cognitively-normal individuals found that positive results for brain Aβ increased from 10% in 50–55 year-olds to 44% in those aged more than 90 years [67].

This demonstrates that the effective use of biomarkers can confirm diagnosis of Alzheimer's disease and rule out other pathologies with an unpredictable therapeutic response to test treatments. Otherwise, it will not be possible to determine if failure of a given trial is due to lack of efficacy of the test compound, an insufficient number of true Alzheimer's disease participants or obscuring of the results due to mixed responses of non-Alzheimer's disease subjects.

5.1 Biomarkers in Body Fluids

Given that CSF is part of the central nervous system, molecular biomarkers in this fluid can be used to monitor brain pathology and treatment response. Higher CSF $A\beta_{42}$ levels appear to be correlated with impaired cognition and measurement of this peptide alone can discriminate individuals with Alzheimer's disease from those with MCI with 90% sensitivity [68]. Furthermore, the $A\beta_{42}/A\beta 1_{40}$ ratio may have stronger specificity for Alzheimer's disease and Aβ PET scans, compared to sole measurements of $A\beta_{42}$ [69–72]. Studies have shown that individuals with MCI tend to progress to dementia more rapidly than age-matched healthy controls and the transition is associated with the CSF levels of $A\beta_{42}$, and total and phosphylated tau [73]. However, these biomarker changes cannot be used to distinguish which patients will progress to Alzheimer's disease compared to the development of other neurodegenerative conditions [74–76]. Other studies have shown that CSF levels of neurogranin are correlated with synaptic degeneration and predictive of hippocampal degeneration and prodromal Alzheimer's disease in cases of MCI [77]. Such findings require validation using cohorts comprised of mixed neurodegenerative conditions.

Some studies have shown that plasma or serum levels of $A\beta_{42}$ and $A\beta_{40}$ may be associated with Alzheimer's disease although there has been some conflicting information on this [78–80] and the circulating levels of these peptides only a low correlation with the corresponding CSF levels [81]. In contrast, a study showed that the circulating levels of phosphorylated tau were correlated with brain Aβ deposition

and neurofibrillary tangles [82] and a prospective study of over 5000 elderly women showed that high circulating levels of tau fragments were correlated with decreased risk of developing dementia [83]. Several other serum of plasma molecules known to have a role in brain function have also been shown to be associated with risk of developing Alzheimer's disease, such as vitamin D [84], thyroid hormone [85], ghrelin [86], sphingolipids [87], and microRNAs [88–90]. Several investigations have also found a link between the levels of serum or plasma cytokine levels with progression of Alzheimer's disease, including interleukin (IL)-1, IL-6, IL-7, IL-8 and tumour necrosis factor receptor 1 [91–93].

5.2 Imaging Biomarkers

A number of tau tracers have been used for early identification of tau pathologies. The use of one tracer called [18F]PI-2620 has shown high affinity for tau deposits in Alzheimer's brain homogenates and specific binding to pathological misfolded tau protein in autoradiographic analysis of brain sections from Alzheimer's disease, Pick's disease and progressive supranuclear palsy, with no specific binding on sections from donors without dementia [94–96]. This compound also showed high selectivity for binding to tau over Aβ or monoamine oxidases A and B. A recent study showed that [18F]PI-2620 also has good brain uptake and rapid washout in preclinical models as well as rapid kinetics, suitable dosimetry and low test-retest variability [96]. Finally, a meta-analysis showed that an imaging technique called optical coherence tomography was capable of detecting losses in the peripapillary retinal nerve layer in patients with MCI and Alzheimer's disease [97], and another study found that this approach can detect Aβ plaques [98]. A recent study found that the reduction in the superficial capillary plexus vessel and perfusion density on optical coherence tomography analysis was significantly correlated with expansion of the inferolateral ventricle in MCI and Alzheimer's disease [99]. In addition, another such study found that the decrease in retinal thickness was correlated with Alzheimer's disease severity [100]. Thus, further research should be conducted to determine the accuracy of this non-invasive means of detecting patients at risk of developing Alzheimer's disease.

6 Conclusions and Future Perspectives

The long-standing strategy of treating Alzheimer's disease as a single pathology and mono-therapeutic approach has met with repeated failures in clinical studies. This is because it is a heterogeneous disorder which may be precipitated by numerous factors including genetics, epigenetics, environmental triggers, and the presence of other diseases. Another factor which could explain the lack of success in this field comes from the fact that most individuals with Alzheimer's disease are only diag-

nosed in the middle to late stages of the pathology, by which time irreversible neuronal damage has already occurred. For these reasons, preventative intervention has become a pressing objective, although this requires the identification and implementation of biomarkers which can used for early and accurate diagnosis during the preclinical or early stages of the disease. In addition, increased success in clinical studies could be achieved using study populations which have been stratified using biomarker-based approaches that may include a combination of imaging, blood, CSF and cognitive analyses.

Although it is clear that Aβ deposition, tau neurofibrillary tangles and neuroinflammation are involved in the disease pathophysiology, a primary catalyst has yet to be identified and will most likely vary with the specific etiological backgrounds. As soluble Aβ and tau oligomers appear to represent critical precipitating steps in the disease process, targeting these forms of the molecules, rather than the respective insoluble plaques and tangles, may lead to greater success in clinical studies. It is also likely that precision medicine approaches will be needed that include combination therapies based on the subtype and stage of the disease.

There are already a number of clinical investigations underway which aim to address some of these questions with the testing of immunotherapeutic approaches against pathologically relevant epitopes in Aβ [55] and tau [101]. Once these results become available, further studies should be performed testing these and other approaches in carefully designed clinical studies consisting of larger biomarker-stratified populations. Such approaches should also include subjects at various stages of the disease including those at high-risk and those with MCI. Considering the critical importance of early detection and treatment, it is hoped that these precision medicine approaches will lead to effective disease-modifying approaches for Alzheimer's disease.

References

1. World Alzheimer Report 2016, Improving healthcare for people living with dementia. https://www.alz.co.uk/research/worldalzheimerreport2016sheet.pdf
2. World Population Forecast (2020–2050). https://www.worldometers.info/world-population/#table-forecast
3. GBD 2016 Dementia Collaborators, Nichols E, Szoeke CEI, Vollset SE, Abbasi N, Abd-Allah F et al (2019) Global, regional, and national burden of Alzheimer's disease and other dementias, 1990–2016: a systematic analysis for the Global Burden of Disease Study 2016. Lancet Neurol 18(1):88–106
4. El-Hayek YH, Wiley RE, Khoury CP, Daya RP, Ballard C, Evans AR et al (2019) Tip of the iceberg: assessing the global socioeconomic costs of Alzheimer's disease and related dementias and strategic implications for stakeholders. J Alzheimers Dis 70(2):323–341
5. Perneczky R (ed) Biomarkers for preclinical Alzheimer's disease (Neuromethods), 1st edn. Humana Press; Totowa, NJ, USA. 2018 edition (3 April 2018). ISBN-10: 1493976737
6. Glenner GG, Wong CW (1984) Alzheimer's disease: initial report of the purification and characterization of a novel cerebrovascular amyloid protein. Biochem Biophys Res Commun 120(3):885–890

7. Grundke-Iqbal I, Iqbal K, Tung YC, Quinlan M, Wisniewski HM, Binder LI (1986) Abnormal phosphorylation of the microtubule-associated protein tau (tau) in Alzheimer cytoskeletal pathology. Proc Natl Acad Sci U S A 83(13):4913–4917
8. Burgess N, Maguire EA, O'Keefe J (2002) The human hippocampus and spatial and episodic memory. Neuron 35(4):625–641
9. Eichenbaum H (1999) The hippocampus and mechanisms of declarative memory. Behav Brain Res 103(2):123–133
10. Larson EB, Shadlen MF, Wang L, McCormick WC, Bowen JD, Teri L et al (2004) Survival after initial diagnosis of Alzheimer disease. Ann Intern Med 140(7):501–509
11. Molinuevo JL, Minguillon C, Rami L, Gispert JD (2018) The rationale behind the new Alzheimer's disease conceptualization: lessons learned during the last decades. J Alzheimers Dis 62(3):1067–1077
12. Armstrong RA (2019) Risk factors for Alzheimer's disease. Folia Neuropathol 57(2):87–105
13. Ford E, Greenslade N, Paudyal P, Bremner S, Smith HE, Banerjee S et al (2018) Predicting dementia from primary care records: a systematic review and meta-analysis. PLoS One 13(3):e0194735. https://doi.org/10.1371/journal.pone.0194735
14. Guest FL (2019) Early detection and treatment of patients with Alzheimer's disease: future perspectives. Adv Exp Med Biol 1118:295–317
15. Cummings JL, Tong G, Ballard C (2019) Treatment combinations for Alzheimer's disease: current and future pharmacotherapy options. J Alzheimers Dis 67(3):779–794
16. Doraiswamy PM (2002) Non-cholinergic strategies for treating and preventing Alzheimer's disease. CNS Drugs 16(12):811–824
17. Buckley JS, Salpeter SR (2015) A risk-benefit assessment of dementia medications: systematic review of the evidence. Drugs Aging 32(6):453–467
18. Tolar M, Abushakra S, Sabbagh M (2019) The path forward in Alzheimer's disease therapeutics: reevaluating the amyloid cascade hypothesis. Alzheimers Dement.. pii: S1552-5260(19)35450-0. https://doi.org/10.1016/j.jalz.2019.09.075
19. Kitching D (2015) Depression in dementia. Aust Prescr 38(6):209–211
20. Baumgart M, Snyder HM, Carrillo MC, Fazio S, Kim H, Johns H (2015) Summary of the evidence on modifiable risk factors for cognitive decline and dementia: a population-based perspective. Alzheimers Dement 11(6):718–726
21. Reitz C, Mayeux R (2014) Alzheimer disease: epidemiology, diagnostic criteria, risk factors and biomarkers. Biochem Pharmacol 88(4):640–651
22. Goate A (2006) Segregation of a missense mutation in the amyloid beta-protein precursor gene with familial Alzheimer's disease. J Alzheimers Dis 9(3 Suppl):341–347
23. Lemere CA, Lopera F, Kosik KS, Lendon CL, Ossa J, Saido TC et al (1996) The E280A presenilin 1 Alzheimer mutation produces increased A beta 42 deposition and severe cerebellar pathology. Nat Med 2(10):1146–1150
24. Rogaev EI, Sherrington R, Rogaeva EA, Levesque G, Ikeda M, Liang Y et al (1995) Familial Alzheimer's disease in kindreds with missense mutations in a gene on chromosome 1 related to the Alzheimer's disease type 3 gene. Nature 376(6543):775–778
25. Scheuner D, Eckman C, Jensen M, Song X, Citron M, Suzuki N et al (1996) Secreted amyloid beta-protein similar to that in the senile plaques of Alzheimer's disease is increased in vivo by the presenilin 1 and 2 and APP mutations linked to familial Alzheimer's disease. Nat Med 2(8):864–870
26. Corder EH, Saunders AM, Strittmatter WJ, Schmechel DE, Gaskell PC, Small GW et al (1993) Gene dose of apolipoprotein E type 4 allele and the risk of Alzheimer's disease in late onset families. Science 261(5123):921–923
27. Farrer LA, Cupples LA, Haines JL, Hyman B, Kukull WA, Mayeux R et al (1997) Effects of age, sex, and ethnicity on the association between apolipoprotein E genotype and Alzheimer disease. A meta-analysis. APOE and Alzheimer Disease Meta Analysis Consortium. JAMA 278(16):1349–1356
28. Giri M, Zhang M, Lu Y (2016) Genes associated with Alzheimer's disease: an overview and current status. Clin Interv Aging 11:665–681

29. Shen L, Jia J (2016) An overview of genome-wide association studies in Alzheimer's disease. Neurosci Bull 32(2):183–190
30. De-Paula VJ, Radanovic M, Diniz BS, Forlenza OV (2012) Alzheimer's disease. Subcell Biochem 5:329–352
31. Huse JT, Doms RW (1991) Closing in on the amyloid cascade: recent insights into the cell biology of Alzheimer's disease. Mol Neurobiol 22(1–3):81–98
32. Braak H, Braak E (1991) Neuropathological stageing of Alzheimer-related changes. Acta Neuropathol 82(4):239–259
33. Kametani F, Hasegawa M (2018) Reconsideration of amyloid hypothesis and tau hypothesis in Alzheimer's disease. Front Neurosci 12:25. https://doi.org/10.3389/fnins.2018.00025
34. Zhu K, Wang X, Sun B, Wu J, Lu H, Zhang X et al (2019) Primary age-related tauopathy in human subcortical nuclei. Front Neurosci 13:529. https://doi.org/10.3389/fnins.2019.00529
35. Rogers J, Mastroeni D, Leonard B, Joyce J, Grover A (2007) Neuroinflammation in Alzheimer's disease and Parkinson's disease: are microglia pathogenic in either disorder? Int Rev Neurobiol 82:235–246
36. Meraz-Ríos MA, Lira-De León KI, Campos-Peña V, De Anda-Hernández MA, Mena-López R (2010) Tau oligomers and aggregation in Alzheimer's disease. J Neurochem 112(6):1353–1367
37. White CS, Lawrence CB, Brough D, Rivers-Auty J (2017) Inflammasomes as therapeutic targets for Alzheimer's disease. Brain Pathol 27(2):223–234
38. Venegas C, Kumar S, Franklin BS, Dierkes T, Brinkschulte R, Tejera D et al (2017) Microglia-derived ASC specks cross-seed amyloid-β in Alzheimer's disease. Nature 552(7685):355–361
39. Braak H, Zetterberg H, Del Tredici K, Blennow K (2013) Intraneuronal tau aggregation precedes diffuse plaque deposition, but amyloid-β changes occur before increases of tau in cerebrospinal fluid. Acta Neuropathol 126(5):631–641
40. Young AL, Oxtoby NP, Daga P, Cash DM, Fox NC, Ourselin S et al (2014) A data-driven model of biomarker changes in sporadic Alzheimer's disease. Brain 137(Pt 9):2564–2577
41. Xiong C, Jasielec MS, Weng H, Fagan AM, Benzinger TL, Head D et al (2016) Longitudinal relationships among biomarkers for Alzheimer disease in the Adult Children Study. Neurology 86(16):1499–1506
42. Alhajraf F, Ness D, Hye A, Strydom A (2019) Plasma amyloid and tau as dementia biomarkers in Down syndrome: systematic review and meta-analyses. Dev Neurobiol 79(7):684–698
43. Fleisher AS, Chen K, Quiroz YT, Jakimovich LJ, Gutierrez Gomez M, Langois CM et al (2015) Associations between biomarkers and age in the presenilin 1 E280A autosomal dominant Alzheimer disease kindred: a cross-sectional study. JAMA Neurol 72(3):316–324
44. Lott IT, Head E (2019) Dementia in Down syndrome: unique insights for Alzheimer disease research. Nat Rev Neurol 15(3):135–147
45. Di Domenico F, Tramutola A, Foppoli C, Head E, Perluigi M, Butterfield DA (2018) mTOR in Down syndrome: role in Aß and tau neuropathology and transition to Alzheimer disease-like dementia. Free Radic Biol Med 114:94–101
46. Tariot PN, Lopera F, Langbaum JB, Thomas RG, Hendrix S, Alzheimer's Prevention Initiative (2018) The Alzheimer's Prevention Initiative Autosomal-Dominant Alzheimer's Disease Trial: a study of crenezumab versus placebo in preclinical PSEN1 E280A mutation carriers to evaluate efficacy and safety in the treatment of autosomal-dominant Alzheimer's disease, including a placebo-treated noncarrier cohort. Alzheimers Dement (N Y) 4:150–160
47. Adolfsson O, Pihlgren M, Toni N, Varisco Y, Buccarello AL, Antoniello K et al (2012) An effector-reduced anti-β-amyloid (Aβ) antibody with unique aβ binding properties promotes neuroprotection and glial engulfment of Aβ. J Neurosci 32(28):9677–9689
48. Josephs K (2017) Current understanding of neurodegenerative diseases associated with the protein tau. Mayo Clin Proc 92(8):1291–1303
49. Cummings J (2019) The role of biomarkers in Alzheimer's disease drug development. Adv Exp Med Biol 1118:29–61

50. Feng J, Wang JX, Du YH, Liu Y, Zhang W, Chen JF et al (2018) Dihydromyricetin inhibits microglial activation and neuroinflammation by suppressing NLRP3 inflammasome activation in APP/PS1 transgenic mice. CNS Neurosci Ther 24(12):1207–1218
51. Münch G, Schinzel R, Loske C, Wong A, Durany N, Li JJ et al (1998) Alzheimer's disease – synergistic effects of glucose deficit, oxidative stress and advanced glycation endproducts. J Neural Transm 105:439–461
52. Wong A, Luth HJ, Deuther-Conrad W, Dukic-Stefanovic S, Gasic-Milenkovic J, Arendt T et al (2001) Advanced glycation endproducts co-localize with inducible nitric oxide synthase in Alzheimer's disease. Brain Res 920:32–40
53. Venigalla M, Sonego S, Gyengesi E, Sharman MJ, Münch G (2016) Novel promising therapeutics against chronic neuroinflammation and neurodegeneration in Alzheimer's disease. Neurochem Int 95:63–74
54. Figueiredo-Pereira ME, Corwin C, Babich J (2016) Prostaglandin J2: a potential target for halting inflammation-induced neurodegeneration. Ann N Y Acad Sci 1363:125–137
55. Camargo CHF, Justus FF, Retzlaff G, Blood MRY, Schafranski MD (2015) Action of anti-TNF-α drugs on the progression of Alzheimer's disease: a case report. Dement Neuropsychol 9(2):196–200
56. Butchart J, Brook L, Hopkins V, Teeling J, Püntener U, Culliford D et al (2015) Etanercept in Alzheimer disease: a randomized, placebo-controlled, double-blind, phase 2 trial. Neurology 84(21):2161–2168
57. Mazzanti G, Di Giacomo S (2016) Curcumin and resveratrol in the management of cognitive disorders: what is the clinical evidence? Molecules 21(9). pii: E1243. https://doi.org/10.3390/molecules21091243
58. Barbara R, Belletti D, Pederzoli F, Masoni M, Keller J, Ballestrazzi A et al (2017) Novel Curcumin loaded nanoparticles engineered for Blood-Brain Barrier crossing and able to disrupt Abeta aggregates. Int J Pharm 526(1–2):413–424
59. Voulgaropoulou SD, van Amelsvoort TAMJ, Prickaerts J, Vingerhoets C (2019) The effect of curcumin on cognition in Alzheimer's disease and healthy aging: a systematic review of pre-clinical and clinical studies. Brain Res 1725:146476. https://doi.org/10.1016/j.brainres.2019.146476
60. McKhann G, Drachman D, Folstein M, Katzman R, Price D, Stadlan EM (1984) Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA Work Group under the auspices of Department of Health and Human Services Task Force on Alzheimer's Disease. Neurology 34(7):939–944
61. McKhann GM, Knopman DS, Chertkow H, Hyman BT, Jack CR Jr, Kawas CH et al (2011) The diagnosis of dementia 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(3):263–269
62. Landau SM, Horng A, Fero A, Jagust WJ (2016) Alzheimer's Disease Neuroimaging Initiative. Amyloid negativity in patients with clinically diagnosed Alzheimer disease and MCI. Neurology 86(15):1377–1385
63. Sevigny J, Suhy J, Chiao P, Chen T, Klein G, Purcell D et al (2016) Amyloid PET screening for enrichment of early-stage Alzheimer disease clinical trials: experience in a phase 1b clinical trial. Alzheimer Dis Assoc Disord 30(1):1–7
64. Ellendt S, Vobeta B, Kohn N, Wagels L, Goerlich KS, Drexler E et al (2017) Predicting stability of mild cognitive impairment (MCI): findings of a community based sample. Curr Alzheimer Res 14(6):608–619
65. Bangen KJ, Clark AL, Werhane M, Edmonds EC, Nation DA, Evangelista N et al (2016) Cortical amyloid burden differences across empirically-derived mild cognitive impairment subtypes and interaction with APOE varepsilon4 genotype. J Alzheimers Dis 52:849–861
66. Jicha GA, Parisi JE, Dickson DW, Johnson K, Cha R, Ivnik RJ et al (2006) Neuropathologic outcome of mild cognitive impairment following progression to clinical dementia. Arch Neurol 63(5):674–681

67. Jansen WJ, Ossenkoppele R, Knol DL, Tijms BM, Scheltens P, Verhey FR et al (2015) Prevalence of cerebral amyloid pathology in persons without dementia: a meta-analysis. JAMA 313(19):1924–1938
68. Hansson O, Zetterberg H, Buchhave P, Londos E, Blennow K, Minthon L (2006) Association between CSF biomarkers and incipient Alzheimer's disease in patients with mild cognitive impairment: a follow-up study. Lancet Neurol 5:228–234
69. Hansson O, Zetterberg H, Buchhave P, Andreasson U, Londos E, Minthon L et al (2007) Prediction of Alzheimer's disease using the CSF Abeta42/Abeta40 ratio in patients with mild cognitive impairment. Dement Geriatr Cogn Disord 23:316–320
70. Wiltfang J, Esselmann H, Bibl M, Hull M, Hampel H, Kessler H et al (2007) Amyloid beta peptide ratio 42/40 but not A beta 42 correlates with phospho-Tau in patients with low- and high-CSF A beta 40 load. J Neurochem 101:1053–1059
71. Lewczuk P, Matzen A, Blennow K, Parnetti L, Molinuevo JL, Eusebi P et al (2017) Cerebrospinal fluid Abeta42/40 corresponds better than Abeta42 to amyloid PET in Alzheimer's disease. J Alzheimers Dis 55:813–822
72. Dumurgier J, Schraen S, Gabelle A, Vercruysse O, Bombois S, Laplanche JL et al (2015) Cerebrospinal fluid amyloid-β 42/40 ratio in clinical setting of memory centers: a multicentric study. Alzheimers Res Ther 7(1):30. https://doi.org/10.1186/s13195-015-0114-5
73. De Roeck EE, Engelborghs S, Dierckx E (2016) Next generation brain health depends on early Alzheimer disease diagnosis: from a timely diagnosis to future population screening. J Am Med Dir Assoc 17(5):452–453
74. Bjerke M, Zetterberg H, Edman Å, Blennow K, Wallin A, Andreasson U (2011) Cerebrospinal fluid matrix metalloproteinases and tissue inhibitor of metalloproteinases in combination with subcortical and cortical biomarkers in vascular dementia and Alzheimer's disease. J Alzheimers Dis 27(3):665–676
75. Slaets S, Le Bastard N, Martin JJ, Sleegers K, Van Broeckhoven C, De Deyn PP et al (2013) Cerebrospinal fluid Aβ1-40 improves differential dementia diagnosis in patients with intermediate P-tau181P levels. J Alzheimers Dis 36(4):759–767
76. Llorens F, Schmitz M, Ferrer I, Zerr I (2016) CSF biomarkers in neurodegenerative and vascular dementias. Prog Neurobiol 138–140:36–53
77. Blennow K, Zetterberg H (2018) The past and the future of Alzheimer's disease fluid biomarkers. J Alzheimers Dis 62(3):1125–1140
78. Soares HD, Chen Y, Sabbagh M, Roher A, Schrijvers E, Breteler M (2009) Identifying early markers of Alzheimer's disease using quantitative multiplex proteomic immunoassay panels. Ann N Y Acad Sci 1180:56–67
79. Perneczky R, Guo LH (2016) Plasma proteomics biomarkers in Alzheimer's disease: latest advances and challenges. Methods Mol Biol 1303:521–529
80. Vogelgsang J, Shahpasand-Kroner H, Vogelgsang R, Streit F, Vukovich R, Wiltfang J (2018) Multiplex immunoassay measurement of amyloid-β_{42} to amyloid-β_{40} ratio in plasma discriminates between dementia due to Alzheimer's disease and dementia not due to Alzheimer's disease. Exp Brain Res 236(5):1241–1250
81. Le Bastard N, Aerts L, Leurs J, Blomme W, De Deyn PP, Engelborghs S (2009) No correlation between time-linked plasma and CSF Abeta levels. Neurochem Int 55(8):820–825
82. Mielke MM, Hagen CE, Xu J, Chai X, Vemuri P, Lowe VJ et al (2018) Plasma phospho-tau181 increases with Alzheimer's disease clinical severity and is associated with tau- and amyloid-positron emission tomography. Alzheimers Dement. pii: S1552-5260(18)30067-0. https://doi.org/10.1016/j.jalz.2018.02.013
83. Neergaard JS, Dragsbæk K, Christiansen C, Karsdal MA, Brix S, Henriksen K (2018) Two novel blood-based biomarker candidates measuring degradation of tau are associated with dementia: a prospective study. PLoS One 13(4):e0194802. https://doi.org/10.1371/journal.pone.0194802
84. Ouma S, Suenaga M, Bölükbaşı Hatip FF, Hatip-Al-Khatib I, Tsuboi Y et al (2018) Serum vitamin D in patients with mild cognitive impairment and Alzheimer's disease. Brain Behav 8(3):e00936. https://doi.org/10.1002/brb3.936

85. Choi HJ, Byun MS, Yi D, Sohn BK, Lee JH, Lee JY et al (2017) Associations of thyroid hormone serum levels with in-vivo Alzheimer's disease pathologies. Alzheimers Res Ther 9(1):64. https://doi.org/10.1186/s13195-017-0291-5
86. Cao X, Zhu M, He Y, Chu W, Du Y, Du H (2018) Increased serum acylated ghrelin levels in patients with mild cognitive impairment. J Alzheimers Dis 61(2):545–552
87. Varma VR, Oommen AM, Varma S, Casanova R, An Y, Andrews RM et al (2018) Brain and blood metabolite signatures of pathology and progression in Alzheimer disease: a targeted metabolomics study. PLoS Med 15(1):e1002482. https://doi.org/10.1371/journal.pmed.1002482
88. Wei H, Xu Y, Xu W, Zhou Q, Chen Q, Yang M et al (2018) Serum Exosomal miR-223 serves as a potential diagnostic and prognostic biomarker for dementia. Neuroscience 379:167–176
89. Yang TT, Liu CG, Gao SC, Zhang Y, Wang PC (2018) The serum exosome derived MicroRNA-135a, −193b, and −384 were potential Alzheimer's disease biomarkers. Biomed Environ Sci 31(2):87–89
90. Wu Y, Xu J, Xu J, Cheng J, Jiao D, Zhou C et al (2017) Lower serum levels of miR-29c-3p and miR-19b-3p as biomarkers for Alzheimer's disease. Tohoku J Exp Med 242(2):129–136
91. Magalhães TNC, Weiler M, Teixeira CVL, Hayata T, Moraes AS, Boldrini VO et al (2017) Systemic inflammation and multimodal biomarkers in amnestic mild cognitive impairment and Alzheimer's disease. Mol Neurobiol. https://doi.org/10.1007/s12035-017-0795-9
92. Lai KSP, Liu CS, Rau A, Lanctôt KL, Köhler CA, Pakosh M et al (2017) Peripheral inflammatory markers in Alzheimer's disease: a systematic review and meta-analysis of 175 studies. J Neurol Neurosurg Psychiatry 88(10):876–882
93. Chen A, Oakley AE, Monteiro M, Tuomela K, Allan LM, Mukaetova-Ladinska EB et al (2016) Multiplex analyte assays to characterize different dementias: brain inflammatory cytokines in poststroke and other dementias. Neurobiol Aging 38:56–67
94. Mueller A, Bullich S, Barret O, Madonia J, Berndt M, Papin C et al (2019) Tau PET imaging with 18F-PI-2620 in patients with Alzheimer's disease and healthy controls: a first-in-human study. J Nucl Med. https://doi.org/10.2967/jnumed.119.236224. pii: jnumed.119.236224. [Epub ahead of print]
95. Kroth H, Oden F, Molette J, Schieferstein H, Capotosti F, Mueller A et al (2019) Discovery and preclinical characterization of [18F]PI-2620, a next-generation tau PET tracer for the assessment of tau pathology in Alzheimer's disease and other tauopathies. Eur J Nucl Med Mol Imaging 46(10):2178–2189
96. Bullich S, Barret O, Constantinescu C, Sandiego C, Mueller A, Berndt M et al (2019) Evaluation of dosimetry, quantitative methods and test-retest variability of 18F-PI-2620 PET for the assessment of tau deposits in the human brain. J Nucl Med. https://doi.org/10.2967/jnumed.119.236240. pii: jnumed.119.236240. [Epub ahead of print]
97. Coppola G, Di Renzo A, Ziccardi L, Martelli F, Fadda A, Manni G et al (2015) Optical coherence tomography in Alzheimer's disease: a meta-analysis. PLoS One 10:e0134750. https://doi.org/10.1371/journal.pone.0134750
98. Koronyo Y, Biggs D, Barron E, Boyer DS, Pearlman JA, Au WJ et al (2017) Retinal amyloid pathology and proof-of-concept imaging trial in Alzheimer's disease. JCI Insight 2(16). https://doi.org/10.1172/jci.insight.93621. pii: 93621. [Epub ahead of print]
99. Yoon SP, Thompson AC, Polascik BW, Calixte C, Burke JR, Petrella JR et al (2019) Correlation of OCTA and volumetric MRI in mild cognitive impairment and Alzheimer's disease. Ophthalmic Surg Lasers Imaging Retina 50(11):709–718
100. Kim JI, Kang BH (2019) Decreased retinal thickness in patients with Alzheimer's disease is correlated with disease severity. PLoS One 14(11):e0224180. https://doi.org/10.1371/journal.pone.0224180
101. Medina M (2018) An overview on the clinical development of tau-based therapeutics. Int J Mol Sci 19(4). pii: E1160. https://doi.org/10.3390/ijms19041160

Chapter 9
Resetting the Aging Clock: Implications for Managing Age-Related Diseases

Aliza K. De Nobrega, Kristine V. Luz, and Lisa C. Lyons

1 Introduction

Medical and scientific advances, combined with changes in public health policies, have increased longevity resulting in demographic shifts in countries around the globe. By 2017, the number of individuals aged 60 and over had doubled from that estimated in 1980 [1]. By 2030, life expectancy in many countries is predicted to be greater than 85 [2], and by 2050, the population of adults over eighty is expected to triple over current numbers [1, 2]. Older individuals in the United States are expected to comprise 22% of the population by 2050 [3, 4]. An even greater percentage of older individuals is predicted for 10 countries including Japan, China and five European countries, with estimates that 40% or more of their populations will be comprised of individuals over 60 by 2050 [1, 2]. As the proportion of aging individuals and life expectancy continue to rise, increasing attention is now focused on healthy aging and the management of age-related diseases and chronic health conditions. In the United States, more than 35% of older individuals are adversely affected by three or more chronic conditions [5]. Consequently, there is a driving need to identify system level factors that exacerbate age-related diseases and chronic conditions as well to find therapeutic and management options that contribute to healthy aging.

Circadian rhythms have been studied for almost 300 years starting with experiments in plants performed by Jean-Jacques de Mairan in 1729. However, the role of the circadian system in health and disease has only risen to prominence in the twenty-first century. The endogenous circadian system provides the ability for organisms from bacteria to humans to coordinate molecular, physiological and

A. K. De Nobrega · K. V. Luz · L. C. Lyons (✉)
Department of Biological Science, Program in Neuroscience, Florida State University, Tallahassee, FL, USA
e-mail: lyons@bio.fsu.edu

P. C. Guest (ed.), *Reviews on New Drug Targets in Age-Related Disorders*,
Advances in Experimental Medicine and Biology 1260,
https://doi.org/10.1007/978-3-030-42667-5_9

behavioral processes in anticipation of regularly occurring environmental events. Similar to setting the time on a clock, the circadian system can be set or entrained by external factors and continue to function even in the absence of time cues (also known as zeitgebers). With the daily rotation of the earth, light-dark cycles provide the primary entrainment signals for almost all organisms [6], although for many animals including humans, meal patterns, social interactions or regular activity can reinforce entrainment of the circadian system [7]. Timing of meals also may be a potent entrainment mechanism for the circadian system in humans [8].

Never before in recorded history have there been so many challenges to proper circadian function. Since the start of the industrial revolution and the rise of factories, individuals have increasingly worked non-standard schedules. Twenty-first century technological advances and globalization have resulted in the exponential rise of circadian and sleep disorders. In this review, we discuss the factors contributing to circadian and sleep disorders, the reciprocal interactions between the circadian clock and aging, and potential interventions for mitigation of age-related pathologies via the circadian system.

2 Modern Challenges and Factors Contributing to Circadian and Sleep Disorders

While there has been growing public awareness regarding the importance of sleep and the prevalence of chronic sleep deprivation and sleep disorders across age groups, there is considerably less public attention paid to circadian disorders. Industrial, technological and societal changes have powered a rise in circadian disorders in countries around the world. Light pollution, irregular work and activity schedules, among other factors, can produce both transient and chronic disruption of the circadian system impacting aging and health.

2.1 Life in Modern Society Undermines Circadian Entrainment

Since the development of the electric light bulb, technological advances have transformed modern societies shifting the percentage of time that individuals spend indoors. With many people spending more than 90% of their work and leisure time indoors, popular references can be found to the “indoor generation”. In the United States and other developed countries, children and adults spend the vast majority of their waking time exposed to artificial light resulting in poor entrainment of the circadian clock. Recent research in North America and Europe found that individuals spend less than 1–2 h outdoors on a daily basis [9–11]. Reliance upon low levels of indoor light (~100–300 lux) compared to bright sunlight (>10,000 lux) or even

cloudy daylight (~1500 lux) results in weak circadian entrainment increasing the incidence of circadian disorders. Although the development of electric light has revolutionized the way we live and work, the unforeseen impacts on circadian entrainment and sleep are taking a toll on individuals of all ages (reviewed in [12]).

Exposure to light at night phase shifts the circadian clock and suppresses production and release of the hormone melatonin which functions to decrease circadian arousal and promote sleep. Urbanization has compounded the issue of poor circadian entrainment with high levels of artificial light at night, i.e. light pollution, dampening the difference in light exposure between the day and the night. Worldwide, 83% of individuals are exposed to significant light pollution at night based on satellite images [13]. Dense urbanization has enlarged the percentage of the population affected by light pollution, with approximately 99% of individuals affected in the United States and Europe [13]. The amount of artificial light at night determined by satellite image analysis has grown more than 2.2% globally per year from 2012 to 2016 with increases in brightness by 1.8% per year over the same period [14]. Artificial light at night not only confounds robust entrainment of the circadian clock, but also contributes to poor sleep quality, shorter sleep duration at night and increased daytime sleep [15] further disrupting circadian function through altered activity patterns of the individual. Ironically, the current shift from the use of incandescent lights to more energy efficient, economical fluorescent and LED lights is predicted to increase light pollution at night by 2.5% [13]. Moreover, as these lights often rely upon shorter wavelength light to increase brightness, the switch to fluorescent and LED lighting increases the potential for interference with circadian entrainment as the circadian system is more sensitive to blue light [16]. Exposure to even low levels of light at night (80–100 lux) can potentially shift the circadian clock and suppress melatonin production [17, 18]. Increased exposure to artificial light at night is associated with increased risk for cancer, metabolic diseases, and mood disorders [19–21]. Additional information on artificial light at night, circadian disruption and the consequences to health can be found in recent reviews [12, 22]. Artificial outdoor light at night and the subsequent circadian costs also have significant ecological consequences for multiple animal species of animals including birds, fish, insects and livestock (reviewed [23, 24]).

In addition to light exposure at night from indoor lighting, the use of personal electronic devices with light emitting screens results in significant light exposures to individuals. Computer screens emit 100 lux of light exposure, while smart phones or handheld devices produce 40 lux light exposure [19, 25–27]. Paradoxically, in an effort to increase efficiency and brightness, handheld devices often use blue-light biased displays that most affect circadian responses to light at night [28]. The use of computers, smartphones and tablets in the late evening significantly suppresses melatonin production and result in delays in the onset of sleep [26, 29, 30]. Adolescents appear even more susceptible to circadian disruption through melatonin suppression from the evening use of computers and hand-held devices [31], potentially initiating lifelong issues with circadian misalignment.

2.2 Working Hours and Non-standard Work Schedules

Although there has been a growing emphasis on achieving a healthy work-life balance in many countries, career pressures and demands for high job performance result in longer work days for many individuals, particularly in the United States [32–34]. According to data from Gallup's Annual Work and Education survey in 2013 and 2014, the average work week for adults in the United States is now estimated at 47 h with almost 40% of the respondents reporting working more than 50 h per week [35]. Data from the 2010 National Health Survey found that 7.2% of U.S. adults worked 60 h or more per week [32, 33]. Longer work days combined with family obligations have contributed to widespread chronic sleep deprivation affecting more than 35% of adults in the United States [36–38]. Although longer work days may be immediately associated with work place errors or accidents [39–41], longer working hours in middle age contribute to aging and age-related pathologies including increased incidence of stroke, cardiovascular disease and cancer [42–45]. Longer work days, shiftwork and irregular hours pressure the circadian system with mistimed cues resulting in desynchronization of the internal circadian system affecting metabolic and physiological processes.

Worldwide, the number of individuals performing shiftwork or engaging in work during non-traditional hours is increasing, with more than one-fifth of the adult population working non-standard hours in industrialized countries [32, 33, 46]. In Europe, surveys have suggested that the majority of the population work non-standard schedules [47, 48]. The rise in service sector jobs, estimated at 80% of jobs in the United States, has driven the increased percentage of individuals working non-standard schedules [49]. Moreover, individuals in white collar jobs now comprise the majority of individuals working non-standard work schedules [49]. Both longer work days and shiftwork significantly increase the risk of injury and accidents when working [50, 51]. Circadian misalignment and circadian disruption whether induced by shiftwork or other factors increase the risk and incidence of numerous diseases including cancer, diabetes, obesity, neurodegenerative diseases, and cardiovascular diseases (reviewed in [6, 52, 53]). In fact, shiftwork and circadian disruption were designated as a probable carcinogen to humans in 2007 by the International Agency on Research on Cancer. Unfortunately, shiftwork may be perceived by the working individual as more of a social and family inconvenience rather than a health issue [54–56], creating potential for long-term health problems. In addition to the adverse health impacts associated with shiftwork and circadian desynchronization, there is a societal and economic cost as well. Non-traditional work schedules are associated with increased occupation and industrial accidents, traffic accidents and health care costs (reviewed in [47, 57]).

2.3 Social Jetlag

Individuals frequently change their sleep/wake schedule on the weekends compared to the work week, increasing social activities in the evenings and sleeping later in the mornings. This twice weekly change in sleep and activity patterns from the workweek to the weekends and back again has been termed social jetlag as it shifts the circadian clock [58, 59]. Given the strain on the circadian system, social jetlag frequently results in circadian misalignment or desynchronization of internal oscillators. Social jetlag has been associated with increased risk of obesity, diabetes, cardiovascular issues and neuropsychiatric disorders [58, 60–62]. Social jetlag and the associated health consequences affect all age groups, including children [63], with potentially long-lasting effects on healthy aging in later decades.

3 Anatomy and Physiology of the Mammalian Clock

3.1 Neural Architecture of the Mammalian Circadian System

In humans and other mammals, the circadian clock is hierarchically structured. Although the molecular machinery that generates rhythms in behavior and physiological processes is present in most mammalian cells [64–67], the suprachiasmatic nucleus (SCN) is considered the master orchestrator of the circadian system, located in the hypothalamus above the optic chiasm flanking the 3rd ventricle [68]. The SCN is the only clock in the mammalian body directly entrained by light and signals from the SCN are responsible for synchronizing rhythms in non-SCN brain and peripheral oscillators to a 24 h cycle [69, 70] (Fig. 9.1). Photic signals are transferred to the SCN via the retinohypothalamic tract (RHT) from melanopsin-containing intrinsically photosensitive retinal ganglion cells (ipRGCs) in the retina [71–75]. Glutamate signaling from the RHT transmits these signals to the core of the SCN, inducing phosphorylation of Cre-binding protein (CREB) by calcium-dependent kinases and subsequent transcription of the core clock genes [75].

The SCN is a heterogenous structure functionally divided into two areas, the core and shell regions, each with distinct afferent and efferent connections, expression of neuropeptides and activation in response to light cues [76–82]. Electrophysiological studies demonstrate that the approximately 20,000 neurons in the SCN contain cell autonomous oscillators with communication between the neurons necessary for maintenance of a 24 h period [83–87]. The ventrolateral “core” neurons of the SCN release vasointestinal peptide (VIP) and gastrin-releasing peptide (GRP) among others, sending direct projections to the dorsomedial “shell” neurons that release arginine vasopressin (AVP) [88–91]. Efferent projections from both the SCN core and shell terminate in the midline thalamus, brain stem and other areas of the hypothalamus, including the dorsomedial hypothalamus (DMH), ventromedial hypothalamus (VMH), arcuate nucleus and paraventricular nucleus of the hypothalamus

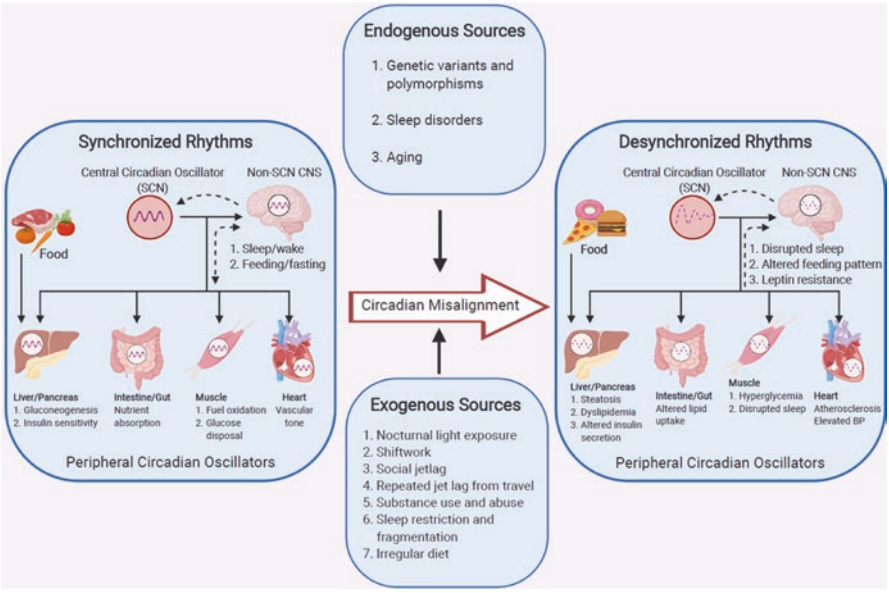


Fig. 9.1 Coordination of central and peripheral circadian oscillators. Synchrony occurs between the Suprachiasmatic Nucleus (SCN) and peripheral oscillators to coordinate metabolic and physiological rhythms. Feedback from non-SCN brain and peripheral oscillators provides information to the SCN. Endogenous factors such as aging, sleep disorders and genetic polymorphisms, or exogenous sources such as shift work, jet lag, nocturnal light exposure, abnormal eating patterns, and social jetlag disrupt behavioral and molecular rhythms causing desynchrony between central and peripheral oscillators. Prolonged misalignment between central and peripheral oscillators increases the risk for a number of pathologies associated with aging including metabolic syndromes, cancer, mood disorders and neurodegenerative diseases

(PVN) [89, 92]. These non-SCN brain regions act in synchrony with the master clock to gate numerous behaviors and physiological processes including sleep-wake cycles, energy expenditure, thermoregulation, feeding behavior, glucose and lipid metabolism [93–101]. SCN projections to the dorsal medial hypothalamus and subsequent signaling from the DMH to the orexinergic/hypocretin neurons of the lateral hypothalamus gate sleep/wake and feeding cycles [102, 103]. A detailed description of the SCN and its connections with other brain regions is reviewed in [104–106].

In the SCN, intercellular communication of phase information is robust, maintaining rhythmicity for days to weeks making this brain region resilient to noise [69, 84, 107, 108]. The strong phase coherence between cells within the SCN explains why mammals can remain rhythmic for extended periods under constant conditions. Although dispersed SCN neurons remain rhythmic, individual cells can vary significantly in period length in the range of 22–30 h, and differ in neuropeptide expression and their response to environmental timing cues [84–86].

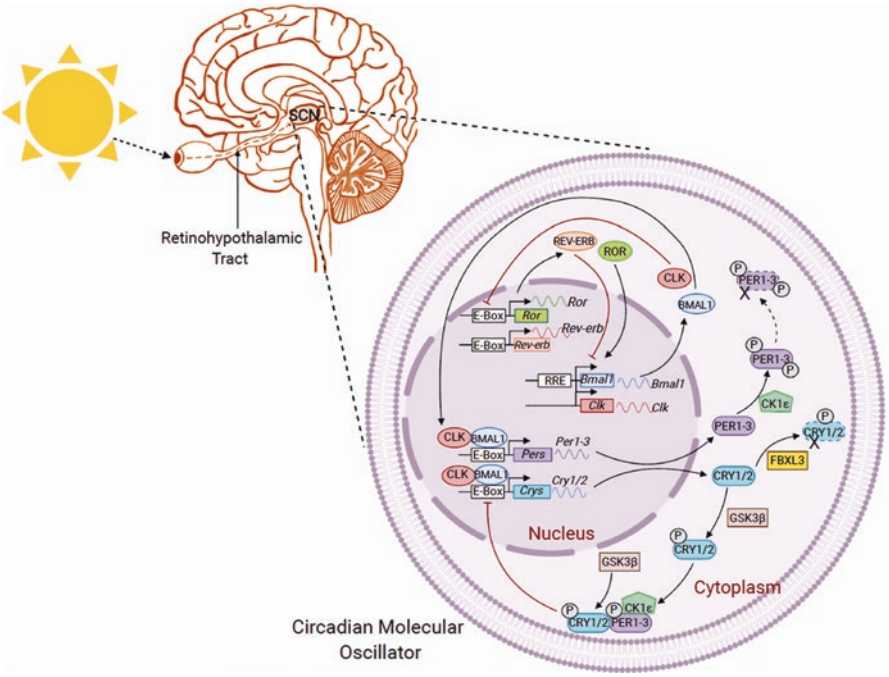


Fig. 9.2 Organization of central and peripheral oscillators in the mammalian circadian system. In mammals, the circadian clock is located in the SCN. Light activates melanopsin in intrinsically photosensitive retinal ganglion cells that project to the SCN via the retinohypothalamic tract. At the molecular level, interlocking transcription/translation feedback loops generate 24 h rhythms of gene expression. The core circadian loop comprises the positive regulators CLK and BMAL1 that form a heterodimer which binds to the promoter region in *Per* and *Cry* genes, facilitating rhythmic transcription of the negative regulators: *Per1*, *Per2*, *Cry1* and *Cry2* and other clock-controlled genes. Accumulated PER and CRY proteins heterodimerize in the cytoplasm, translocate to the nucleus and inhibit their own transcription. Post-translational modifications fine tune circadian timing through the kinase CK1ε and the F-box protein FBXL21 involved in tagging PER and CRY monomers respectively for ubiquitin-dependent degradation. CK1ε and GSK3β also control the rate at which PER:CRY complexes enter the nucleus. A second transcriptional/translational loop requires the binding of CLOCK:BMAL1 heterodimers to activate transcription of the nuclear receptors *Ror*α and *Rev-erbβ*. REV-ERBα and REV-ERBβ repress transcription of *Bmal1* driven by RORα and RORβ. These interlocking feedback loops buffer against environmental noise thus generating rhythmic timing of behavior and physiology

3.2 Molecular Machinery of the Cellular Clock

Across species, the molecular circadian oscillator functions as an interlocking set of positive and negative autoregulatory feedback loops of transcription and translation (Fig. 9.2). Identification of the molecular mechanism of the mammalian circadian system began in 1997 with the cloning of *Clock*, the first known mammalian circadian gene, soon followed by *Per1* and *Bmal1* [77, 107, 109–115]. At the core of the primary loop are the basic-helix-loop-helix (BHLH)/PAS proteins: aryl

hydrocarbon receptor nuclear translocator-like protein 1 (ARNTL1; more commonly known as BMAL1) and Circadian Locomotor Output Cycles Kaput (CLOCK). BMAL1 and CLOCK are considered the positive transcription factors forming the heterodimeric CLOCK-BMAL1 complex that binds to the E-box enhancer (CACGTG) upstream of the *Per* gene at the beginning of the light cycle [110, 111, 116]. Specifically, CLOCK-BMAL1 heterodimers activate the negative limb of the core loop by inducing the transcription of the repressor genes *Period* 1–3 (*mPer*1–3) and *Cryptochrome* 1 and 2 (*mCry* 1–2) [77, 114, 117, 118]. CRY was initially identified the plant *Arabidopsis thaliana* as a blue light-dependent photoreceptor but CRY proteins only function as transcriptional regulators in mammals and not as photoreceptors [119–125]. *mPer* and *mCry*genes are translated, and their corresponding proteins mPERs 1–3 and mCRY1 and mCRY2 accumulate in the cytoplasm. Monomers of mPER and mCRY are phosphorylated by *Casein-kinase Epsilon* 1 (CK1ε) and FBXL21 respectively, rendering them unstable and subsequent targets of ubiquitination and proteasome degradation [126–128]. When the concentrations of mPER and mCRY proteins increase to critical activity levels, they dimerize and form a complex with CK1ε, translocate back to the nucleus and suppress their own expression by interrupting the DNA binding of the CLOCK/BMAL1 complex, therein removing them from the E-box sequences within promoters of the *mPer* and *mCry* genes [117, 128–132]. Degradation of the PER/CRY complexes and thus disinhibition of BMAL1/CLOCK towards the end of the night phase facilitates the start of another 24 h cycle. In the absence of *Clock* as seen in *Clock* −/− mice, NPAS2 (MPO4) act as a substitute and binds BMAL1 to drive locomotor behavior under constant darkness conditions [133–135]. Recent *in vitro* and *in vivo* studies have shown that NPAS2 can substitute for CLOCK in other peripheral oscillators including in the liver, vascular smooth muscle cells and fibroblast cells [136, 137].

An opposing regulatory circuit is established by two families of nuclear orphan receptors, retinoic-acid-related orphan receptors (RORs) and nuclear receptor subfamily 1, group D, member 1 (NR1D1) also known as REV-ERBs. The transcriptional activators RORα, RORβ and RORγbind to the RORE elements in the promoters of the *Bmal1* gene, thereby increasing the rhythmic transcription of *Bmal1* [138–140]. Conversely, the transcriptional repressors, REV-ERBα and REV-ERBβ are rhythmically expressed and compete with the ROR activators for binding at the RORE promoter sites [139–141]. REV-ERBα and REV-ERBβ recruit corepressor complexes to reduce transcription of *Bmal1* and, to a lesser extent, *Clock*, in a manner antiphase to *Per1* and *Per2* [106, 139, 142–144]. These sophisticated feedback loops generate rhythms with a period of approximately 24 h. Clock factors also bind to cell-specific enhancers and establish complexes with various regulators to drive rhythmic expression of target genes outside the core clock mechanism, i.e., clock-controlled genes (CCGs) [145–148]. Finally, a number of post-transcriptional and translational elements regulate the core clock proteins to influence their cellular localization and nuclear stability thereby fine-tuning period length [149]. These include kinases such as *casein kinase 1 epsilon* (CK1ε) that regulate the phosphorylation of PER targeting it for ubiquitination and proteasome degradation and

glycogen synthase kinase 3β (GSK3β) [150]. A more exhaustive description of the circadian molecular machinery can be found in the following recent review articles [105, 131, 151–156].

Our understanding of the molecular components of the circadian clock and the workings of the transcription/translation feedback loops arose from widely divergent model systems including *Drosophila* and the fungus *Neurosporacrassa* [151, 156–162]. Many of the genes comprising the core circadian machinery were initially discovered in *Drosophila* [163, 164]. The *Drosophila* circadian clock demonstrates a high degree of conservation with the mammalian oscillator with only a few notable exceptions. In the positive limb, *dClock* (*dClk*) and *dCycle* (*dCyc*) form a dimer and bind to the E-box sequences in the promoter of the *period* (*per*) and *timeless* (*tim*) genes, resulting in their transcription [113, 165–170]. When they've reached maximum activity levels, PER and TIM proteins interact to form heterodimers with DOUBLETIME (DBT) and enter the nucleus, halting their own transcription by inhibiting CLK-CYC activity [113, 165–172]. It should be noted that in *Drosophila*, CRY also acts as a photoreceptive molecule in central pacemaker neurons [173–176]. The high degree of similarity combined with the relatively short lifespan of *Drosophila* has made *Drosophila* a practical model for aging and circadian studies.

3.3 Coupling Between Central and Peripheral Oscillators in Mammals

Peripheral circadian oscillators rely upon the SCN for synchronization and may be found in non-SCN brain regions and numerous tissues throughout the body. Peripheral oscillatory tissues, including the liver and adrenal glands, may provide feedback about physiological state to hypothalamic nuclei and to the SCN [177–179]. The liver is also an independent oscillator that can be driven by systemic cues such as feeding/fasting cycles in the absence of signals from the central SCN pacemaker [180]. The ability of the SCN to synchronize peripheral oscillators depends upon endocrine signaling as well as connections through sympathetic and parasympathetic pre-ganglionic neurons of the autonomic nervous system [181–184]. Initial evidence establishing SCN control of rhythms arose from experiments demonstrating reinstatement of rhythms in behavior following transplantation of SCN grafts in SCN-lesioned rats, mice and hamsters [185–189]. Parabiosis experiments between SCN-lesioned and intact mice confirmed that SCN regulation of circadian oscillations in peripheral tissues required circulating factors [190]. The circadian regulation of glucocorticoid secretion is one of the best examples of bi-directional communication between the SCN and peripheral oscillators. SCN-mediated activation of CRH secretion from the PVN controls the rhythmic release of adrenocorticotropin hormone (ACTH) from the pituitary gland, which in turn regulates the rhythmic production of glucocorticoids in the adrenal cortex [191–194].

Glucocorticoids are important for entraining peripheral clocks and maintaining energy balance through the regulation of glucose, fat and protein metabolism, anti-inflammatory actions as well as modulating mood and cognition [195–199]. In turn, the peripheral clocks in the adrenal gland gate the circadian production of glucocorticoids in response to signals from the SCN [193, 198, 200–202]. The effects of glucocorticoids are exerted via binding with the Glucocorticoid receptor [GR], a nuclear hormone receptor widely expressed throughout the body and the brain with the exception of the SCN [203–205]. Upon glucocorticoid binding, GRs translocate from the cytosol to the nucleus, bind to G-response elements (GREs), DNA motifs in the regulatory regions of CCGs and clock genes, thereby activating the transcription of *Bmal1*, *mPer1*, *mPer2*, *mCry1* [196–199]. In the liver, glucocorticoids may synchronize the circadian expression of target genes through interactions REV-ERBα [206, 207]. These multiple bi-directional interactions demonstrate the SCN's control of glucocorticoids as a major entrainment signal for peripheral oscillators. As will be discussed in the following sections, the intricacies of circadian synchronization between neurons within the SCN as well as between the SCN and peripheral oscillators are strongly affected by aging and present potential targets for reinforcement of the circadian system.

3.4 Non-photic Entrainment of Peripheral Oscillators

Non-photic environmental cues such as food, temperature and induced activity can entrain the peripheral oscillators in mammals independent of light entrainment through the SCN [208]. The two most common non-photic zeitgebers are food and temperature. Daily cycles in feeding and fasting during active and rest periods respectively, are strong entrainment cues for peripheral oscillators, such as the liver [180, 209]. In nocturnal animals, in which the SCN is entrained to a dark-light cycle, permitting food access only during the day (the inactive period of rodents) strongly shifts the expression profile of circadian genes in the liver and other peripheral tissues, uncoupling circadian gene expression in peripheral oscillators from that of the SCN [180, 209, 210]. However, the speed and degree to which synchrony is achieved with food entrainment differs across tissues and organs [211]. As with continuous light shifts, if the feeding time is continuously changed, animals exhibit arrhythmic behaviors and fail to anticipate food [212–214]. Even for animals in which the SCN has been lesioned, timed-feeding induces rhythms in locomotor activity and body temperature [212–214]. This rhythmic behavior persists on days of total food deprivation, indicating that this is not a transient phenomenon but rather driven by an underlying oscillator, now termed the Food Entrainable Oscillator (FEO) [215]. Importantly, SCN-lesioned animals with desynchrony among peripheral tissue oscillators can be entrained with timed feeding, resulting in stable phase relationships between these oscillators [180, 216, 217]. In intact mice, timed feeding out of phase with light dark cycles differentially induces phase changes in peripheral oscillators, whereby some peripheral oscillators remain in phase with the

SCN and others such as those in the liver strongly entrain to the timing of food [180, 211, 217]. The cellular mechanism through which the FEO informs components of the central oscillator about metabolic state is still unclear, but evidence suggests that the FEO uses similar pathways as the SCN including hormones and metabolites to organize peripheral tissues [218–220].

Changes in feeding habits that uncouple peripheral tissues from the master clock result in metabolic alterations similar to that observed in circadian misalignment, a condition often associated with shift work [221, 222]. Fixed feeding cycles and nutritionally balanced foods are important for maintaining robust peripheral rhythms and metabolic fitness [223]. For example, mice housed on high fat diets exhibit blunted rhythms in feeding behavior, with the majority of their food intake occurring during their rest period [224, 225]. Mice with global mutations in *Clock* consume more food during the day (their inactive period) and are more pre-disposed to metabolic syndrome compared to wild-type mice [226]. Rescuing CLOCK in the liver decreases the sensitivity of mice to the pathologies associated with high fat diets [227]. Imposing time-restricted feeding on *Cry1*- and *Cry2*-deficient mice rescues rhythms in expression of hepatic transcripts [580]. Interestingly, changes in the timing of eating, in humans and animal models, associated with the onset of obesity and other metabolic diseases suggest a role for circadian disruption as a key factor in facilitating these disease states [225, 228–233]. For the years 2014–2016, 15% of adults aged 20 and over had Diabetes and 93.3 million (39.8%) were obese [234, 235]. Thus, changes in either timing of food consumption or food fat content precipitate circadian dysfunction, increasing the incidence of obesity and metabolic diseases, chronic conditions frequently associated with unhealthy aging.

4 Reciprocal Interactions Between the Circadian System and Aging

Across species from invertebrates to humans, aging weakens the circadian system by decreasing the robustness of circadian rhythms and increasing fragmentation of these rhythms [236–239]. At the behavioral level, age-dependent changes can be observed in decreased robustness and amplitude of the rhythms, increased fragmentation and changes in the free-running period in rodent models [237, 240–243] as well as non-human primates [244]. In humans, age-related changes in amplitude or fragmentation have been shown for rhythms in behavior and physiology including activity, sleep-wake, hormone and body temperature rhythms [245–247]. Shortening of the period for molecular rhythms has also been observed in novel cell culture experiments in which serum factors were added from older individuals [248], similar to the changes in free-running period associated with aging in animal models. Circadian dysfunction, desynchronization or weakening of the circadian system can accelerate cellular aging and aggravate many age-related diseases and chronic disorders.

4.1 *Effects of Aging on the Central Clock and Circuit Connectivity*

As discussed in Sect. 3.1, the SCN is considered the master circadian clock in mammals synchronizing most tissue specific peripheral oscillators [249]. In animal models and *in vitro* studies, aging has been shown to affect SCN oscillatory neurons decreasing the amplitude of the rhythm in neuronal firing rate [241, 250–253]. It has been difficult to definitively establish the mechanism through which aging affects SCN oscillatory neurons as aging does not uniformly affect all circadian genes. However, numerous studies have shown age-dependent effects on the core oscillator. In the SCN, age-related decreases in the rhythm amplitude or expression levels have been found for *Bmal1*, *Clk* and *Per2* [254–256]. The photic induction of *Per1* and *Per2* in the SCN is also significantly reduced in older animals [257]. One confound that may explain differences between studies examining age-dependent effects on core clock gene expression may be whether the experiments were conducted under light-dark cycles or constant conditions [255]. As numerous studies have shown in both *Drosophila* and rodent models, animals may maintain driven behavioral and molecular rhythms under light-dark cycles even when a necessary core oscillator gene is mutated. In rats housed in LD cycles, no differences were found for *Per1*, *Per2* or *Cry1* cycling in the SCN [257]. Similarly, in rhesus macaques, no differences were found in the diurnal rhythms of core clock genes in the SCN between young and old males [259].

Age-dependent changes in SCN oscillators, whether occurring through changes in core circadian gene cycling or SCN neural activity, are transmitted to circadian gene expression in non-SCN brain regions. In the hippocampus, cingulate cortex and prefrontal cortex of rodent models, age-dependent decreases have been observed for clock genes including *Bmal1*, *Clk* and *Per2* as well as for clock controlled genes involved in learning and memory in the prefrontal cortex [256, 260–262]. In a groundbreaking study examining the impact of aging on circadian gene expression using human brain tissue from the prefrontal cortex, age-dependent changes in circadian rhythmicity were found for almost 1200 genes including genes associated with cognition, sleep and mood regulation [263].

4.2 *Aging Impairs Resynchronization of Circadian Oscillators*

Synchronization of neurons both within the SCN and between the SCN and peripheral oscillators is a necessary component of circadian function. Resetting oscillators to a new phase is essential following phase shifts, i.e. changes in time zone with travel or changes from daylight savings time to standard time. Aging may affect synchronization and resynchronization after perturbation by: (1) decreasing sensitivity of the circadian clock to entrainment signals; (2) decreasing the connectivity between SCN neurons; and (3) altering the synchronization between peripheral

oscillators and the SCN. In humans, aging decreases the sensitivity of the eye to light thereby weakening circadian entrainment [239, 264–266]. Age-related decreases in clarity and light transmittance of the lens, i.e. the development of cataracts, are hypothesized to be responsible for much of the decreased light sensitivity [267]. Cataract surgery does not necessarily improve circadian function [268], but based on patient questionnaires, the surgery has a long-term effect on increasing the responses of the melanopsin-containing intrinsically photosensitive retinal ganglion cells and improving circadian rhythms [269, 270]. Improvements in sleep-activity rhythms and sleep quality have also been found, although the mechanism through which this occurs remains unclear [269, 271, 272]. In rodent studies, significantly higher light intensities are needed to induce high amplitude circadian rhythms in older rats compared to young animals, suggesting decreased sensitivity to photoentrainment [273]. Similarly, research in nocturnal non-human primates found that older animals had decreased responsiveness to light intensity and light wavelengths compared to young animals [274].

Within the SCN, synchronization is necessary to maintain 24 h periods and to set the phase of pacemaker neurons as the period and phase of the rhythms vary significantly in individual pacemaker neurons [86]. Aging weakens neural activity and decreases the synchronization between SCN neurons [275–277]. In addition to the intracellular changes with the SCN, aging affects neural connectivity by decreasing the rhythmic expression of circadian neuropeptides as outputs from the SCN. In rodent models, the circadian rhythm in VIP mRNA levels in the SCN, and in its receptor, are reduced with aging [261, 278–281]. In studies using mice with a luciferase reporter to measure *Per* gene expression (PER2::LUC), phase shifts induced more erratic changes and desynchrony between SCN pacemaker neurons in older mice than in SCNs from younger mice prior to the establishment of stable re-entrainment [282]. Soluble factors from the SCN can partially reverse the effects of aging on molecular and behavioral rhythms as shown by transplant experiments in hamsters and rats in which SCN tissue from young animals was transplanted into older animals [252, 283–285]. Similarly, in non-human primates, age-dependent phase shifts are seen in both the circadian neuropeptides VIP and AVP [244, 286]. The age-related changes in pacemaker connectivity are a conserved impact of aging across species. Older *Drosophila* also display reduced expression of the circadian neuropeptide PDF indicating decreased communication between circadian neurons or targets [287]. These studies strongly suggest that aging affects synchronization between SCN pacemaker neurons by adversely altering the organized output of the SCN to peripheral oscillators and tissues. Signaling from the melanopsin-containing intrinsically photosensitive retinal ganglion cells to the SCN is necessary for photoentrainment of peripheral oscillators [288]. Although peripheral oscillators may be entrained by non-photic zeitgebers such as rest-activity cycles, feeding-fasting rhythms or body temperature rhythms, these cues are governed either directly or indirectly by the SCN [289] making phase coherence of the SCN of primary importance for synchronization of peripheral oscillators.

In peripheral tissues, the rate of entrainment and resynchronization after a phase shift varies between tissues and organs [71]. Aging differentially affects peripheral

oscillators with some tissues seemingly unaffected by phase shifts while other tissues exhibit phase discordance with the light cycle or the absence of rhythms [243]. Aged mice and rats subjected to simulated jet-lag require significantly longer for locomotor rhythms to reflect re-entrainment with new light-dark cycles [282, 290]. Moreover, peripheral oscillators from older transgenic reporter mice (PER2::LUC) exhibited slower re-entrainment in the esophagus, lung, thymus and liver [282, 290]. As discussed previously, circadian desynchronization, such as seen in aging, results in many adverse health effects. In rodents, persistent phase-shifts of the circadian system with a chronic jet lag model increased mortality in aged animals [291] demonstrating the ultimate harm of age-induced circadian dysfunction. Similarly, constant light conditions or light at night that presumably promulgate circadian desynchronization was found to shorten life span in mice and rats [292, 293].

4.3 Aging Differentially Affects Peripheral Oscillators and Tissues

Just as aging affects muscles and joints, aging also affects peripheral circadian oscillators and subsequent tissue function. The development of age-associated impairments in circadian function and the severity of those impairments vary across tissues. Age-related disturbances in peripheral oscillators can be observed in core clock gene expression, regulation of output pathways that affect tissue or organ function, and synchronization between oscillators.

Aging Affects Core Oscillator Gene Expression in a Tissue Dependent Manner

Although many studies using rodent models have identified age-related changes in core clock gene expression, it has been difficult to pinpoint which genes are affected and when they are affected. Importantly, studies in rodents suggest that aging may affect the core molecular mechanisms of peripheral oscillators in an organ-specific manner [148]. Bonaconsa and colleagues (2014) conducted a comparative analysis of the circadian expression of core clock genes at the mRNA level and found significantly attenuated rhythm amplitudes for *mPer1* and *mPer2* levels in the livers and *mPer2* levels in hearts of aged [22 months] mice compared to young [2 months] mice, with no significant changes in *Bmal1* and *Clock* mRNA levels [294]. However, a transcriptome wide analysis found that circadian profiles of core clock genes in the liver for 14–18 month old animals were not significantly different than observed for younger animals, although significant phase shifts and reorganization of clock-controlled genes was observed [148]. *In vivo* whole-body imaging in mice using a peripheral PER2::luciferase reporter system to monitor circadian rhythms found no significant dampening of the rhythms in the kidney, liver and submandibular glands

in either light-dark cycles or constant darkness of aged compared to young mice, although phase alterations were detected (18 months and 3–6 months respectively) [295]. Similarly, in older *Per1*-luciferase transgenic rats [24–26 months], aging had little effect on the strength of *Per1* rhythms in the kidneys and liver but rendered the rhythms in the lung arrhythmic compared to young rats [243]. It should be noted that although the strength of *Per1* rhythms in the kidneys was unaffected, the phase of the rhythms in the kidneys was significantly phase advanced 4 h in older rats compared to young rats [243]. Thus, while peripheral oscillators may continue to function with rhythmic expression of core clock genes, aging may induce phase shifts and reorganization of rhythms in clock controlled genes.

Examination of other tissues, such as white and brown adipose tissue, reveal that aging causes arrhythmicity and a two-fold increase in *mCry1* expression in mice aged 24–28 months compared to young mice at 9 months of age [296]. Other studies using bioluminescence recordings to assess *mPer1* expression in primary fibroblast cultures report that aged rats [18–24 months] exhibit a shortened period of ~22 h with a significantly reduced rhythm amplitude compared to the robust 24 h period observed in young rats [297]. Finally, significantly decreased levels of *Bmal1* are observed in peripheral blood cells of aged human females (40–79 years) [298].

Age-related changes in the cellular environment can also affect the rhythms in the expression of core clock genes [248]. When a *Bmal1::luciferase* reporter system was used assess changes in circadian expression of *Bmal1* in skin fibroblasts, no significant changes were observed in the amplitude or phase of *Bmal1* rhythms in aged adults (60–88 years) compared to young adults (21–30 years) [248]. However, when the standardized fibroblast serum used to incubate the cells was replaced by human serum of aged participants, cells from both young and aged donors exhibited a significantly shorter period in *Bmal1* expression along with a 2–2.5 h phase advance in temperature-entrained cells [248]. Heat inactivation of old serum was sufficient to restore the 24.5 h period of *Bmal1* expression in young and old cells [248]. These results suggest that aging alters circulating factors that may influence the expression of core clock genes.

The effect of aging on peripheral oscillators can be observed across phylogeny. In *Drosophila*, studies assessing the transcription of *per* using a *per*-luciferase reporter in peripheral oscillators revealed robust circadian rhythms in young flies (11 days) but significantly dampened reporter cycling in older flies (51 days) [299]. Additional analysis of mRNA levels in *per*, *Clk*, *cry* and *Pdp1ε* in fly bodies showed significantly reduced rhythms in older flies (>50 days) [299, 300]. Moreover, age-related decreases in the amplitude of circadian gene expression can even be seen in middle-aged flies (25–35 days) [300]. At the protein level, significant dampening is observed in PER rhythms in retinal photoreceptors that are also peripheral oscillators. Unlike mammals, *Drosophila* have peripheral oscillators that can be independently entrained by light and function separately from the central brain pacemaker neurons [301, 302]. Nuclear PER cycling in the independent peripheral oscillators of the Malphigian tubules, gut and abdominal fat bodies was found to be unaffected with aging [300]. Thus, aging induces tissue-specific changes in circadian function even in a relatively simple model system.

Aging Affects Clock-Controlled Gene Expression

The core circadian oscillator regulates output pathways through the rhythmic transcription of hundreds of clock-controlled genes (CCGs) comprising between 5% and 15% of genes in a tissue specific manner [146, 147, 303–305]. Impairments in circadian regulation, as seen with aging, disrupt the oscillations of many CCGs affecting tissue function. For example, the circadian clock regulates many genes involved in the responses to oxidative stress and cellular redox [305–309]. In *Drosophila*, aging significantly alters the rhythmic expression of CCGs that are necessary for regulating the cellular redox cycle [310]. Young flies (5 days) display strong diurnal patterns in glutathione biosynthesis with rhythms apparent in both *Gclc* and *Gclm* mRNA and protein levels, the genes encoding the catalytic and modulatory subunits of glutamate cysteine ligase (GCL) [311]. However, aging significantly increases *Gclc* and decreases *Gclm* mRNA and protein in older flies (50 days), disrupting the rhythms in downstream oxidative genes and compromising glutathione homeostasis [311]. Similarly, in mice, aging disturbs the temporal regulation of mitochondrial gene expression in the liver, altering the phase and amplitude of mRNA and protein rhythms [312, 313]. Furthermore, the rise in expression of mitochondrial redox genes observed during the resting phase in young mice is abolished in older mice [312]. These results suggest that aging significantly alters the expression profile of clock-controlled genes by altering the phases and amplitudes of their circadian rhythms.

4.4 The Circadian Clock Mediates Lifespan and Health Span

The circadian system and aging interact bidirectionally, with aging affecting circadian oscillators and synchronization, and circadian impairments, in turn, hastening cellular aging and disease pathologies. As the circadian clock modulates metabolic, immune and endocrine processes, it is hypothesized that alterations in circadian function impact longevity and health span. Research studies using animal models have shown that system wide knock down of core circadian genes accelerates aging phenotypes and mortality [307, 314–317]. Mice with mutations in *mPer1* and *mPer2* are morphologically identical to wild-type animals at birth but show signs of premature aging including early decline in fertility, soft tissue loss and kyphosis (an abnormally curved spine reminiscent of osteoporosis) as early as 2–4 months of age [317]. Moreover, *mPer2* mutant mice develop tissue abnormalities, and when challenged with gamma radiation, develop tumors earlier and at a higher rate than wild type mice [316]. The role of normal circadian function and the *per* gene in delaying the onset of aging pathologies are observed across species as per^{01} mutant flies, as well as flies with altered period length, have shorter lifespans. These flies also exhibit signs of accelerated aging including increased accumulation of oxidative damage and early signs of neurodegeneration compared to age-matched wild-type flies [314, 315, 318].

The impact of the loss of individual core clock components on the development of age-related pathologies varies in severity as illustrated with *Clock* and *Bmal1* mutants [319, 320]. Mice deficient in *Bmal1* have significantly shorter lifespans, approximately 37 weeks, compared to wild-type mice with lifespans reaching approximately 120 weeks [320]. Moreover, *Bmal1* mutants exhibit the premature appearance of multiple age-related pathologies including early signs of cataracts and cornea changes, sarcopenia, osteoporosis, and organ shrinkage [320]. Decreased *Bmal1* levels in mice also result in profound neurodegeneration accompanied by astrocyte proliferation and chronic inflammation, contributing to accelerated aging of the brain [321–323]. Compared to the premature aging phenotypes and the severity of the aging pathologies observed in *Bmal1* mutants, $Clock^{-/-}$ mice exhibit only a 15–20% decline in lifespan [319]. However, $Clock^{-/-}$ mice do exhibit age-related pathologies such as cataracts and dermatitis earlier than wild-type mice [319]. The differences in the observed severity of aging phenotypes between the circadian mutants may be due to the redundancy present in the circadian system as NPAS2 can form heterodimers with BMAL1 to activate transcription [133, 134, 136]. However, caution is necessary when inferring results from system wide knockdown of genes as genes may function in multiple pathways or processes and null mutants of circadian genes may exhibit defects independent of circadian clock disruption [324–326]. Nevertheless, these studies suggest a need for robust expression of clock genes for both the circadian regulation of behavior and physiology as well as more broadly in overall healthy aging.

The circadian clock also affects longevity and health span through the regulation of CCGs. Dysregulation of CCGs may affect immune system function shortening lifespan. For example, wild-type rats and mice exposed to repeated shifts of the light-dark cycle exhibit significantly lowered levels of immune factors such as leukocyte, lymphocyte and hemolysin concentrations in the blood, and a corresponding decrease in lifespan compared to wild-type litter mates that were not repeatedly phase shifted [327]. Moreover, mice injected with tumor cells and subjected to photoperiod shifts had significantly decreased survival rates and accelerated tumor growth compared to tumor-injected mice that were not phase-shifted [327]. In the nocturnal mouse lemur, a non-human primate, the degree of circadian disruption that occurs during aging correlates with the level of the proinflammatory cytokine, interferon-gamma (IFN-γ), a biomarker of aging, and is associated with decreased lifespan [286]. Similarly, humans subjected to a simulated night time shiftwork schedule exhibited desynchronized cycling of plasma levels of immune factors including IFN-γ and TNFα [328]. The circadian regulation of immune processes that affect longevity and health span appears to be a function of evolution as similar observations are made in lower organisms [329, 330]. For instance, in *Drosophila*, the *Achilles* gene regulates immune function, acting as a link between neurons and immune tissues. *Achilles* is highly rhythmic in the brain, peaking in the late dark phase with trough levels in the late light phase [329]. These studies suggest that disrupting circadian expression of clock-controlled genes exacerbate susceptibility to disease and reduce longevity.

Another hypothesis to explain the adverse effects of circadian disruption on longevity and health span in aged animals is the cost of a mismatch between the endogenous circadian period of the animal and the period imposed by the LD cycle in the environment to which the animal is entrained. For example, wild-type mice with free running rhythms close to 24 h exhibit 20% increase in lifespan compared to littermates with longer and shorter periods housed in a 24 h LD cycle [256, 331]. In a test of the mismatch hypothesis, wild-type mice kept under extreme 4 h: 4 h LD cycles exhibited significantly higher mortality [332]. Similarly, hamsters deficient in *tau*, an allele of CK1ε, have short endogenous periods of 20 h and fail to entrain to 14 h: 10 h LD cycles, with a reduction in lifespan of ~7 months compared to wild-type hamsters [333]. However, when the homozygous $tau^{-/-}$, heterozygous $tau^{+/-}$ and wild-type hamsters were housed under constant dim red light conditions (allows endogenous clock to free-run) immediately after weaning, the $tau^{-/-}$ hamsters had ~17% longer lifespan compared to $tau^{+/-}$ and wild-type hamsters [334]. Even in fruit flies, artificially dissociating the period of the LD cycle from the 24 h period of the endogenous clock by exposing young flies to a shorter or longer LD cycle significantly reduces lifespan [335–338]. These studies support the circadian resonance hypothesis that the environmental cycles to which animals are exposed need to match the period of the internal pacemaker for optimal physiological outcomes.

Conversely, individuals with robust circadian function throughout their life appear to live longer. Reports from human population studies indicate enhanced insulin sensitivity and more robust rhythms in glucose, cortisol and non-HDL cholesterol cycling in centenarians and long-lived individuals, with rhythms appearing more similar to those seen in middle-aged adults [339–342]. The benefits of a robust circadian clock appear to transcend generations as offspring of nonagenarians also exhibit higher amplitudes in cholesterol rhythms, specifically non-HDL cholesterol, and lower lipid accumulation in the skeletal muscle [339, 340, 342]. Similar observations were made in long-lived animal models [343]. For example, the extracellular serine protease, *urokinase-type plasmin activator* (uPA) appears necessary for tissue remodeling, brain plasticity and neuroprotection [344–346]. Both young and old [8 and 18 months] αMUPA transgenic mice have significantly longer lifespans and exhibited free running periods of 24 h with robust rhythms in food intake, body temperature and hepatic clock gene expression compared to wild-type mice with a lengthened period of ~25 h [343]. The necessity for coordination between the endogenous period and daily synchronization to a 24 h LD cycle may result from the metabolic cost of maintaining circadian organization when entrainment is compromised and the cost that stems from disrupting physiological processes that were temporally coordinated over evolutionary time.

4.5 Aging and Circadian Disruption Affect the Response to Physiological Challenges

Despite the medical and scientific advances that have significantly increased longevity, challenges remain for healthy physical and cognitive aging including the ability to respond to acute physiological challenges such as stress or infection. It has

long been known that the circadian clock affects behavioral and hormonal responses to stress [347, 348]. Changes in circadian function with age can affect hormonal responses altering physical and cognitive processes in managing stress. Older rats (22-months) have higher amplitude ACTH rhythms and lower amplitude corticosterone rhythms with narrower peaks compared to younger rats (3 months old) [349, 350]. In non-human primate studies, biochemical analysis of stress response molecules from the adrenal cortex of female rhesus monkeys following a restrained stress protocol show significantly lowered peak levels of cortisol and decreased amplitudes of the steroid hormones necessary for buffering the stress response, dehydroepiandrosterone sulfate (DHEAS) and glutathione reductase, in older monkeys compared to younger monkeys (26–27 and 6–8 years old respectively) [351]. Similarly, in humans, women aged 49–75 exhibit significant dampening in rhythmic cycling of salivary cortisol [352, 353].

At the cellular level, the circadian clock regulates enzymes and the expression of genes involved in cellular resistance to stress. Microarray studies report circadian rhythms in the expression of genes involved in stress resistance [303, 354, 355] and rhythmic cycling of antioxidant enzymes important for protecting against high levels of reactive oxygen species such as catalase, super-oxide dismutases and glutathione-S-transferase [356–358]. Aging significantly alters the circadian regulation of stress response genes in the liver [312]. Older mice (19 months old) exhibit a 6–12 h phase difference in the expression of *Gpd1* and *Hmgcs2* and 70–90% attenuation in the *Sirt3*, *Hmgcs2*, *Txnip* and *Ndufv3*, genes important for mitochondrial activity compared to young mice (3 months old) [312]. Numerous studies demonstrating the effect of circadian dysfunction on the mismanagement of cellular stress can be found across model systems. In *Drosophila*, flies exposed to a 2–3 week chronic circadian misalignment paradigm demonstrate accelerated aging, reduced lifespan and downregulation in genes necessary for lipid metabolism and biosynthesis [337]. *Bmal1* mutant mice exhibit increased oxidative damage and fatty livers and increased insulin resistance [359]. Mice with $Clock^{\Delta 19}$ mutations exhibit amplified endoplasmic reticulum (ER) stress and upregulated unfolded protein response, resulting in increased sensitivity to oxidative challenges and premature aging of the liver [360]. In fact, the ER stress response is implicated in multiple age-related disorders including metabolic syndrome [361–364], sleep disruption [365] and atherosclerosis [364, 366]. Therefore, an intact and functional circadian clock appears necessary as a buffer to increased sensitivity to stress with aging while disrupting the circadian clock likely exacerbates the sensitivity to stressors.

Circadian regulation of cellular and oxidative stress has implications for the response to physiological challenges in aging such as alcohol or drug use, high fat diets, smoking and other lifestyle choices. It has been estimated that genetic factors governing healthy aging and longevity account for 25% and lifestyle choices are responsible for 75% [367]. For example, aging and circadian disruption negatively affect the liver's drug metabolizing actions thereby increasing drug sensitivity and the time necessary to recover from the effects of drugs [368–370]. Early studies in rodent models demonstrate a time-of-day specificity in the sensitivity and toxicity of mice to alcohol, methamphetamines and other drugs [371–374]. In animal studies, aged mice (22–30 months) exhibit a loss of the diurnal rhythms in sensitivity to opiates such as morphine and

opiate agonists compared to young [1–2 months] and mature mice (8–12 months) [375–377]. Also, older rats exhibit dampened rhythms in locomotor behavior in response to methamphetamine and these rhythms had opposite phases of young rats treated with methamphetamine [378]. Finally, in animal models and humans, aging has also been shown to significantly increase the sensitivity to nicotine and benzodiazepines [379–381]. As with other interactions of the circadian clock and aging, age-related changes in circadian modulation of drug sensitivity appears conserved across species. Studies from our lab have shown that the circadian clock regulates alcohol-induced sensitivity and toxicity in *Drosophila* [382–384]. In wild-type flies, aging increases the behavioral sensitivity to alcohol and slows the recovery following alcohol exposure [383]. Genetic or environmental perturbations of clock function in young flies exacerbate behavioral sensitivity and toxicity similar to that seen in older flies [383]. In humans, the consequences of alcohol use disorders appear higher in aging populations in which circadian and sleep disruption is common [385]. Although additional research is needed to identify the mechanisms through which the circadian clock mediates drug and alcohol sensitivity, it appears that age-induced changes in circadian function may contribute to age-related increases in drug and alcohol sensitivity.

The decreased resilience of the stress response with aging may be more complex than simply blunting the rhythmic expression of molecular responses. The aging process may invoke a reprogramming of circadian regulation of gene expression. Microarray analysis of gene expression in aged stem cells from 25 to 29 month old mice reveal a loss of rhythmic expression of genes necessary for cellular homeostasis and a corresponding gain in rhythmic expression of *de novo* genes responsible for mitigating different types of tissue-specific stresses, including inefficient autophagy and DNA replicative stress such as *Brca2* and *Tipin* [148, 386]. These observations are conserved as deep sequencing studies in *Drosophila* show that aging diminishes the circadian regulation of some stress response genes and augments the rhythmic expression of others [387]. Aged flies (55 and 75 days old) exhibit robust circadian cycling of some genes not rhythmic in young flies (5 days old) including *Hsp22*, *Impl3*, *bnl*, *Hsp40-like* [387]. These studies suggest a more complex relationship between the circadian clock and aging whereby aging reprograms the circadian regulation of the stress response mechanism comparable to what is seen with the metabolic clock in the liver, upregulating molecules involved in inflammation, cytokine production and mitochondrial DNA repair. A robust circadian clock and age-related changes in the genes regulated by the clock may be necessary for organisms to meet the physiological challenges associated with increased longevity.

4.6 Circadian Disruption as a Predictor of Neurodegenerative Pathologies

Corresponding to the increase in human longevity, there has been a dramatic rise in the number of individuals with neurodegenerative disorders. In 2019, an estimated 5.8 million Americans were living with Alzheimer's disease and the number of people expected to be living with Parkinson's disease is predicted to be 930,000 by the

year 2020 and 1.2 million by 2030 [388, 389]. Worldwide the numbers of individuals living with dementia are predicted to nearly double every 20 years with estimates of 75 million affected individuals by 2030 [390].

Circadian dysfunction is considered a hallmark symptom of Alzheimer's disease and other neurodegenerative diseases. Patients with Alzheimer's disease have disrupted rhythms in rest-activity behavior such as fragmented activity patterns with increased activity at night and decreased activity in the day, as well as lower amplitude rhythms [391–394]. Sundowning, a common symptom of AD and other types of dementia whereby individuals exhibit increased severity of the behavioral issues and symptoms of AD around sunset, may be partially attributed to the phase delays of temperature and hormone rhythms in patients [395, 396]. Alzheimer's patients also exhibit rhythm disruptions in melatonin secretion and *Bmal1* oscillations [397, 398]. Post-mortem analysis of Alzheimer's patients found altered circadian expression of clock genes in the bed nucleus of the striaterminalis (BNST), cingulate cortex and pineal gland, brain regions commonly affected in Alzheimer's patients [399]. While significant diurnal rhythms in *Per1*, *Per2* and *Bmal1* oscillations were found in these brain regions, the phases of the rhythms were advanced by 4 h in the BNST compared to the controls [399]. Aberrant rhythms in epigenetic modifications for *Bmal1* have also been found in brain tissue samples and fibroblast cell cultures from Alzheimer's patients [400]. Recently, a large scale genome wide association study using a gene-based analysis approach was performed with more than 37,000 Alzheimer's samples and 17,000 controls [401]. Of the three novel significant genes identified as associated with Alzheimer's disease, two were the circadian genes, RORα and PPARGC1A [401]. Not only do circadian rhythm disturbances affect patients with Alzheimer's disease but they are also one of the most challenging aspects to caregivers who frequently also suffer from disturbed circadian and sleep patterns [402].

Disruptions in circadian gene expression have also been associated with Parkinson's disease. Modifications of clock gene expression including decreased *Per2* in the striatum and abolished *Per1*, *Cry1* and *Bmal1* rhythms in the SCN have been reported in rodent models of Parkinson's disease [403, 404]. Similarly, human studies have found correlations between genetic polymorphisms of circadian disruption and increased susceptibility to the pathologies of Parkinson's disease [405, 406]. Gu and colleagues (2015) analyzed single nucleotide polymorphisms of patients with and without Parkinson's disease for variants in 8 clock genes [405]. Individuals with a polymorphism in *Bmal1* were more likely to have motor tremors whereas *Per1* variant individuals were more likely to express postural instability and difficulties with gait [405]. Polymorphisms in *Clock* have also been associated with symptoms of Parkinson's disease in a Chinese population in which it was found that $Clock^{3111T/C}$ variant carriers were more likely to have motor fluctuations, a decline in the uninterrupted control of symptoms following L-dopa administration [406].

Circadian disturbances have emerged as a vital feature in the progression of neurodegenerative disorders [405, 407–410]. Fragmentation of rest-activity rhythms appears correlated with increases in Alzheimer's disease pathologies in early preclinical Alzheimer's disease patients (~66.6 years) compared with age-matched healthy controls [411]. Delayed rising time and increases in sleep-wake

disturbances are also correlated with increases in biomarkers of Alzheimer's disease including amplified degeneration of the hippocampus, increased Aβ levels in cerebrospinal fluid and tau activation [412, 413]. In animal models, deletion of *Bmal1* alone or deletion of *Clock* and *Npas2* in the brain exacerbates neurodegenerative pathologies such as astrogliosis in the cortex and hippocampus [323]. Mice with deletion of *Bmal1* in neurons and glia also exhibit degeneration of synaptic terminals, impairment of functional connectivity in the cortex, alteration of oxidative redox defense genes and increased oxidative damage [323]. Thus, there appear to be reciprocal interactions between circadian dysfunction and neurodegenerative disease pathology.

As circadian rhythm and sleep disturbances are often identified in retrospect as one of the early symptoms of Alzheimer's disease, the question has been debated as to whether circadian disruption is a result of the neurodegenerative process affecting clock mechanisms or whether disruptions in circadian rhythms precede the progression of neurodegenerative diseases. If circadian disruption is a harbinger to neurodegenerative disease onset, disruptions in behavioral and molecular rhythms may precede the onset of the disease. Longitudinal studies have assayed the behavioral indicators of circadian rhythm disruption to assess the connection between circadian disruption and the development or progression of neurodegenerative pathologies [53, 414–416]. For example, wrist actigraphy measurements of older women (83 years) show that weakened and delayed circadian rhythms in daytime activity increased the onset of dementia 5 years later at follow up [53, 415]. In cognitively normal older men and women (~83 years), lowered amplitude circadian activity rhythms and fragmented daytime activity correlated with increased deterioration of cognitive performance as measured by the California Verbal Learning Task, Mini-mental Status Exam, Trail Making Test and categorical and letter fluency tests 5 years at follow-up [416, 417]. Extended or shortened sleep along with delayed rising times appear to be predictors of dementia in healthy older individuals (60–73 years) when followed up 17 years later [418, 419]. Finally, increased daytime sleepiness with as little as 1 h naps during the daytime has been associated with increased risk of developing early motor symptoms of Parkinson's disease in healthy and prediagnostic individuals (76 years) [414, 420]. Evidence suggests that in groups of individuals in which circadian disruption is common such as those on shiftwork schedules, there is an increased cognitive decline compared to age-matched controls, increased risk of dementia, and an increased risk for developing Parkinson's disease [53, 421, 422]. These studies support the hypothesis that circadian disruption is a risk factor and potential predictor for the development of Alzheimer's disease, Parkinson's disease and dementia.

One question that arises is how do circadian disturbances or circadian dysfunction promote the progression of neurodegeneration? One hypothesis focuses on sleep with disturbances in circadian function disrupting the timing of sleep resulting in less consolidated sleep at night and increased daytime napping as seen in Parkinson's disease and Alzheimer's disease patients. Disrupted sleep in both rodent models and in humans exacerbates Aβ and tau pathologies, increases CSF markers of inflammation and neuronal injury and disrupts protein clearance [423–429]. Potentially, addressing sleep issues in Alzheimer's disease and Parkinson's disease

patients can ameliorate some of the symptoms or slow progression of neurodegeneration. However, sleep disturbances appear to only be one part of the circadian influence on neurodegeneration, the other part being the effect of the core components of the clock itself.

Another possible mechanism linking the circadian system with neurodegeneration could be the degeneration of the central clock circuitry and subsequent effects on downstream signaling pathways implicated in neurodegenerative disorders. Changes in the SCN can result in disturbances in rhythms and potentiate pathologies characteristic of neurodegenerative disorders. For example, mouse models of Huntington's disease exhibit significantly decreased spontaneous cell firing in the SCN compared to age-matched controls, although no change occurs in SCN cell number [430–432]. In humans, post-mortem studies of Alzheimer's disease patients show a loss of hypothalamic neurons, including in the SCN, along with decreased AVP and VIP neuropeptide levels [433, 434]. Another mechanism through which circadian dysfunction can promote degeneration is through disrupted regulation of genes and serum factors implicated in neurodegenerative pathologies. For example, *Presenilin2* regulates the levels of B-amyloid peptide and mutations in *Presenilin1* and *Presenilin2* are risk factors for early onset Alzheimer's disease [435]. Circadian rhythms in *Presenilin2* can be observed in the SCN and in the liver [436]. Therefore, there may be a causal link between the cycling of clock genes and genes that pose a risk for neurodegenerative disorders. More detailed analyses of the relationship between circadian disruption and neurodegeneration can be found in the reviews [322, 437–442].

5 Potential Interventions and Treatments

Circadian robustness is necessary for optimal physiological function and depends on strong intercellular coupling and a balance between the positive and negative arms of the core oscillator. As seen in the previous sections, aging correlates with a decreased response to entrainment signals and disintegration of the synchrony between central and peripheral oscillators. Chronic disturbances to circadian oscillations decrease the amplitude of behavioral and molecular rhythms, making resynchronization following either small or large perturbations more difficult [443–445]. The rise in circadian disorders across age groups, as well as the increasing aging population, makes it necessary to explore and develop new strategies to treat aging-related pathologies and manage chronic conditions. The bidirectional interactions between the circadian system and aging processes suggest that reinforcing circadian function may potentially mitigate the onset and/or progression of age-related diseases. Targeting the circadian system for treatment of pathologies associated with aging can be done by directly affecting oscillator function and synchronization using behavioral paradigms to reinforce entrainment of circadian rhythms, or pharmacologically to increase the robustness and amplitude of the core oscillator (Fig. 9.3). Additionally, implementing a chronopharmacological approach for drug delivery to coincide with the rhythmic regulation of the processes targeted

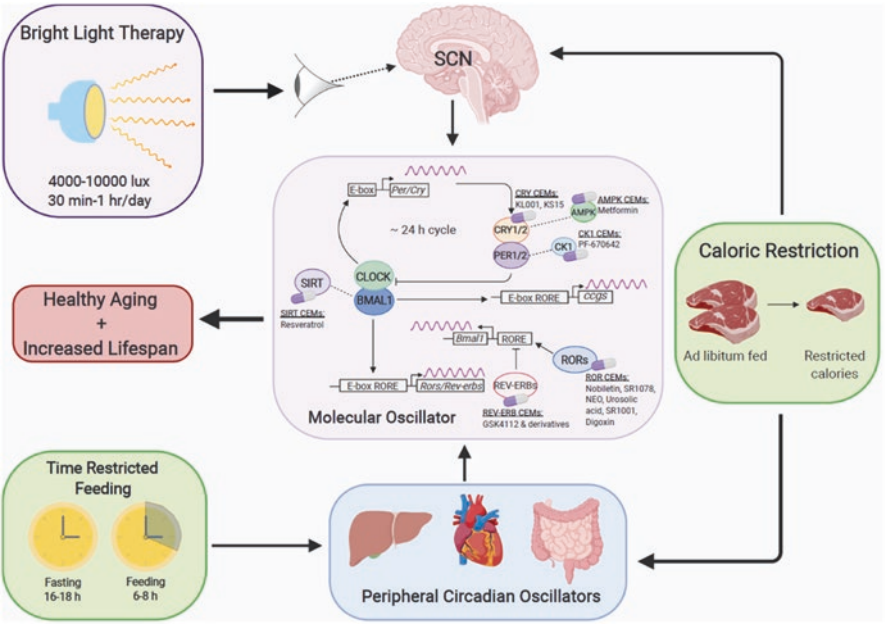


Fig. 9.3 Reinforcement strategies to improve circadian function. The circadian system can be strengthened using behavioral interventions such as bright light therapy and dietary restriction or pharmacologically using clock enhancing molecules (CEMs). Bright Light Therapy targets entrainment of SCN oscillators. Dietary regimens such as time-restricted feeding, intermittent fasting and caloric restriction reinforce rhythms in peripheral oscillators of the liver, heart and gut. Pharmacological agents have the potential for specific targeting of key oscillator components to adjust the phase, period or amplitude of molecular oscillations. Potentially, healthy aging may be facilitated by interventions that increase the synchronization and robustness of circadian function

may improve clinical outcomes for many diseases and reduce adverse side effects [446]. Studies that have targeted the circadian system to manage chronic conditions or pathologies associated with aging are briefly reviewed in the following sections. More comprehensive analyses of these interventions are described in the following reviews (for drug targets and biomarkers [447–452], for time-restricted feeding: [453–460], and for bright light interventions [461–465]).

5.1 Behavioral Reinforcement of the Circadian System

Targeting Symptoms of Aging Using Bright Light Therapy (Table 9.1)

As light is the primary zeitgeber for the SCN, researchers have used increasing light intensity or extended light duration to modify the responses of the SCN. Early studies focused on aging and the circadian clock in rats demonstrated that increased light intensity amplified the firing rate of SCN neurons and decreased the

Table 9.1 Therapeutic applications of bright light therapy

Problem	Parameters	Outcomes	Reference
Alzheimer's Disease			
Sleep disturbances and reduced performance on cognitive tests in Alzheimer's patients	Bright light exposure for 2 h during early-mid day for 4 weeks	1. Improved sleep wake cycles 2. Enhanced performance on cognitive tests	[466]
Poor performance in cognition and daily living activities on the Alzheimer's Disease Rating scale	45 min-2 h morning light therapy (5000–8000 lux) for 2–4 weeks	1. Strengthened sleep wake cycles, enhanced sleep quality and sleep efficiency 2. Decreased daytime sleep	[381, 467–474]
Decreased daytime activity and ineffective regulation of sleep wake cycle by melatonin	2500 lux morning light exposure for 10 weeks coupled with 6 mg melatonin in the evening	1. Strengthened rest-activity rhythms 2. Increased daytime wake activity 3. Enhanced melatonin activity	[472, 475]
Parkinson's Disease			
Insomnia, tremors, and depression in Parkinson's patients	Daily 30 min bright light exposure(7500 lux) for 2–5 weeks	1. Improved motor function 2. Decreased intensity of tremors 3. Normalized latency of sleep onset	[472, 476, 477]
Disturbed rest-activity rhythms in Parkinson's patients	2 times daily exposure to 1 h bright light therapy exposure for 14 days	1. Decreased sleep fragmentation 2. Improved sleep quality and ease of falling asleep 3. Increased daily physical activity	[478]
Primary symptoms – bradykinesia, rigidity, tremors and altered gate Secondary symptoms – depression, insomnia, involuntary movement and mood disturbances	Daily exposure of 1 h bright light (3000 lux) for 4 months to 5 years	1. Improved performance on standardized motor tests 2. Decreased anxiety and mood disturbances 3. Lowered insomnia	[479]
Mood disturbances in Parkinson's patients and patients with Major Depression	30 min light exposure (10,000 lux)twice daily for 3 months	1. Subjective improvements in mood and sleep quality	[480]
Disturbances of Healthy Aging			
Sleep disturbances in older adults (61–78 years)	4000–10,000 lux for 0.5–1 h daily for 1–3 weeks	1. Improved daytime alertness 2. Enhanced daily sleep wake cycles 3. Decreased daytime sleep 4. Decreased difficulty falling asleep prior to bed time	[481–486]

(continued)

Table 9.1 (continued)

Problem	Parameters	Outcomes	Reference
Increased depression in older adults	4000–7000 lux bright light exposure in the morning for 4 weeks	1. Reduced depression scores 2. Improved mood regulation	[487–490]
Resistance to antidepressants	5000–10,000 lux bright light exposure daily coupled with an antidepressant.	3. Augmented response to antidepressants: fluoxetine, setraline, venlaxfaxine hydrochloride	[491–493]
Increased cardiac stress	Single 5000–8000 lux exposure to bright light therapy	1. Decreased heart rate 2. Increased vagal tone	[494]
Arrhythmic temperature and melatonin rhythms	Single 5000–8000 lux exposure to bright light therapy	1. Enhanced rhythms in thermoregulation 2. Restored melatonin rhythms	[495]
Mood disturbances and headaches	Bright light therapy coupled with melatonin for 3.5 years	3. Reduced sleep fragmentation 4. Lowered irritability 5. Reduced dizziness and headaches 6. Lowered constipation	[496]

age-related loss of AVP neurons [497, 498]. In the past 25 years, bright light therapy has been used as an effective treatment to reinforce circadian function in conditions such as delayed sleep phase syndrome, jetlag, shift work, seasonal affective disorder and depression [461, 465, 499–501]. Numerous studies have assayed the efficacy and conditions necessary for using bright light therapy as a supplemental tool to manage circadian disturbances in older adults and individuals with neurodegenerative diseases [467, 502, 503].

Light therapy (~4000–10,000 lux for ~0.5 to 1 h) given in the morning enhances daily sleep wake cycles, improving daytime alertness and decreasing daytime sleep in older individuals [481–485, 487, 500]. Bright light exposure in older adults also improves overall sleep quality compared to age-matched controls [481, 485, 504–508]. Short morning or mid-day exposures to bright light (30 min) in aged adults (61–78 years) decreases evening alertness prior to bedtime reducing the difficulty in falling asleep [486, 507, 509]. High intensity(5000–8000 lux) bright light therapy also has been shown effective in resolving disorganized rhythms in thermoregulation and circulating neuroendocrine factors as measured by melatonin levels [495]. Even a single, short exposure to bright light decreases heart rate and increases vagal tone, markers of cardiac stress reactions in older individuals during resting and cognitive stimulation tests [494]. Bright light therapy is applicable for managing circadian disorders across all age groups as young adults with sleep disturbances also benefited with exposure to natural bright light, as evidenced by 2 h phase shifts in their melatonin rhythms [510, 511].

In addition to the efficacy of bright light therapy for sleep, this therapy also may be beneficial in improving cognitive function and mood disorders. Older adults (60–82 years) demonstrated improved scores on cognitive tasks including Stroop Congruency, Two Letter Visual Search and Wilkinson Four-Choice Reaction Time as measured by logical reasoning, memory recall and reaction time following 7 consecutive days of 2 h bright light exposure in the daytime [512]. The Centers for Disease Control and Prevention reports that more than seven million adults aged 65 and older experience depression every year, with this age group accounting for 16% of recorded suicides in 2004 [513–515]. While antidepressant medications can be helpful, one complication in older adults is potential drug interactions with other medications taken which may render pharmacological treatment of depression less effective [488]. However, light therapy avoids these potential complications making it a low risk effective management tool. The delivery of 4000–7000 lux of morning bright light therapy significantly lowered depression scores in patients aged 60–90 years when compared to dim bright light or no-treatment control groups [487, 489, 515]. A 1 h daily regimen of bright light exposure for older adults (59–80 years) for 4 weeks improved their ratings on mood scales compared to age-matched controls [488]. Light therapy can augment the responses to medications for the treatment of mood and sleep disturbances. A pilot study found that 5000–10,000 lux of morning and afternoon exposure of bright fluorescent light ameliorated mood disturbances in depressed patients with resistance to antidepressant medications [488, 516–518]. Schuchardt and colleagues found that bright light exposure (5000 lux) with fluoxetine administration in the daytime significantly augmented the antidepressant responses in patients compared to dim light and fluoxetine administration. Similarly, combining the antidepressants, Sertraline or Venlaxfaxine hydrochloride with bright light therapy (10,000 lux and 7000 lux in the morning respectively) significantly decreased depressive symptoms compared to the drug alone [491–493]. However, light therapy does not augment the effects of all drug regimens as there have been studies in which light therapy alone has been more effective than in combination with medications. For example, 66% of the patients given 10,000 lux bright light therapy for 3 weeks exhibited significantly decreased depressive symptoms compared to patients given the antidepressant imipramine [33.3%] or combined light therapy and imipramine (35.4%) [519]. No differences were observed when bright light therapy was paired with trimipramine or other antidepressants compared to the drug therapy alone [488, 520]. While the effect of light on mood disorders appears to occur through the melanopsin-containing ipRCGs, this process may be independent of the SCN's circadian function [521, 522].

The effectiveness of bright light therapy extends to deleterious neurological conditions including neurodegenerative disorders. Bright light therapy has been shown to ameliorate the motor symptoms and improve cognitive abilities in neurodegenerative disorders such as dementia, Parkinson's and Alzheimer's disease [463, 465, 466, 480, 496]. Patients with Alzheimer's disease exhibit more disrupted circadian rhythms in rest activity cycles and bright light therapy strengthens sleep-wake cycles, reduces sleep disturbances, and increases sleep quality [467–472, 505, 523]. Exposure to 2 h bright light in the morning for 2 weeks significantly decreased

daytime sleep in Alzheimer's disease patients [473]. As with depression, bright light therapy can increase the effectiveness of other sleep aids as researchers found that Alzheimer's disease patients exposed to bright light (>2500 lux) for 10 weeks coupled with 5 mg melatonin in the evening increased daytime wake and activity levels and strengthened the rest-activity rhythm [472]. In group comparisons, individuals with higher daily light exposures (>417 lux) had significantly increased positive feelings and daily alertness, as well as overall higher quality of life with less time spent in bed, and later onset of sleep episodes for Alzheimer's disease patients [524]. Whether light therapy is more effective at mild or severe stages of the disease appears to be a matter for debate [475, 525]. Bright light exposure (2500 lux) for 2–3 h in the morning has been shown to significantly increase nighttime sleep in patients with advanced Alzheimer's disease [526]. But, other studies have shown that bright light therapy more effectively reduced sleep disturbances in patients with mild to moderate Alzheimer's disease [475]. In a third study comparing across patient groups, researchers found that while light treatment was effective regardless of the disease severity, it was more effective in managing behavior and sleep issues in patients with more advanced disease progression [527]. For Parkinson's disease, clinical trials of patients (~62 years) found that 1 h bright light therapy daily lowered sleep disturbances including sleep fragmentation and improved overall sleep quality, ease of falling asleep and increased daily physical activity [478].

Several important considerations need to be addressed when considering bright light therapy as a management tool. First, the daily timing of the bright light exposure is critical if it serves as a reinforcement tool for entrainment of the circadian clock, with morning light delivery optimal and no therapeutic effect of administering light therapy in the evening [520]. Second, the effect of light on other medications or supplements needs to be considered. As research has shown, if bright light is paired with melatonin in the late evening, negative mood effects were found presumably due to light-induced melatonin suppression, whereas bright light alone for 2 weeks (10,000 lux bright light therapy for 30 min twice daily) was effective in decreasing restless behavior and increasing calmness [528]. Third, the daily duration of the bright light therapy needs to be considered as part of a long-term management tool. Daily light exposure (10,000 lux) of 45 min for 3 months appears to significantly ameliorate sleep disturbances and improve sleep quality in geriatric patients (~60 years) compared to age-matched individuals receiving 20 min of the same treatment [529]. The positive effects of the longer 45 min exposure persisted for 3 and 6 months following treatment, an effect not observed in individuals receiving the 20 min light regimen [529]. Fourth, the wavelength and intensity of the light is an important factor given the tuning of circadian photoreceptive molecules. Administration of bright green light (1200 lux) is more effective than dim red light (<10 lux) in ameliorating depressive symptoms in older adults (59–80 years) [530]. Finally, although bright light therapy as a long-term management tool is relatively low risk, there have been cases in which bright light interventions increased irritability, anxiety and agitation in normal elderly men and women not experiencing any of the above-mentioned problems [469, 531]. Nevertheless, with a well-designed paradigm, bright light therapy may be an attractive option to improve sleep cycles

and cognitive performance, and decrease depression in older adults and individuals with neurodegenerative and mood disorders.

Time-Restricted Feeding Reinforces Synchrony Among Peripheral Oscillators and Mitigates the Onset and Progression of Metabolic Disorders (Table 9.2)

Misalignment of circadian rhythms or circadian desynchronization exacerbates metabolic pathologies including decreased glucose tolerance, weight gain and dysregulated glycemic control as well as increasing the risk of diabetes, metabolic syndrome and cardiovascular disease [545–548]. Time-restricted feeding (TRF), in which access to food is limited to 6–12 h of the active period is a form of intermittent fasting (IF) and represents one dietary approach that shows promise in mitigating the onset and development of metabolic disturbances [457, 532, 540, 544, 555, 556]. Numerous studies have shown that restricting food consumption to specified time intervals significantly enhances metabolic health and facilitates healthy aging [457, 546, 548–554].

Timed feeding and fasting cycles appear to delay the onset or reduce the risk of metabolic disorders in middle-aged and older healthy adults and in animal models [532, 538, 555–557]. Mechanistically, it appears that time restricted feeding bolsters the circadian system and reduces inflammatory markers associated with metabolic disease. In rodent studies, young mice exposed to a daytime TRF paradigm for 12 weeks exhibited more robust circadian cycling of clock genes, drastically decreased mRNA and protein levels of proinflammatory cytokines and chemokines in the liver, jejunum, and white adipose tissue, and increased the anti-inflammatory cytokine *Il-10* in the liver and jejunum [556, 557]. Similarly, in healthy middle-aged adults (45–55 years), 8 h TRF cycles significantly decreased body weight, energy intake and systolic blood pressure although little to no changes were found in heart rate, cholesterol, triglycerides, glucose or insulin sensitivity [558, 559]. The duration of the window to which food intake is restricted appears important with shorter periods of food availability having more positive outcomes on health indices [560]. For example, Jamshead and colleagues (2019) subjected healthy young and middle-aged adults [20–45 years] to either 12 h [8 am–8 pm] TRF or a 6 h (8 am–2 pm) early time restricted feeding paradigm for 4 days [560]. Individuals on the early restricted feeding schedule exhibited significantly reduced levels of circulating glucose over a 24 h period compared to those on the 12 h feeding schedule [560]. Moreover, individuals on the early time-restricted feeding paradigm had significantly increased glycemic markers such as ketones, cholesterol, stress response SIRT1 and the autophagic gene LC3A in the morning and elevated levels of BDNF and mTOR [important for nutrient sensing and cell growth] at night compared to individuals on the longer feeding fasting paradigm [560]. Finally, more robust circadian rhythms in cortisol, BMAL1, PER, CRY and RORα across the 24 h cycle were observed in the individuals on the early compared to the longer feeding fasting schedules [560]. The effects of TRF on reinforcing circadian rhythms and

Table 9.2 Benefits of time-restricted feeding

Model	Problem	Manipulation	Outcome	Reference
Drosophila	Reduced sleep in older flies and deterioration of cardiac function	Food restricted to 12 h/day during light cycle	Improved sleep/activity rhythms and decreased cardiac aging	[532]
	Disturbed metabolic and neural signaling	8 h/day alternate day fasting during the light cycle	Reprogramming of metabolic and neural transcriptome	[533]
Rats	Disrupted entrainment to light	Restricted feeding for 2 h during the light cycle for 14 days	Aged rats entrained to feeding rhythms	[534]
Mice	Disrupted circadian rhythms in metabolism in Huntington's disease	Restricted feeding for 5 h/day for 12 days in R6/2 Huntington's disease mouse model	Restoration of behavioral rhythms and hepatic circadian gene expression	[535]
		Restricted feeding for 6 h/day in mHTT Huntington's disease model	Decreased mTOR phosphorylation, increased SIRT1 levels, increased mRNA expression of autophagic markers	[536]
	Disrupted signaling in metabolic factors in HFD mice	Restricted feeding for 6 h/day in Q175 Huntington's disease model for 3 months	Improved rhythms in locomotorbehavior, sleep awakening time and heart function. Reduced levels of HD markers in brain	[537]
	Glasgow osteosarcoma	Restricted feeding for 6 h/day in HFD mice for 8 weeks	Decreased weight gain, liver triglycerides, plasma leptin and cholesterol and reduced inflammation in adipose tissue	[538]
		Restricted feeding for 12 h/day for 6 weeks	Increased lifespan, circadian reprogramming of carcinogenic and tumor suppression (*c-myc* and *p53*)	[539]
Humans	Overweight individuals	4 h feeding window every other day for 2 weeks	Increased glucose uptake and increased insulin suppression of lipolysis	[540]
	Overweight males and females 29–70 years	8–10 h/ day for 4 weeks	Decreased body weight, decreased lipid factors and increased glucose metabolism	[541]
	Increased risk marker HbA1c for breast cancer in middle-aged women ~46 years	Restricted feeding schedule in line with circadian phase	Reduced levels of HbA1c risk marker and slower onset or risk of developing cancer	[542, 543]

ameliorating age-related pathologies can be observed across species. Studies in *Drosophila* found that 7 days of timed feeding in late middle-age flies [35 days old] significantly improved sleep-wake activity cycles and cardiac function [532]. Moreover, the suppression of cardiac aging was associated with temporal gene expression and was dependent upon a functional circadian clock [532]. A long-term TRF schedule with short 8 h fasting periods on alternate days reprogrammed the transcriptome in the brains and muscles of middle-aged and old flies, improving age-dependent expression of genes involved in the stress response, metabolic and neural processes and chromatin remodeling [533]. TRF strategies in young adulthood may contribute to more robustness in old age as seen in *Drosophila* in which young adult flies subjected to a 2-day fed:5-day fasted intermittent feeding regimen had extended lifespans, and increased the resistance to starvation, oxidative and xenobiotic stress in later adulthood [555]. Early-life exposure to intermittent feeding also increased the lipid content and enhanced the gut barrier function in older flies (60 days) [555]. These studies suggest that eating patterns established with social jetlag or shiftwork in young and middle age adults may contribute to poorer health outcomes with aging.

Apart from delaying the onset of age-related pathologies that contribute to development of metabolic disorders, restricted feeding paradigms may reduce the severity of existing disease symptoms associated with aging as seen in animal studies and humans [536, 537, 542, 543, 561–567]. Timed feeding schedules have beneficial effects in animal models prone to developing pathologies associated with metabolic disorders [538, 556]. Young wild-type mice given a high fat diet and placed on 6 h:18 h feeding fasting schedule for 8 weeks gained less weight and had lower body fat percentage, liver triglycerides, plasma leptin and cholesterol levels compared to *ad libitum* HFD littermates [538]. Although no reduction in systemic TNFα levels (a proinflammatory cytokine) was observed, restricted feeding schedules significantly reduced inflammation of adipose tissue in the HFD-TRF mice compared to *ad libitum* fed mice on a high fat diet [538]. Rodent studies assessing the effects of timed feeding paradigms administered after long-term exposure to a high fat diet appeared inconsistent with regard to body weight changes compared to *ad libitum* fed mice, but the studies consistently reported improved circadian rhythms in metabolic markers and lowered glucose tolerance in HFD mice on restricted feeding schedules [568–571]. Similarly, in humans, restricting high calorie intake to the daytime in obese individuals significantly decreased body weight, ghrelin levels, insulin resistance and increases satiation [572]. In older, overweight individuals (65 years and older), restricting food access to 8 h during the day for 4 weeks significantly lowers body weight, cognitive and physical function, the incidence of adverse falling events and the overall self-reported quality of life [541]. These effects of timed feeding on existing metabolic pathologies are consistent across species as young obesogenic flies raised on diets high in fat and/or sugar and subjected to a 12 h:12 h TRF schedule exhibit significant decreases in the obesity-induced pathologies and increased muscle performance, mitochondrial aberrations and markers of insulin resistance [573].

Timed feeding regimens also show high efficacy in decreasing the severity or delaying pathologies associated with neurodegenerative disorders and cancer. Rodent studies indicate that time restricted feeding paradigms in the early phases of carcinogenesis have a beneficial effect on disease severity [556]. Young mice given a high fat diet on a TRF schedule for 18 months followed by a transplantation of pre-neoplastic liver cells exhibit reduced cell death and fat accumulation coupled with upregulation of SIRT1, an anti-aging factor in the liver [556]. Restricted feeding increases lifespan and appears to reprogram the rhythmic expression of genes involved in carcinogenesis and tumor progression such as *c-myc* and *p53* in mouse models of Glasgow osteosarcoma [539]. Similarly, middle-aged women (mean age ~46 years) at risk for breast cancer following a restricted feeding schedule in line with their circadian phase report significantly reduced levels of the HbA1c breast cancer marker, thereby slowing the onset or risk of developing breast cancer [542, 543, 574, 575]. The results from these studies support restricted feeding models as a therapeutic intervention to augment health indices, increase lifespan and stave off the increased risk of cancer associated with old age.

The underlying mechanism through which time restricted feeding enhances circadian synchronization appears to occur directly through the peripheral oscillators' coordination of metabolic activity rhythms to feeding fasting cycles and not the SCN [576]. Studies have shown that feeding fasting paradigms enhance the oscillations of circadian genes, clock controlled genes and their corresponding proteins in the liver, kidney and pancreas without affecting these oscillations in the SCN [180, 209, 216, 577]. Even in the absence of a functional SCN clock, timed feeding still appears to drive oscillations in transcription, downstream metabolites and even gut microbial activities [578–580]. This reinforcement of rhythms in peripheral oscillators is long-lasting as mice exhibit food anticipatory behavior 2–4 h before a meal with increased locomotor activity, corticosterone secretion, gastrointestinal motility and digestive enzyme activity [106, 215, 581, 582]. Disrupting the rhythms in peripheral clocks has adverse effects on metabolic processes as seen by decreased glucose absorption in the gut, insulin sensitivity in the liver and increased insulin secretion in the pancreas [583]. Apart from timed feeing, caloric restriction itself has been shown to increase longevity in many model systems [455, 456, 458, 459, 584–588]. However, it is unclear as to whether this occurs through the circadian system.

5.2 Pharmacological Targeting of Clock Components for Age-Related Pathologies (Table 9.3)

Bright light therapy, time-restricted feeding and other behavioral interventions not described here show encouraging potential for strengthening synchronization of the central and peripheral circadian system to manage chronic conditions. However, older individuals may have difficulty adhering to the treatment plans or may be

Table 9.3 Small molecule clock modifiers with potential for age-related diseases

CEM/Drug	Circadian Activities	Potential/Physiological Applications	Reference
CRY			
KL001 (carbazole derivative)	1. Stabilizes CRY 2. Lengthens period 3. Reduces*Bmal1* amplitude	1. Improves glucose tolerance in obese mice 2. Enhances liver gluconeogenesis	[589, 590]
KS15	1. Inhibits CRY 2. Shortens period 3. Reduces amplitude	1. Slows proliferation and increase chemosensitivity of breast cancer cells	[591–593]
CK1δ/ε			
PF-670462	1. Inhibit CKIδ/ε 2. Inhibit PER nuclear translocation 3. Lengthen period	1. Enhances locomotor behavioral rhythms 2. Attenuates methamphetamine-stimulated locomotion *in vivo.*	[594]
CKI-7, D4476, Longdaysin, Compounds 1–3 and others	1. Inhibits CK1δ/ε 2. Inhibits PER1 phosphorylation 3. Lengthens period	1. Suppresses proliferation and migration of breast cancer cells 2. Induces cell death in tumor cells of multiple myeloma	[595, 596]
GSK3β			
Indirubin	1. Shortens period 2. Inhibits GSK3β	1. Induces cell cycle arrest and inhibits cell proliferation 2. Decreases lipid buildup and glucose in cells 3. Increases antioxidant activity	[150, 597, 598]
Chir99021, 1-azakenpaullone	1. Shortens period 2. Inhibits GSK3β	1. Improves glucose metabolism 2. Anti-inflammatory effects 3. Increases cell viability and decrease apoptosis in liver cells 4. Reduces proliferation of malignant gliomas	[599–601]
Lithium	1. GSK3 β inhibitor 2. Lengthens period of *Bmal1*expression	1. Regulates mood 2. Prevents myelin fragmentation and reduces inflammation 3. Partially reduces mitochondrial damage and apoptosis	[602–605]

(continued)

Table 9.3 (continued)

CEM/Drug	Circadian Activities	Potential/Physiological Applications	Reference
RORα/γ			
Nobiletin	1. Activates RORα/γ 2. Increases *Bmal1* expression	1. Improves metabolic homeostasis in obese/diabetic mice 2. Decreases apoptosis in insulin-producing cells 3. Enhances lipid biogenesis in the liver 4. Mitigates memory impairment in amnesia models 5. Beneficial effects against tumors, inflammation, and cardiovascular disease	[258, 606–616]
SR1078	1. Activate RORα/γ 2. Increases *Bmal1* expression	1. Decreases apoptosis and increase autophagy to lower cardiomyopathy 2. Enhances cell response to ROS and inhibit hepatoma cell growth 3. Ameliorates autistic behavior in mice	[606, 617]
Neoruscogenin	1. ROR agonist 2. Promotes ROR interaction with NCOA2/TIF2 3. Activates *Bmal1* expression	1. Anti-inflammatory effects in liver in obesity models 2. Reduces symptoms of pulmonary hypertension and pulmonary disease	[618–620]
SR1001	1. T0901317 derivative 2. Selective inverse agonist for RORα and RORγ	1. Inhibits Th17 cell differentiation 2. Anti-inflammatory response 3. Delays onset of autoimmune disorders	[621]
SR2211, SR1555 Digoxin, Ursolic acid	1. RORγ inverse agonist	1. Inhibits Th17 cell differentiation	[450]
Compound 1a	1. RORγagonist	1. Promotes Th17 cell differentiation	[622, 623]
SR3335	1. RORα inverse agonist	1. Reduces and regulate blood glucose levels in obese mice	[624]
REV-ERBα/β			
GSK4112	1. REV-ERB agonist 2. Enhances interaction between REV-ERB and NCOR peptide	1. Inhibits gluconeogenesis 2. Inhibits inflammatory response in cortical and spinal astrocytes	[450, 625, 626]

(continued)

Table 9.3 (continued)

CEM/Drug	Circadian Activities	Potential/Physiological Applications	Reference
SR9009 SR9011	1. Selective agonists for REV-ERB 2. Alters circadian behavior and gene expression 3. Reduces BMAL1 expression	1. Improve glucose homeostasis in obese mice 2. Promotes wakefulness 3. Reduces anxiety 4. Cardiac remodeling and anti-inflammatory effects	[621, 627]
ARN5187	1. REV-ERBβ agonist	1. Cytotoxic against cancer cells	[628]
SIRT1			
Resveratrol	1. Activates Sirt1 2. Synchronizes locomotor and temperature rhythms	1. Broad physiological and antiaging efficacies 2. Protect against cancer and heart disease 3. Anti-inflammatory effects	[629, 630]
SRT2183, SRT1720, SRTCD1023, SRTCL1015	1. Activates Sirt1 2. Reduces amplitude of *Per2* rhythms	1. Improves circadian clock gene expression 2. Reduces inflammation in chronic obstructive pulmonary disease 3. Represses glioma cell growth	[631–633]

non-responsive to behavioral reinforcement therapies. Potentially, drugs that target the circadian system with tissue specific effects may be effective in combination with behavioral interventions to improve healthy aging. In recent years, high throughput unbiased and target-based screens in preclinical models identified numerous promising synthetic compounds that modulate circadian physiology. These drugs, termed clock enhancing molecules (CEMS), enhance cellular or tissue rhythms either directly by targeting core clock proteins, post-translational regulators such as the kinases that phosphorylate core clock components, or indirectly by acting on membrane receptors, ion channels or nuclear receptors coupled to clock components [450, 452, 625, 634, 635].

Small-molecule clock modifiers that alter the molecular dynamics of the core oscillator provide a novel way to target the circadian clock. For example, KL001, a carbazole derivative was among the first small molecules identified in a cell-based screen that acted directly on the core components of the mammalian clock [589]. In SCN explants and fibroblasts, continuous treatment with KL001 increases the activation of CRY proteins and potentiates transcriptional repression of *Bmal1* activity at the *Per2* promoter, thereby lengthening period and reducing the amplitude of *Bmal1* rhythms [589]. Structural studies demonstrate that KL001 stabilizes CRY proteins by binding to the FAD-binding pocket of CRY disrupting its recognition by FBXL3, thus blocking the ubiquitin-dependent degradation of CRY 1 and 2 [589, 590, 636, 637]. KL001 shows potential as an anti-diabetic agent as administration

of KL001 in mouse liver cells dose dependently attenuates the expression of genes involved with liver gluconeogenesis necessary for the production of glucose from glucagon in fasting states [590, 638]. CRY has also been identified as a molecule that may be targeted for circadian disruption and cancer interactions. Circadian disruption, especially in shift workers, is a potent risk factor for breast cancer [639–641]. Small molecule modulators of CRY show potential for treating some types of breast cancer [591–593]. For instance, pharmacological inhibition of CRY using a derivative of 2-ethoxypropanoic acid KS15 impedes proliferation and increases chemosensitivity of human breast cancer cells [591–593]. KS15 binds the c-terminus of CRY inhibiting the interaction between CRY and BMAL1 in MCF-7 breast cancer cells, thereby shortening the period and attenuating the amplitude of molecular rhythms in these cells [591, 592]. More detailed explanations of pharmacological compounds that affect circadian function with therapeutic potential for anticancer therapy are reviewed in [447].

Independent chemical screens have identified compounds that indirectly target the core circadian loop by affecting post-translational modifiers, usually protein kinases, that control the timing of the oscillator [589, 622, 642–645], including numerous inhibitors of *casein kinase 1 delta and epsilon* (CK1δ and CK1ε) [150, 646, 647]. CK1δ inhibitors lengthen the period of the molecular oscillator at the molecular and behavioral levels [594]. For example, in SCN slice preparations from arrhythmic *Vipr2*$^{-/-}$mice, application of the CK1δ inhibitor, PF-670462 restores robust cycling of *Per2* luciferase reporter rhythms [594]. Oral administration of PF-670462 induces more robust rhythms in locomotor behavior in arrhythmic *Vipr2−/−* mice and wild-type mice housed in constant light conditions, an environmental perturbation that disrupts locomotor behavioral rhythms [594]. The potential of PF-670462 to synchronize and restore behavioral rhythms in aberrant light conditions makes this clock modifier an attractive target for therapeutically regulating sleep and circadian abnormalities in individuals with disrupted circadian rhythms such as shift workers and aged individuals. Other clock modifiers for CK1δ have been identified with similar inhibitory effects including Longdaysin, DH4476, CK1–7 and Compounds 1–3 [150, 642, 644–647]. Interestingly, cases of familial delayed sleep phase syndrome are linked directly to mutations in CK1δ and CK1ε, making these small molecule modifiers a potential therapeutic target [648, 649]. Clock modifiers have also been shown to affect glycogen-synthase kinase 3 beta (GSK3β)], a broad acting kinase involved in circadian regulation and linked to numerous age-related diseases including Alzheimer's disease, diabetes, cancer and neuropsychiatric disorders [650–652]. In the molecular oscillator, GSK3β is important for phosphorylation and stabilization of REV-ERBα, regulation of BMAL1 protein stability, phosphorylation of CLOCK in a BMAL1-dependent manner, and PER phosphorylation facilitating its translocation into the nucleus [627, 653–656]. Research studies in animal models report the anti-aging potential of the GSK3β inhibitors indirubin, Chir99021 and 1-azakenpaullone in reprogramming cellular bioenergetic pathways and improving glucose metabolism and anti-inflammatory responses [597, 642]. Pharmacological inhibition of GSK3β using indirubin shortens the period of molecular rhythms in mammalian cell cultures [150, 598].

Selectively inhibiting GSK3β activity using the indirubin derivative 6-BIO decreases the cellular buildup of lipids and glucose and activates antioxidant molecules by upregulating Nrf2 [150, 598]. Elevated GSK3β activity appears to disrupt mitochondrial activity and increase oxidative injury, thereby facilitating the progression of liver cirrhosis [657]. Another GSK3β inhibitor, Chir99021 was shown to significantly increase cell viability, decrease apoptosis and ROS levels and restore normal mitochondrial activity in mouse liver cells exposed to hydrogen peroxide for 6 h [599]. As the majority of liver disease deaths occur in individuals aged 45 and above [658], Chir99021 provides a potential therapeutic avenue for protecting against liver cirrhosis deaths in these age groups. Research across species supports the hypothesis that GSK3β inhibitors may aid with healthy aging as elevated GSK3β activity suppresses protein folding and decreases longevity in *Drosophila* [597].

The nuclear receptor families, RORs and REV-ERBs represent perhaps the most pursued pharmacological targets as these molecules are implicated in multiple pathologies associated with aging including diabetes, cancer and circadian dysfunction [450, 659, 660]. These receptors appear to facilitate the crosstalk between the circadian clock and many cellular processes involved in inflammation, cell proliferation and metabolism, making them ideal targets for small molecule modifiers [450, 659, 660]. Studies using mammalian one-hybrid assays and radioligand assays identified a natural flavonoid Nobiletin [NOB] directly binding and activating RORα and RORγ receptors [606, 661]. NOB appears to act on pathways that connect metabolic and circadian fitness ameliorating the symptoms of metabolic syndrome, oxidative stress, inflammation and cancer with little known pharmacokinetic toxicity [606–608, 661–665]. In genetic obesity models and wild-type mice subjected to a high-fat diet, NOB administration has been found to increase energy expenditure, limit body weight gain, increase glucose and insulin tolerance, and enhance circadian rhythms in locomotor activity, thereby restoring metabolic and circadian resonance in the liver [606, 666–668]. NOB also decreases apoptosis of islet cells necessary for the production of insulin by regulating ER stress pathways [609, 610]. More recent studies demonstrate a role for the NOB modulator in regulating the metabolism of cholesterol and bile acids in aged animals [611]. In older mice (22 months) given a high fat diet, NOB administration improved the serum markers of cholesterol and bile acids, regulated expression of genes necessary for bile acid production, remodeled the gut microbiota landscape and reprogrammed genes involved in circadian and lipid homeostasis, thereby enhancing the overall health of the livers [611]. Administration of NOB also appears to promote healthy aging in older animals [612]. NOB supplementation in older mice (22 months) on a regular diet reduces glucose levels and restores rhythms in locomotor activity and temperature [612]. A combined high fat diet and NOB supplement in older mice restores rhythmic locomotor activity, enhances rhythmic expression of core clock genes and genes necessary for mitochondrial respiration and energy expenditure in the skeletal muscle [612].

Nobiletin may also be effective in ameliorating age-related memory impairments. Recent research in multiple mouse models found that NOB mitigated memory impairments in amnesia induced models, reduced the pathological features of

Alzheimer's disease in a mouse model including Aβ pathology, hyperphosphorylation of tau and oxidative stress, and improved motor and cognitive deficits of Parkinson's disease in a mouse model [258]. Given the critical role of ROR receptors in the circadian clock and regulation of multiple output pathways, it is not surprising that NOB may affect other systems such as the cardiovascular system in addition to metabolism and cognitive function. Recent research suggests that NOB can facilitate the reduction of adverse impacts of cardiomyopathies [613–615]. Myocardial ischemia represents the most common complication in cardiovascular surgery and PER2 appears to protect against this complication [669, 670]. Anesthetics such as pentobarbital, fentanyl, ketamine and isofluorane among others, reduce the *Per2* mRNA levels and increase the infarct size and troponin 1 levels [613]. In a mouse model of myocardial injury, treatment with NOB abolished the deleterious effects of the anesthetics by decreasing the infarct size and troponin 1 levels and increasing *Per2* levels [613]. Treatment with NOB prior to surgery in rat models of cardiomyocyte injury appears to protect against myocardial injuries by inhibiting apoptosis of cardiac muscle cells, decreasing the total area of dead tissue and restoring systolic cardiac function [614, 615]. Altogether, these studies suggest a strong potential for NOB as a therapeutic agent for enhancing circadian regulation and alleviating the pathologies associated with neurodegeneration and metabolic dysfunction [666].

Since the identification of NOB, numerous small molecules have been identified that target the RORα and RORγ receptors to influence cellular responses to chronic inflammation common in cancer and metabolic syndromes. Diabetic cardiomyopathy is a complication that significantly contributes to morbidity and mortality in diabetics [671]. RORα is significantly downregulated in hearts of diabetic mouse models [606, 672]. Transgenic mice with mutations in RORα exhibit myocardial apoptosis, disruption of autophagy and decreased antioxidant gene expression [606]. Transgenic overexpression of RORα increases cardiac function 8 weeks following streptozocin-induced diabetes. Pharmacological activation of RORα with the SR1078 agonist significantly slows the development of cardiomyopathy by reducing apoptosis of heart cells and fibrosis as well as increasing autophagy in the RORα mutant mice compared to controls [606]. As seen with NOB, the positive effects of the SR1078 RORα agonist appear to extend to cancer models, specifically models of liver cancer [617]. In mouse liver cancer cells, restricting glutamine levels corresponded to an increase in RORα levels, amplifying the cellular responses to oxidative stress and thus reducing proliferation of tumor cells. Administration of SR1078 decreased biosynthetic pathways in these cancer cells reducing glycolysis and increasing *p21* rather than *p53*, thereby inhibiting the growth of the liver cancer cells [617]. Finally, ruscogenins, a third family of compounds targeting RORα, have been shown to reduce the symptoms of pulmonary disease by preventing pulmonary hypertension and remodeling the pulmonary vasculature [618–620]. As with other drugs targeting RORα, ruscogenins increase expression of *Bmal1*. In mice, treatment with a small dose of neoruscogenin for 7 days exerted strong anti-inflammatory effects in the liver significantly upregulating RORα-targeted gene expression of *Bmal1*, *Cyp7b1* and *G6pase* in the liver, making neoruscogenin a potential molecule for regulating inflammation in obesity and hepatic steatosis [673, 674]. Interestingly, inverse agonists of RORγ also appear to mediate

the cellular inflammatory response [450, 624]. Targeting RORγ with an inverse agonist may reduce the adverse symptoms of autoimmune diseases [675]. SR1001 is a strong selective inverse agonist of RORα and RORγ and has been shown to inhibit murine Helper T cell differentiation [621]. Additionally, in a mouse model of multiple sclerosis, treatment with SR1001 was found to delay the onset and severity of experimental autoimmune encephalitis [621]. Altogether, these results highlight the tractability of the ROR nuclear receptors as molecular targets for therapeutic interventions to manage the pathologies associated with age-related diseases.

6 Future Perspectives

Over the past 50 years, innovations in networking, communication and personal electronics have transformed the nature of society driving social and cultural changes. Fueled by urbanization, career pressures, irregular work hours, and other factors, the incidence of circadian and sleep disorders has dramatically risen in countries around the globe. Circadian and sleep disorders have become particularly prominent in young adults, teenagers and even elementary school age children correlated to the exponential rise in the use of smart phones, engagement in social media and social jetlag. Given the prevailing indoor lifestyle and the firm establishment of smartphones and other electronic devices in everyday life, it is unlikely that the incidence of circadian and sleep disorders will decrease as quickly as they have arisen. Advances in circadian research highlight the necessity of proper circadian function for good health. Physiological issues and behavioral patterns established during young adulthood or middle age provide the roots for lifelong chronic conditions and age-related diseases. With the expected rise in the elderly population, identifying behavioral and pharmacological options for long-term management of multiple chronic conditions and age-related diseases will be paramount. Thus, it is essential for continuing research to identify and understand circadian and aging interactions.

Acknowledgments We would like to thank Victoria L. Bagent and Katherine N. Lyons for their assistance with manuscript proofreading and final preparation. Research support provided by a grant from the National Institute of Aging R016062398 (L.C.L.), a grant from the Council on Research and Creativity at Florida State University (L.C.L.) and a Graduate Women in Science National Fellowship (A.K.D.)

References

1. United Nations DoEaSAPD (2017) World population ageing 2017 – highlights (ST/ESA/SER.A/397)
2. Kontis V, Bennett JE, Mathers CD, Li G, Foreman K, Ezzati M (2017) Future life expectancy in 35 industrialised countries: projections with a Bayesian model ensemble. Lancet 389(10076):1323–1335

3. Ortman JM, Velkoff VA, Hogan A (2014) An aging nation: the older population in the United States, population estimates and projections (U.S. Census Bureau), (commerce Do). https://www.census.gov/prod/2014pubs/p25-1140.pdf
4. Vincent GK, Velkoff VA (2010) The next four decades the older population in the United States: 2010 to 2050 (U.S. Census Bureau, economics and statistics administration), (commerce Do). https://www.census.gov/prod/2010pubs/p25-1138.pdf
5. Osborn R, Doty MM, Moulds D, Sarnak DO, Shah A (2017) Older Americans were sicker and faced more financial barriers to health care than counterparts in other countries. Health Aff (Millwood) 36(12):2123–2132
6. Roenneberg T, Merrow M (2016) The circadian clock and human health. Curr Biol 26(10):R432–R443
7. Webb IC, Antle MC, Mistlberger RE (2014) Regulation of circadian rhythms in mammals by behavioral arousal. Behav Neurosci 128(3):304–325
8. Wehrens SMT, Christou S, Isherwood C, Middleton B, Gibbs MA, Archer SN et al (2017) Meal timing regulates the human circadian system. Curr Biol 27(12):1768–1775.e1763
9. Diffey BL (2011) An overview analysis of the time people spend outdoors. Br J Dermatol 164(4):848–854
10. Matz CJ, Stieb DM, Davis K, Egyed M, Rose A, Chou B et al (2014) Effects of age, season, gender and urban-rural status on time-activity: Canadian Human Activity Pattern Survey 2 (CHAPS 2). Int J Environ Res Public Health 11(2):2108–2124
11. Smolensky MH, Sackett-Lundeen LL, Portaluppi F (2015) Nocturnal light pollution and underexposure to daytime sunlight: complementary mechanisms of circadian disruption and related diseases. Chronobiol Int 32(8):1029–1048
12. Lunn RM, Blask DE, Coogan AN, Figueiro MG, Gorman MR, Hall JE et al (2017) Health consequences of electric lighting practices in the modern world: a report on the National Toxicology Program's workshop on shift work at night, artificial light at night, and circadian disruption. Sci Total Environ 607-608:1073–1084
13. Falchi F, Cinzano P, Duriscoe D, Kyba CC, Elvidge CD, Baugh K et al (2016) The new world atlas of artificial night sky brightness. Sci Adv 2(6):e1600377. https://doi.org/10.1126/sciadv.1600377
14. Kyba CCM, Kuester T, Sánchez de Miguel A, Baugh K, Jechow A, Hölker F et al (2017) Artificially lit surface of earth at night increasing in radiance and extent. Sci Adv 3(11):e1701528. https://doi.org/10.1126/sciadv.1701528
15. Ohayon MM, Milesi C (2016) Artificial outdoor nighttime lights associate with altered sleep behavior in the American general population. Sleep 39(6):1311–1320
16. Hattar S, Liao HW, Takao M, Berson DM, Yau KW (2002) Melanopsin-containing retinal ganglion cells: architecture, projections, and intrinsic photosensitivity. Science 295(5557):1065–1070
17. Zeitzer JM, Dijk DJ, Kronauer R, Brown E, Czeisler C (2000) Sensitivity of the human circadian pacemaker to nocturnal light: melatonin phase resetting and suppression. J Physiol 526(Pt 3):695–702
18. Glickman G, Levin R, Brainard GC (2002) Ocular input for human melatonin regulation: relevance to breast cancer. Neuro Endocrinol Lett 23(Suppl 2):17–22
19. Bedrosian TA, Nelson RJ (2017) Timing of light exposure affects mood and brain circuits. Transl Psychiatry 7(1):e1017. https://doi.org/10.1038/tp.2016.262
20. Fonken LK, Workman JL, Walton JC, Weil ZM, Morris JS, Haim A et al (2010) Light at night increases body mass by shifting the time of food intake. Proc Natl Acad Sci U S A 107(43):18664–18669
21. Navara KJ, Nelson RJ (2007) The dark side of light at night: physiological, epidemiological, and ecological consequences. J Pineal Res 43(3):215–224
22. Touitou Y, Reinberg A, Touitou D (2017) Association between light at night, melatonin secretion, sleep deprivation, and the internal clock: health impacts and mechanisms of circadian disruption. Life Sci 173:94–106

23. Hölker F, Wolter C, Perkin EK, Tockner K (2010) Light pollution as a biodiversity threat. Trends Ecol Evol 25(12):681–682
24. Russart KLG, Nelson RJ (2018) Light at night as an environmental endocrine disruptor. Physiol Behav 190:82–89
25. Chang AM, Aeschbach D, Duffy JF, Czeisler CA (2015) Evening use of light-emitting eReaders negatively affects sleep, circadian timing, and next-morning alertness. Proc Natl Acad Sci U S A 112(4):1232–1237
26. Oh JH, Yoo H, Park HK, Do YR (2015) Analysis of circadian properties and healthy levels of blue light from smartphones at night. Sci Rep 5:11325. https://doi.org/10.1038/srep11325
27. Wood B, Rea MS, Plitnick B, Figueiro MG (2013) Light level and duration of exposure determine the impact of self-luminous tablets on melatonin suppression. Appl Ergon 44(2):237–240
28. Gringras P, Middleton B, Skene DJ, Revell VL (2015) Bigger, brighter, bluer-better? Current light-emitting devices - adverse sleep properties and preventative strategies. Front Public Health 3:233. https://doi.org/10.3389/fpubh.2015.00233
29. Cajochen C, Frey S, Anders D, Späti J, Bues M, Pross A et al (2011) Evening exposure to a light-emitting diodes (LED)-backlit computer screen affects circadian physiology and cognitive performance. J Appl Physiol (1985) 110(5):1432–1438
30. Heo JY, Kim K, Fava M, Mischoulon D, Papakostas GI, Kim MJ et al (2017) Effects of smartphone use with and without blue light at night in healthy adults: a randomized, double-blind, cross-over, placebo-controlled comparison. J Psychiatr Res 87:61–70
31. Touitou Y, Touitou D, Reinberg A (2016) Disruption of adolescents' circadian clock: the vicious circle of media use, exposure to light at night, sleep loss and risk behaviors. J Physiol Paris 110(4 Pt B):467–479
32. Alterman T, Luckhaupt SE, Dahlhamer JM, Ward BW, Calvert GM (2013) Job insecurity, work-family imbalance, and hostile work environment: prevalence data from the 2010 National Health Interview Survey. Am J Ind Med 56(6):660–669
33. Alterman T, Luckhaupt SE, Dahlhamer JM, Ward BW, Calvert GM (2013) Prevalence rates of work organization characteristics among workers in the U.S.: data from the 2010 National Health Interview Survey. Am J Ind Med 56(6):647–659
34. Knutson KL, Van Cauter E, Rathouz PJ, DeLeire T, Lauderdale DS (2010) Trends in the prevalence of short sleepers in the USA: 1975-2006. Sleep 33(1):37–45
35. Saad L (2014) The "40-hour" workweek is actually longer -- by seven hours. In: Full-time U.S. workers, on average, report working 47 hours weekly (Gallup). https://news.gallup.com/poll/175286/hour-workweek-actually-longer-seven-hours.aspx
36. Ford ES, Cunningham TJ, Croft JB (2015) Trends in self-reported sleep duration among US Adults from 1985 to 2012. Sleep 38(5):829–832
37. Liu Y, Wheaton AG, Chapman DP, Cunningham TJ, Lu H, Croft JB (2016) Prevalence of healthy sleep duration among adults--United States, 2014. MMWR Morb Mortal Wkly Rep 65(6):137–141
38. National Center for Chronic Disease Prevention and Health Promotion DoPH (2014) Short sleep duration among US Adults. Centers for Disease Control and Prevention
39. Adams RJ, Appleton SL, Taylor AW, Gill TK, Lang C, McEvoy RD et al (2017) Sleep health of Australian adults in 2016: results of the 2016 Sleep Health Foundation national survey. Sleep Health 3(1):35–42
40. Rajaratnam SM, Arendt J (2001) Health in a 24-h society. Lancet 358(9286):999–1005
41. Swanson LM, Arnedt JT, Rosekind MR, Belenky G, Balkin TJ, Drake C (2011) Sleep disorders and work performance: findings from the 2008 National Sleep Foundation Sleep in America poll. J Sleep Res 20(3):487–494
42. Conway SH, Pompeii LA, Gimeno Ruiz de Porras D, Follis JL, Roberts RE (2017) The identification of a threshold of long work hours for predicting elevated risks of adverse health outcomes. Am J Epidemiol 186(2):173–183
43. Fadel M, Sembajwe G, Gagliardi D, Pico F, Li J, Ozguler A et al (2019) Association between reported long working hours and history of stroke in the CONSTANCES cohort. Stroke 50(7):1879–1882

44. Kivimäki M, Jokela M, Nyberg ST, Singh-Manoux A, Fransson EI, Alfredsson L et al (2015) Long working hours and risk of coronary heart disease and stroke: a systematic review and meta-analysis of published and unpublished data for 603,838 individuals. Lancet 386(10005):1739–1746
45. Kivimäki M, Virtanen M, Kawachi I, Nyberg ST, Alfredsson L, Batty GD et al (2015) Long working hours, socioeconomic status, and the risk of incident type 2 diabetes: a meta-analysis of published and unpublished data from 222 120 individuals. Lancet Diabetes Endocrinol 3(1):27–34
46. Luckhaupt SE, Tak S, Calvert GM (2010) The prevalence of short sleep duration by industry and occupation in the National Health Interview Survey. Sleep 33(2):149–159
47. Costa G (2010) Shift work and health: current problems and preventive actions. Saf Health Work 1(2):112–123
48. Costa G, Akerstedt T, Nachreiner F, Baltieri F, Carvalhais J, Folkard S et al (2004) Flexible working hours, health, and well-being in Europe: some considerations from a SALTSA project. Chronobiol Int 21(6):831–844
49. Winkler MR, Mason S, Laska MN, Christoph MJ, Neumark-Sztainer D (2018) Does non-standard work mean non-standard health? Exploring links between non-standard work schedules, health behavior, and well-being. SSM Popul Health 4:135–143
50. Hänecke K, Tiedemann S, Nachreiner F, Grzech-Sukalo H (1998) Accident risk as a function of hour at work and time of day as determined from accident data and exposure models for the German working population. Scand J Work Environ Health 24(Suppl 3):43–48
51. Lombardi DA, Folkard S, Willetts JL, Smith GS (2010) Daily sleep, weekly working hours, and risk of work-related injury: US National Health Interview Survey (2004-2008). Chronobiol Int 27(5):1013–1030
52. Golombek DA, Casiraghi LP, Agostino PV, Paladino N, Duhart JM, Plano SA et al (2013) The times they're a-changing: effects of circadian desynchronization on physiology and disease. J Physiol Paris 107(4):310–322
53. Schlosser Covell GE, Dhawan PS, Lee Iannotti JK, Hoffman-Snyder CR, Wellik KE, Caselli RJ et al (2012) Disrupted daytime activity and altered sleep-wake patterns may predict transition to mild cognitive impairment or dementia: a critically appraised topic. Neurologist 18(6):426–429
54. Åkerstedt T, Hallvig D, Kecklund G (2017) Normative data on the diurnal pattern of the Karolinska Sleepiness Scale ratings and its relation to age, sex, work, stress, sleep quality and sickness absence/illness in a large sample of daytime workers. J Sleep Res 26(5):559–566
55. Åkerstedt T, Kecklund G (2017) What work schedule characteristics constitute a problem to the individual? A representative study of Swedish shift workers. Appl Ergon 59(Pt A):320–325
56. Åkerstedt T, Narusyte J, Svedberg P, Kecklund G, Alexanderson K (2017) Night work and prostate cancer in men: a Swedish prospective cohort study. BMJ Open 7(6):e015751. https://doi.org/10.1136/bmjopen-2016-015751
57. Koritala BSC, Çakmaklı S (2018) The human circadian clock from health to economics. Psych J 7(4):176–196
58. Roenneberg T, Allebrandt KV, Merrow M, Vetter C (2012) Social jetlag and obesity. Curr Biol 22(10):939–943
59. Wittmann M, Paulus M, Roenneberg T (2010) Decreased psychological well-being in late 'chronotypes' is mediated by smoking and alcohol consumption. Subst Use Misuse. 45 (1-2): 15–30.
60. Koopman ADM, Rauh SP, van't Riet E, Groeneveld L, van der Heijden AA, Elders PJ et al (2017) The association between social jetlag, the metabolic syndrome, and type 2 diabetes mellitus in the general population: the New Hoorn Study. J Biol Rhythm 32(4):359–368
61. Rutters F, Lemmens SG, Adam TC, Bremmer MA, Elders PJ, Nijpels G et al (2014) Is social jetlag associated with an adverse endocrine, behavioral, and cardiovascular risk profile? J Biol Rhythm 29(5):377–383

62. Wong PM, Hasler BP, Kamarck TW, Muldoon MF, Manuck SB (2015) Social jetlag, chronotype, and cardiometabolic risk. J Clin Endocrinol Metab 100(12):4612–4620
63. Stoner L, Castro N, Signal L, Skidmore P, Faulkner J, Lark S et al (2018) Sleep and adiposity in preadolescent children: the importance of social jetlag. Child Obes 14(3):158–164
64. Balsalobre A, Damiola F, Schibler U (1998) A serum shock induces circadian gene expression in mammalian tissue culture cells. Cell 93(6):929–937
65. Nagoshi E, Saini C, Bauer C, Laroche T, Naef F, Schibler U (2004) Circadian gene expression in individual fibroblasts: cell-autonomous and self-sustained oscillators pass time to daughter cells. Cell 119(5):693–705
66. Welsh DK, Yoo SH, Liu AC, Takahashi JS, Kay SA (2004) Bioluminescence imaging of individual fibroblasts reveals persistent, independently phased circadian rhythms of clock gene expression. Curr Biol 14(24):2289–2295
67. Yoo SH, Yamazaki S, Lowrey PL, Shimomura K, Ko CH, Buhr ED et al (2004) PERIOD2::LUCIFERASE real-time reporting of circadian dynamics reveals persistent circadian oscillations in mouse peripheral tissues. Proc Natl Acad Sci U S A 101(15):5339–5346
68. Eastman C, Rechtschaffen A (1983) Circadian temperature and wake rhythms of rats exposed to prolonged continuous illumination. Physiol Behav 31(4):417–427
69. Mohawk JA, Green CB, Takahashi JS (2012) Central and peripheral circadian clocks in mammals. Annu Rev Neurosci 35:445–462
70. Reppert SM, Weaver DR (2002) Coordination of circadian timing in mammals. Nature 418(6901):935–941
71. Yamazaki S, Numano R, Abe M, Hida A, Takahashi R, Ueda M et al (2000) Resetting central and peripheral circadian oscillators in transgenic rats. Science 288(5466):682–685
72. Freedman MS, Lucas RJ, Soni B, von Schantz M, Muñoz M, David-Gray Z et al (1999) Regulation of mammalian circadian behavior by non-rod, non-cone, ocular photoreceptors. Science 284(5413):502–504
73. Hattar S, Lucas RJ, Mrosovsky N, Thompson S, Douglas RH, Hankins MW et al (2003) Melanopsin and rod-cone photoreceptive systems account for all major accessory visual functions in mice. Nature 424(6944):76–81
74. Provencio I, Jiang G, De Grip WJ, Hayes WP, Rollag MD (1998) Melanopsin: an opsin in melanophores, brain, and eye. Proc Natl Acad Sci U S A 95(1):340–345
75. Berson DM, Dunn FA, Takao M (2002) Phototransduction by retinal ganglion cells that set the circadian clock. Science 295(5557):1070–1073
76. Moore RY, Eichler VB (1972) Loss of a circadian adrenal corticosterone rhythm following suprachiasmatic lesions in the rat. Brain Res 42(1):201–206
77. Tei H, Okamura H, Shigeyoshi Y, Fukuhara C, Ozawa R, Hirose M et al (1997) Circadian oscillation of a mammalian homologue of the Drosophila period gene. Nature 389(6650):512–516
78. Aton SJ, Colwell CS, Harmar AJ, Waschek J, Herzog ED (2005) Vasoactive intestinal polypeptide mediates circadian rhythmicity and synchrony in mammalian clock neurons. Nat Neurosci 8(4):476–483
79. Harmar AJ, Marston HM, Shen S, Spratt C, West KM, Sheward WJ et al (2002) The VPAC(2) receptor is essential for circadian function in the mouse suprachiasmatic nuclei. Cell 109(4):497–508
80. Mieda M, Okamoto H, Sakurai T (2016) Manipulating the cellular circadian period of arginine vasopressin neurons alters the behavioral circadian period. Curr Biol 26(18):2535–2542
81. Mieda M, Ono D, Hasegawa E, Okamoto H, Honma K, Honma S et al (2015) Cellular clocks in AVP neurons of the SCN are critical for interneuronal coupling regulating circadian behavior rhythm. Neuron 85(5):1103–1116
82. Park J, Zhu H, O'Sullivan S, Ogunnaike BA, Weaver DR, Schwaber JS et al (2016) Single-cell transcriptional analysis reveals novel neuronal phenotypes and interaction networks involved in the central circadian clock. Front Neurosci 10:481. https://doi.org/10.3389/fnins.2016.00481
83. Herzog ED, Aton SJ, Numano R, Sakaki Y, Tei H (2004) Temporal precision in the mammalian circadian system: a reliable clock from less reliable neurons. J Biol Rhythm 19(1):35–46

84. Ko CH, Yamada YR, Welsh DK, Buhr ED, Liu AC, Zhang EE et al (2010) Emergence of noise-induced oscillations in the central circadian pacemaker. PLoS Biol 8(10):e1000513. https://doi.org/10.1371/journal.pbio.1000513
85. Liu C, Weaver DR, Strogatz SH, Reppert SM (1997) Cellular construction of a circadian clock: period determination in the suprachiasmatic nuclei. Cell 91(6):855–860
86. Welsh DK, Logothetis DE, Meister M, Reppert SM (1995) Individual neurons dissociated from rat suprachiasmatic nucleus express independently phased circadian firing rhythms. Neuron 14(4):697–706
87. Yamaguchi S, Isejima H, Matsuo T, Okura R, Yagita K, Kobayashi M et al (2003) Synchronization of cellular clocks in the suprachiasmatic nucleus. Science 302(5649):1408–1412
88. Abrahamson EE, Moore RY (2001) Suprachiasmatic nucleus in the mouse: retinal innervation, intrinsic organization and efferent projections. Brain Res 916(1–2):172–191
89. Abrahamson EE, Moore RY (2001) The posterior hypothalamic area: chemoarchitecture and afferent connections. Brain Res 889(1–2):1–22
90. Mai JK, Kedziora O, Teckhaus L, Sofroniew MV (1991) Evidence for subdivisions in the human suprachiasmatic nucleus. J Comp Neurol 305(3):508–525
91. Mieda M (2019) The network mechanism of the central circadian pacemaker of the SCN: do AVP neurons play a more critical role than expected? Front Neurosci 13:139. https://doi.org/10.3389/fnins.2019.00139
92. Leak RK, Card JP, Moore RY (1999) Suprachiasmatic pacemaker organization analyzed by viral transynaptic transport. Brain Res 819(1–2):23–32
93. Guzmán-Ruiz M, Saderi N, Cazarez-Márquez F, Guerrero-Vargas NN, Basualdo MC, Acosta-Galván G et al (2014) The suprachiasmatic nucleus changes the daily activity of the arcuate nucleus α-MSH neurons in male rats. Endocrinology 155(2):525–535
94. Myers MG, Olson DP (2012) Central nervous system control of metabolism. Nature 491(7424):357–363
95. Williams KW, Elmquist JK (2012) From neuroanatomy to behavior: central integration of peripheral signals regulating feeding behavior. Nat Neurosci 15(10):1350–1355
96. Chao PT, Yang L, Aja S, Moran TH, Bi S (2011) Knockdown of NPY expression in the dorsomedial hypothalamus promotes development of brown adipocytes and prevents diet-induced obesity. Cell Metab 13(5):573–583
97. Wiater MF, Mukherjee S, Li AJ, Dinh TT, Rooney EM, Simasko SM et al (2011) Circadian integration of sleep-wake and feeding requires NPY receptor-expressing neurons in the mediobasal hypothalamus. Am J Physiol Regul Integr Comp Physiol 301(5):R1569–R1583
98. Akabayashi A, Levin N, Paez X, Alexander JT, Leibowitz SF (1994) Hypothalamic neuropeptide Y and its gene expression: relation to light/dark cycle and circulating corticosterone. Mol Cell Neurosci 5(3):210–218
99. Akabayashi A, Wahlestedt C, Alexander JT, Leibowitz SF (1994) Specific inhibition of endogenous neuropeptide Y synthesis in arcuate nucleus by antisense oligonucleotides suppresses feeding behavior and insulin secretion. Brain Res Mol Brain Res 21(1–2):55–61
100. Li AJ, Wiater MF, Oostrom MT, Smith BR, Wang Q, Dinh TT et al (2012) Leptin-sensitive neurons in the arcuate nuclei contribute to endogenous feeding rhythms. Am J Physiol Regul Integr Comp Physiol 302(11):R1313–R1326
101. Xu B, Kalra PS, Farmerie WG, Kalra SP (1999) Daily changes in hypothalamic gene expression of neuropeptide Y, galanin, proopiomelanocortin, and adipocyte leptin gene expression and secretion: effects of food restriction. Endocrinology 140(6):2868–2875
102. Chou TC, Scammell TE, Gooley JJ, Gaus SE, Saper CB, Lu J (2003) Critical role of dorsomedial hypothalamic nucleus in a wide range of behavioral circadian rhythms. J Neurosci 23(33):10691–10702
103. Deurveilher S, Semba K (2005) Indirect projections from the suprachiasmatic nucleus to major arousal-promoting cell groups in rat: implications for the circadian control of behavioural state. Neuroscience 130(1):165–183

104. Greco CM, Sassone-Corsi P (2019) Circadian blueprint of metabolic pathways in the brain. Nat Rev Neurosci 20(2):71–82
105. Hastings MH, Maywood ES, Brancaccio M (2019) The mammalian circadian timing system and the suprachiasmatic nucleus as its pacemaker. Biology (Basel) 8(1). pii: E13. https://doi.org/10.3390/biology8010013
106. Honma S, Ikeda M, Abe H, Tanahashi Y, Namihira M, Honma K et al (1998) Circadian oscillation of BMAL1, a partner of a mammalian clock gene clock, in rat suprachiasmatic nucleus. Biochem Biophys Res Commun 250(1):83–87
107. Herzog ED (2007) Neurons and networks in daily rhythms. Nat Rev Neurosci 8(10):790–802
108. O'Neill JS, Maywood ES, Hastings MH (2013) Cellular mechanisms of circadian pacemaking: beyond transcriptional loops. Handb Exp Pharmacol 217:67–103
109. Bunger MK, Wilsbacher LD, Moran SM, Clendenin C, Radcliffe LA, Hogenesch JB et al (2000) Mop3 is an essential component of the master circadian pacemaker in mammals. Cell 103(7):1009–1017
110. Gekakis N, Staknis D, Nguyen HB, Davis FC, Wilsbacher LD, King DP et al (1998) Role of the CLOCK protein in the mammalian circadian mechanism. Science 280(5369):1564–1569
111. Hogenesch JB, Gu YZ, Jain S, Bradfield CA (1998) The basic-helix-loop-helix-PAS orphan MOP3 forms transcriptionally active complexes with circadian and hypoxia factors. Proc Natl Acad Sci U S A 95(10):5474–5479
112. King DP, Zhao Y, Sangoram AM, Wilsbacher LD, Tanaka M, Antoch MP et al (1997) Positional cloning of the mouse circadian clock gene. Cell 89(4):641–653
113. Kloss B, Price JL, Saez L, Blau J, Rothenfluh A, Wesley CS et al (1998) The Drosophila clock gene double-time encodes a protein closely related to human casein kinase Iepsilon. Cell 94(1):97–107
114. Jin X, Shearman LP, Weaver DR, Zylka MJ, de Vries GJ, Reppert SM (1999) A molecular mechanism regulating rhythmic output from the suprachiasmatic circadian clock. Cell 96(1):57–68
115. Albrecht U, Sun ZS, Eichele G, Lee CC (1997) A differential response of two putative mammalian circadian regulators, mper1 and mper2, to light. Cell 91(7):1055–1064
116. Lee C, Etchegaray JP, Cagampang FR, Loudon AS, Reppert SM (2001) Posttranslational mechanisms regulate the mammalian circadian clock. Cell 107(7):855–867
117. Kume K, Zylka MJ, Sriram S, Shearman LP, Weaver DR, Jin X et al (1999) mCRY1 and mCRY2 are essential components of the negative limb of the circadian clock feedback loop. Cell 98(2):193–205
118. Vitaterna MH, Selby CP, Todo T, Niwa H, Thompson C, Fruechte EM et al (1999) Differential regulation of mammalian period genes and circadian rhythmicity by cryptochromes 1 and 2. Proc Natl Acad Sci U S A 96(21):12114–12119
119. Horwitz BA, Gressel J, Malkin S, Epel BL (1985) Modified cryptochrome in vivo absorption in dim photosporulation mutants of Trichoderma. Proc Natl Acad Sci U S A 82(9):2736–2740
120. Griffin EA, Staknis D, Weitz CJ (1999) Light-independent role of CRY1 and CRY2 in the mammalian circadian clock. Science 286(5440):768–771
121. Sponga F, Deitzer GF, Mancinelli AL (1986) Cryptochrome, phytochrome, and the photoregulation of anthocyanin production under blue light. Plant Physiol 82(4):952–955
122. Ahmad M, Cashmore AR (1993) HY4 gene of A. thaliana encodes a protein with characteristics of a blue-light photoreceptor. Nature 366(6451):162–166
123. Ahmad M, Cashmore AR (1996) Seeing blue: the discovery of cryptochrome. Plant Mol Biol 30(5):851–861
124. Ahmad M, Lin C, Cashmore AR (1995) Mutations throughout an Arabidopsis blue-light photoreceptor impair blue-light-responsive anthocyanin accumulation and inhibition of hypocotyl elongation. Plant J 8(5):653–658
125. Selby CP, Sancar A (2006) A cryptochrome/photolyase class of enzymes with single-stranded DNA-specific photolyase activity. Proc Natl Acad Sci U S A 103(47):17696–17700

126. Eide EJ, Woolf MF, Kang H, Woolf P, Hurst W, Camacho F et al (2005) Control of mammalian circadian rhythm by CKIepsilon-regulated proteasome-mediated PER2 degradation. Mol Cell Biol 25(7):2795–2807
127. Hirano A, Yumimoto K, Tsunematsu R, Matsumoto M, Oyama M, Kozuka-Hata H et al (2013) FBXL21 regulates oscillation of the circadian clock through ubiquitination and stabilization of cryptochromes. Cell 152(5):1106–1118
128. Koike N, Yoo SH, Huang HC, Kumar V, Lee C, Kim TK et al (2012) Transcriptional architecture and chromatin landscape of the core circadian clock in mammals. Science 338(6105):349–354
129. Brown SA, Ripperger J, Kadener S, Fleury-Olela F, Vilbois F, Rosbash M et al (2005) PERIOD1-associated proteins modulate the negative limb of the mammalian circadian oscillator. Science 308(5722):693–696
130. Kim JY, Kwak PB, Gebert M, Duong HA, Weitz CJ (2015) Purification and analysis of PERIOD protein complexes of the mammalian circadian clock. Methods Enzymol 551:197–210
131. Partch CL, Green CB, Takahashi JS (2014) Molecular architecture of the mammalian circadian clock. Trends Cell Biol 24(2):90–99
132. Ye R, Selby CP, Chiou YY, Ozkan-Dagliyan I, Gaddameedhi S, Sancar A (2014) Dual modes of CLOCK:BMAL1 inhibition mediated by cryptochrome and period proteins in the mammalian circadian clock. Genes Dev 28(18):1989–1998
133. DeBruyne JP, Weaver DR, Reppert SM (2007) CLOCK and NPAS2 have overlapping roles in the suprachiasmatic circadian clock. Nat Neurosci 10(5):543–545
134. DeBruyne JP, Weaver DR, Reppert SM (2007) Peripheral circadian oscillators require CLOCK. Curr Biol 17(14):R538–R539
135. Reick M, Garcia JA, Dudley C, McKnight SL (2001) NPAS2: an analog of clock operative in the mammalian forebrain. Science 293(5529):506–509
136. Landgraf D, Wang LL, Diemer T, Welsh DK (2016) NPAS2 compensates for loss of CLOCK in peripheral circadian oscillators. PLoS Genet 12(2):e1005882. https://doi.org/10.1371/journal.pgen.1005882
137. McNamara P, Seo SB, Rudic RD, Sehgal A, Chakravarti D, FitzGerald GA (2001) Regulation of CLOCK and MOP4 by nuclear hormone receptors in the vasculature: a humoral mechanism to reset a peripheral clock. Cell 105(7):877–889
138. Crumbley C, Burris TP (2011) Direct regulation of CLOCK expression by REV-ERB. PLoS One 6(3):e17290. https://doi.org/10.1371/journal.pone.0017290
139. Preitner N, Damiola F, Lopez-Molina L, Zakany J, Duboule D, Albrecht U et al (2002) The orphan nuclear receptor REV-ERBalpha controls circadian transcription within the positive limb of the mammalian circadian oscillator. Cell 110(2):251–260
140. Zhang Y, Fang B, Emmett MJ, Damle M, Sun Z, Feng D et al (2015) GENE REGULATION. Discrete functions of nuclear receptor Rev-erbα couple metabolism to the clock. Science 348(6242):1488–1492
141. Ueda HR, Hayashi S, Chen W, Sano M, Machida M, Shigeyoshi Y et al (2005) System-level identification of transcriptional circuits underlying mammalian circadian clocks. Nat Genet 37(2):187–192
142. Feng D, Liu T, Sun Z, Bugge A, Mullican SE, Alenghat T et al (2011) A circadian rhythm orchestrated by histone deacetylase 3 controls hepatic lipid metabolism. Science 331(6022):1315–1319
143. Sato TK, Panda S, Miraglia LJ, Reyes TM, Rudic RD, McNamara P et al (2004) A functional genomics strategy reveals Rora as a component of the mammalian circadian clock. Neuron 43(4):527–537
144. Guillaumond F, Dardente H, Giguère V, Cermakian N (2005) Differential control of Bmal1 circadian transcription by REV-ERB and ROR nuclear receptors. J Biol Rhythm 20(5):391–403

145. Asher G, Schibler U (2011) Crosstalk between components of circadian and metabolic cycles in mammals. Cell Metab 13(2):125–137
146. Bozek K, Relógio A, Kielbasa SM, Heine M, Dame C, Kramer A et al (2009) Regulation of clock-controlled genes in mammals. PLoS One 4(3):e4882. https://doi.org/10.1371/journal.pone.0004882
147. Lehmann R, Machné R, Herzel H (2014) The structural code of cyanobacterial genomes. Nucleic Acids Res 42(14):8873–8883
148. Sato S, Solanas G, Peixoto FO, Bee L, Symeonidi A, Schmidt MS et al (2017) Circadian reprogramming in the liver identifies metabolic pathways of aging. Cell 170(4):664–677. e611. https://doi.org/10.1016/j.cell.2017.07.042
149. Ko CH, Takahashi JS (2006) Molecular components of the mammalian circadian clock. Hum Mol Genet 15 Spec No 2:R271–R277
150. Hirota T, Lewis WG, Liu AC, Lee JW, Schultz PG, Kay SA (2008) A chemical biology approach reveals period shortening of the mammalian circadian clock by specific inhibition of GSK-3beta. Proc Natl Acad Sci U S A 105(52):20746–20751
151. Yoshii T, Hermann-Luibl C, Helfrich-Förster C (2016) Circadian light-input pathways in Drosophila. Commun Integr Biol 9(1):e1102805. https://doi.org/10.1080/19420889.2015.1102805
152. Tataroglu O, Emery P (2015) The molecular ticks of the Drosophila circadian clock. Curr Opin Insect Sci 7:51–57
153. Mendoza-Viveros L, Bouchard-Cannon P, Hegazi S, Cheng AH, Pastore S, Cheng HM (2017) Molecular modulators of the circadian clock: lessons from flies and mice. Cell Mol Life Sci 74(6):1035–1059
154. Carmona-Alcocer V, Rohr KE, Joye DAM, Evans JA (2018) Circuit development in the master clock network of mammals. Eur J Neurosci. https://doi.org/10.1111/ejn.14259. [Epub ahead of print]
155. Hegazi S, Lowden C, Rios Garcia J, Cheng AH, Obrietan K, Levine JD et al (2019) A symphony of signals: intercellular and intracellular signaling mechanisms underlying circadian timekeeping in mice and flies. Int J Mol Sci 20(9). pii: E2363. https://doi.org/10.3390/ijms20092363
156. Kozlov A, Nagoshi E (2019) Decoding Drosophila circadian pacemaker circuit. Curr Opin Insect Sci 36:33–38
157. Artiushin G, Sehgal A (2017) The Drosophila circuitry of sleep-wake regulation. Curr Opin Neurobiol 4:243–250
158. Cha J, Zhou M, Liu Y (2015) Methods to study molecular mechanisms of the Neurospora circadian clock. Methods Enzymol 551:137–151
159. Cha J, Zhou M, Liu Y (2015) Mechanism of the Neurospora circadian clock, a FREQUENCY-centric view. Biochemistry 54(2):150–156
160. Hurley J, Loros JJ, Dunlap JC (2015) Dissecting the mechanisms of the clock in Neurospora. Methods Enzymol 551:29–52
161. Hurley JH, Dasgupta A, Andrews P, Crowell AM, Ringelberg C, Loros JJ et al (2015) A tool set for the genome-wide analysis of Neurospora crassa by RT-PCR. G3 (Bethesda) 5(10):2043–2049
162. Jarabo P, Martin FA (2017) Neurogenetics of Drosophila circadian clock: expect the unexpected. J Neurogenet 31(4):250–265
163. Dubowy C, Sehgal A (2017) Circadian rhythms and sleep in Drosophila melanogaster. Genetics 205(4):1373–1397
164. Franco DL, Frenkel L, Ceriani MF (2018) The underlying genetics of Drosophila circadian behaviors. Physiology (Bethesda) 33(1):50–62
165. He Q, Wu B, Price JL, Zhao Z (2017) Circadian rhythm neuropeptides in Drosophila: signals for normal circadian function and circadian neurodegenerative disease. Int J Mol Sci 18(4). pii: E886. https://doi.org/10.3390/ijms18040886

166. Glossop NR, Lyons LC, Hardin PE (1999) Interlocked feedback loops within the Drosophila circadian oscillator. Science 286(5440):766–768
167. Reppert SM, Sauman I (1995) Period and timeless tango: a dance of two clock genes. Neuron 15(5):983–986
168. Sehgal A, Price JL, Man B, Young MW (1994) Loss of circadian behavioral rhythms and per RNA oscillations in the Drosophila mutant timeless. Science 263(5153):1603–1606
169. Price JL, Blau J, Rothenfluh A, Abodeely M, Kloss B, Young MW (1998) Double-time is a novel Drosophila clock gene that regulates PERIOD protein accumulation. Cell 94(1):83–95
170. Rutila JE, Suri V, Le M, So WV, Rosbash M, Hall JC (1998) CYCLE is a second bHLH-PAS clock protein essential for circadian rhythmicity and transcription of Drosophila period and timeless. Cell 93(5):805–814
171. Abruzzi KC, Rodriguez J, Menet JS, Desrochers J, Zadina A, Luo W et al (2011) Drosophila CLOCK target gene characterization: implications for circadian tissue-specific gene expression. Genes Dev 25(22):2374–2386
172. Menet JS, Abruzzi KC, Desrochers J, Rodriguez J, Rosbash M (2010) Dynamic PER repression mechanisms in the Drosophila circadian clock: from on-DNA to off-DNA. Genes Dev 24(4):358–367
173. Ozturk N, Selby CP, Annayev Y, Zhong D, Sancar A (2011) Reaction mechanism of Drosophila cryptochrome. Proc Natl Acad Sci U S A 108(2):516–521
174. Stanewsky R, Kaneko M, Emery P, Beretta B, Wager-Smith K, Kay SA et al (1998) The cryb mutation identifies cryptochrome as a circadian photoreceptor in Drosophila. Cell 95(5):681–692
175. Benito J, Houl JH, Roman GW, Hardin PE (2008) The blue-light photoreceptor CRYPTOCHROME is expressed in a subset of circadian oscillator neurons in the Drosophila CNS. J Biol Rhythm 23(4):296–307
176. Collins B, Mazzoni EO, Stanewsky R, Blau J (2006) Drosophila CRYPTOCHROME is a circadian transcriptional repressor. Curr Biol 16(5):441–449
177. Buijs FN, León-Mercado L, Guzmán-Ruiz M, Guerrero-Vargas NN, Romo-Nava F, Buijs RM (2016) The circadian system: a regulatory feedback network of periphery and brain. Physiology (Bethesda) 31(3):170–181
178. Buijs RM, Kalsbeek A (2001) Hypothalamic integration of central and peripheral clocks. Nat Rev Neurosci 2(7):521–526
179. Yuan XS, Wei HH, Xu W, Wang L, Qu WM, Li RX et al (2018) Whole-brain monosynaptic afferent projections to the cholecystokinin neurons of the suprachiasmatic nucleus. Front Neurosci 12:807. https://doi.org/10.3389/fnins.2018.00807
180. Damiola F, Le Minh N, Preitner N, Kornmann B, Fleury-Olela F, Schibler U (2000) Restricted feeding uncouples circadian oscillators in peripheral tissues from the central pacemaker in the suprachiasmatic nucleus. Genes Dev 14(23):2950–2961
181. Buijs RM, la Fleur SE, Wortel J, Van Heyningen C, Zuiddam L, Mettenleiter TC et al (2003) The suprachiasmatic nucleus balances sympathetic and parasympathetic output to peripheral organs through separate preautonomic neurons. J Comp Neurol 464(1):36–48
182. Buijs RM, van Eden CG, Goncharuk VD, Kalsbeek A (2003) The biological clock tunes the organs of the body: timing by hormones and the autonomic nervous system. J Endocrinol 177(1):17–26
183. Kalsbeek A, Yi CX, la Fleur SE, Buijs RM, Fliers E (2010) Suprachiasmatic nucleus and autonomic nervous system influences on awakening from sleep. Int Rev Neurobiol 93:91–107
184. Ueyama T, Krout KE, Nguyen XV, Karpitskiy V, Kollert A, Mettenleiter TC et al (1999) Suprachiasmatic nucleus: a central autonomic clock. Nat Neurosci 2(12):1051–1053
185. LeSauter J, Romero P, Cascio M, Silver R (1997) Attachment site of grafted SCN influences precision of restored circadian rhythm. J Biol Rhythm 12(4):327–338
186. Meyer-Bernstein EL, Morin LP (1999) Electrical stimulation of the median or dorsal raphe nuclei reduces light-induced FOS protein in the suprachiasmatic nucleus and causes circadian activity rhythm phase shifts. Neuroscience 92(1):267–279

187. Lehman MN, Lesauter J, Silver R (1998) Fiber outgrowth from anterior hypothalamic and cortical xenografts in the third ventricle. J Comp Neurol 391(1):133–145
188. Lehman MN, Silver R, Gladstone WR, Kahn RM, Gibson M, Bittman EL (1987) Circadian rhythmicity restored by neural transplant. Immunocytochemical characterization of the graft and its integration with the host brain. J Neurosci 7(6):1626–1638
189. LeSauter J, Lehman MN, Silver R (1996) Restoration of circadian rhythmicity by transplants of SCN "micropunches". J Biol Rhythm 11(2):163–171
190. Guo H, Brewer JM, Champhekar A, Harris RB, Bittman EL (2005) Differential control of peripheral circadian rhythms by suprachiasmatic-dependent neural signals. Proc Natl Acad Sci U S A 102(8):3111–3116
191. Kaneko M, Hiroshige T, Shinsako J, Dallman MF (1980) Diurnal changes in amplification of hormone rhythms in the adrenocortical system. Am J Phys 239(3):R309–R316
192. Kaneko M, Kaneko K, Shinsako J, Dallman MF (1981) Adrenal sensitivity to adrenocorticotropin varies diurnally. Endocrinology 109(1):70–75
193. Oster H, Damerow S, Kiessling S, Jakubcakova V, Abraham D, Tian J et al (2006) The circadian rhythm of glucocorticoids is regulated by a gating mechanism residing in the adrenal cortical clock. Cell Metab 4(2):163–173
194. Simpson ER, Waterman MR (1988) Regulation of the synthesis of steroidogenic enzymes in adrenal cortical cells by ACTH. Annu Rev Physiol 50:427–440
195. Balsalobre A, Marcacci L, Schibler U (2000) Multiple signaling pathways elicit circadian gene expression in cultured Rat-1 fibroblasts. Curr Biol 10(20):1291–1294
196. Reddy AB, Maywood ES (2007) Circadian rhythms: per2bations in the liver clock. Curr Biol 17(8):R292–R294
197. Reddy AB, Maywood ES, Karp NA, King VM, Inoue Y, Gonzalez FJ et al (2007) Glucocorticoid signaling synchronizes the liver circadian transcriptome. Hepatology 45(6):1478–1488
198. So AY, Bernal TU, Pillsbury ML, Yamamoto KR, Feldman BJ (2009) Glucocorticoid regulation of the circadian clock modulates glucose homeostasis. Proc Natl Acad Sci U S A 106(41):17582–17587
199. Yamamoto T, Nakahata Y, Tanaka M, Yoshida M, Soma H, Shinohara K et al (2005) Acute physical stress elevates mouse period1 mRNA expression in mouse peripheral tissues via a glucocorticoid-responsive element. J Biol Chem 280(51):42036–42043
200. Buijs RM, Wortel J, Van Heerikhuize JJ, Feenstra MG, Ter Horst GJ, Romijn HJ et al (1999) Anatomical and functional demonstration of a multisynaptic suprachiasmatic nucleus adrenal (cortex) pathway. Eur J Neurosci 11(5):1535–1544
201. Mahoney MM, Ramanathan C, Hagenauer MH, Thompson RC, Smale L, Lee T (2009) Daily rhythms and sex differences in vasoactive intestinal polypeptide, VIPR2 receptor and arginine vasopressin mRNA in the suprachiasmatic nucleus of a diurnal rodent, Arvicanthis niloticus. Eur J Neurosci 30(8):1537–1543
202. Son GH, Chung S, Choe HK, Kim HD, Baik SM, Lee H et al (2008) Adrenal peripheral clock controls the autonomous circadian rhythm of glucocorticoid by causing rhythmic steroid production. Proc Natl Acad Sci U S A 105(52):20970–20975
203. Balsalobre A, Marcacci L, Schibler U (2000) Multiple signaling pathways elicit circadian gene expression in cultured Rat-1 fibroblasts. Curr Biol 10 (20):1291-1294
204. Rosenfeld P, Van Eekelen JA, Levine S, De Kloet ER (1988) Ontogeny of the type 2 glucocorticoid receptor in discrete rat brain regions: an immunocytochemical study. Brain Res 470(1):119–127
205. Rosenfeld P, van Eekelen JA, Levine S, de Kloet ER (1993) Ontogeny of corticosteroid receptors in the brain. Cell Mol Neurobiol 13(4):295–319
206. Caratti G, Iqbal M, Hunter L, Kim D, Wang P, Vonslow RM et al (2018) REVERBa couples the circadian clock to hepatic glucocorticoid action. J Clin Invest 128(10):4454–4471
207. Schmutz I, Ripperger JA, Baeriswyl-Aebischer S, Albrecht U (2010) The mammalian clock component PERIOD2 coordinates circadian output by interaction with nuclear receptors. Genes Dev 24(4):345–357

208. Wams EJ, Riede S, van der Laan I, Bulte TT, Hut RA (2017) Mechanisms of non-photic entrainment. In: Biological timekeeping: clocks, rhythms and behavior, 1st edn. Springer, New Delhi. ISBN-10: 9788132236863
209. Stokkan KA, Yamazaki S, Tei H, Sakaki Y, Menaker M (2001) Entrainment of the circadian clock in the liver by feeding. Science 291(5503):490–493
210. Cuninkova L, Brown SA (2008) Peripheral circadian oscillators: interesting mechanisms and powerful tools. Ann N Y Acad Sci 1129:358–370
211. Le Minh N, Damiola F, Tronche F, Schütz G, Schibler U (2001) Glucocorticoid hormones inhibit food-induced phase-shifting of peripheral circadian oscillators. EMBO J 20(24):7128–7136
212. Krieger DT, Hauser H, Krey LC (1977) Suprachiasmatic nuclear lesions do not abolish food-shifted circadian adrenal and temperature rhythmicity. Science 197(4301):398–399
213. Stephan FK, Swann JM, Sisk CL (1979) Entrainment of circadian rhythms by feeding schedules in rats with suprachiasmatic lesions. Behav Neural Biol 25(4):545–554
214. Stephan FK, Swann JM, Sisk CL (1979) Anticipation of 24-hr feeding schedules in rats with lesions of the suprachiasmatic nucleus. Behav Neural Biol 25(3):346–363
215. Stephan FK (2002) The "other" circadian system: food as a Zeitgeber. J Biol Rhythm 17(4):284–292
216. Hara R, Wan K, Wakamatsu H, Aida R, Moriya T, Akiyama M et al (2001) Restricted feeding entrains liver clock without participation of the suprachiasmatic nucleus. Genes Cells 6(3):269–278
217. Pezuk P, Mohawk JA, Yoshikawa T, Sellix MT, Menaker M (2010) Circadian organization is governed by extra-SCN pacemakers. J Biol Rhythm 25(6):432–441
218. Konturek SJ, Konturek JW, Pawlik T, Brzozowski T (2004) Brain-gut axis and its role in the control of food intake. J Physiol Pharmacol 55(1 Pt 2):137–154
219. Nakahata Y, Sahar S, Astarita G, Kaluzova M, Sassone-Corsi P (2009) Circadian control of the NAD+ salvage pathway by CLOCK-SIRT1. Science 324(5927):654–657
220. Ramsey KM, Yoshino J, Brace CS, Abrassart D, Kobayashi Y, Marcheva B et al (2009) Circadian clock feedback cycle through NAMPT-mediated NAD+ biosynthesis. Science 324(5927):651–654
221. Mukherji A, Kobiita A, Chambon P (2015) Shifting the feeding of mice to the rest phase creates metabolic alterations, which, on their own, shift the peripheral circadian clocks by 12 hours. Proc Natl Acad Sci U S A 112(48):E6683–E6690
222. Mukherji A, Kobiita A, Damara M, Misra N, Meziane H, Champy MF et al (2015) Shifting eating to the circadian rest phase misaligns the peripheral clocks with the master SCN clock and leads to a metabolic syndrome. Proc Natl Acad Sci U S A 112(48):E6691–E6698
223. Asher G, Sassone-Corsi P (2015) Time for food: the intimate interplay between nutrition, metabolism, and the circadian clock. Cell 161(1):84–92
224. Kentish SJ, Vincent AD, Kennaway DJ, Wittert GA, Page AJ (2016) High-fat diet-induced obesity ablates gastric vagal afferent circadian rhythms. J Neurosci 36(11):3199–3207
225. Kohsaka A, Laposky AD, Ramsey KM, Estrada C, Joshu C, Kobayashi Y et al (2007) High-fat diet disrupts behavioral and molecular circadian rhythms in mice. Cell Metab 6(5):414–421
226. Turek FW, Joshu C, Kohsaka A, Lin E, Ivanova G, McDearmon E et al (2005) Obesity and metabolic syndrome in circadian clock mutant mice. Science 308(5724):1043–1045
227. Meyer-Kovac J, Kolbe I, Ehrhardt L, Leliavski A, Husse J, Salinas G et al (2017) Hepatic gene therapy rescues high-fat diet responses in circadian. Mol Metab 6(6):512–523
228. de Assis MA, Kupek E, Nahas MV, Bellisle F (2003) Food intake and circadian rhythms in shift workers with a high workload. Appetite 40(2):175–183
229. de Assis MA, Nahas MV, Bellisle F, Kupek E (2003) Meals, snacks and food choices in Brazilian shift workers with high energy expenditure. J Hum Nutr Diet 16(4):283–289
230. Manenschijn L, van Kruysbergen RG, de Jong FH, Koper JW, van Rossum EF (2011) Shift work at young age is associated with elevated long-term cortisol levels and body mass index. J Clin Endocrinol Metab 96(11):E1862–E1865

231. Schiavo-Cardozo D, Lima MM, Pareja JC, Geloneze B (2013) Appetite-regulating hormones from the upper gut: disrupted control of xenin and ghrelin in night workers. Clin Endocrinol 79(6):807–811
232. Waterhouse J, Nevill A, Edwards B, Godfrey R, Reilly T (2003) The relationship between assessments of jet lag and some of its symptoms. Chronobiol Int 20(6):1061–1073
233. Yildiz BO, Suchard MA, Wong ML, McCann SM, Licinio J (2004) Alterations in the dynamics of circulating ghrelin, adiponectin, and leptin in human obesity. Proc Natl Acad Sci U S A 101(28):10434–10439
234. Hales CM, Carroll MD, Fryar CD, Ogden CL (2017) Prevalence of obesity among adults and youth: United States, 2015-2016. NCHS Data Brief 288:1–8
235. Mendola ND, Chen TC, Gu Q, Eberhardt MS, Saydah S (2018) Prevalence of Total, diagnosed, and undiagnosed diabetes among adults: United States, 2013-2016. NCHS Data Brief 319:1–8
236. Gibson EM, Williams WP, Kriegsfeld LJ (2009) Aging in the circadian system: considerations for health, disease prevention and longevity. Exp Gerontol 44(1–2):51–56
237. Weinert D (2000) Age-dependent changes of the circadian system. Chronobiol Int 17(3):261–283
238. Mattis J, Sehgal A (2016) Circadian rhythms, sleep, and disorders of aging. Trends Endocrinol Metab 27(4):192–203
239. Zuurbier LA, Luik AI, Hofman A, Franco OH, Van Someren EJ, Tiemeier H (2015) Fragmentation and stability of circadian activity rhythms predict mortality: the Rotterdam study. Am J Epidemiol 181(1):54–63
240. Froy O (2011) Circadian rhythms, aging, and life span in mammals. Physiology (Bethesda) 26(4):225–235
241. Nakamura TJ, Takasu NN, Nakamura W (2016) The suprachiasmatic nucleus: age-related decline in biological rhythms. J Physiol Sci 66(5):367–374
242. Weinert H, Weinert D, Schurov I, Maywood ES, Hastings MH (2001) Impaired expression of the mPer2 circadian clock gene in the suprachiasmatic nuclei of aging mice. Chronobiol Int 18(3):559–565
243. Yamazaki S, Straume M, Tei H, Sakaki Y, Menaker M, Block GD (2002) Effects of aging on central and peripheral mammalian clocks. Proc Natl Acad Sci U S A 99(16):10801–10806
244. Aujard F, Cayetanot F, Bentivoglio M, Perret M (2006) Age-related effects on the biological clock and its behavioral output in a primate. Chronobiol Int 23(1–2):451–460
245. Youngstedt SD (2001) Ceiling and floor effects in sleep research. Sleep Med Rev 5(1):79–81
246. Hofman MA, Swaab DF (2006) Living by the clock: the circadian pacemaker in older people. Ageing Res Rev 5(1):33–51
247. Yoon IY, Kripke DF, Elliott JA, Youngstedt SD, Rex KM, Hauger RL (2003) Age-related changes of circadian rhythms and sleep-wake cycles. J Am Geriatr Soc 51(8):1085–1091
248. Pagani L, Schmitt K, Meier F, Izakovic J, Roemer K, Viola A et al (2011) Serum factors in older individuals change cellular clock properties. Proc Natl Acad Sci U S A 108(17):7218–7223
249. Albrecht U (2012) Timing to perfection: the biology of central and peripheral circadian clocks. Neuron 74(2):246–260
250. Nakamura TJ, Nakamura W, Yamazaki S, Kudo T, Cutler T, Colwell CS et al (2011) Age-related decline in circadian output. J Neurosci 31(28):10201–10205
251. Nygård M, Hill RH, Wikström MA, Kristensson K (2005) Age-related changes in electrophysiological properties of the mouse suprachiasmatic nucleus in vitro. Brain Res Bull 65(2):149–154
252. Satinoff E, Li H, Tcheng TK, Liu C, McArthur AJ, Medanic M et al (1993) Do the suprachiasmatic nuclei oscillate in old rats as they do in young ones? Am J Phys 265(5 Pt 2):R1216–R1222
253. Watanabe A, Shibata S, Watanabe S (1995) Circadian rhythm of spontaneous neuronal activity in the suprachiasmatic nucleus of old hamster in vitro. Brain Res 695(2):237–239

254. Kolker DE, Fukuyama H, Huang DS, Takahashi JS, Horton TH, Turek FW (2003) Aging alters circadian and light-induced expression of clock genes in golden hamsters. J Biol Rhythm 18(2):159–169
255. Nakamura TJ, Nakamura W, Tokuda IT, Ishikawa T, Kudo T, Colwell CS et al (2015) Age-related changes in the circadian system unmasked by constant conditions. eNeuro 2(4). pii: ENEURO.0064-15.2015. https://doi.org/10.1523/ENEURO.0064-15.2015
256. Wyse CA, Coogan AN (2010) Impact of aging on diurnal expression patterns of CLOCK and BMAL1 in the mouse brain. Brain Res 1337:21–31
257. Asai M, Yoshinobu Y, Kaneko S, Mori A, Nikaido T, Moriya T et al (2001) Circadian profile of per gene mRNA expression in the suprachiasmatic nucleus, paraventricular nucleus, and pineal body of aged rats. J Neurosci Res 66(6):1133–1139
258. Nakajima A, Aoyama Y, Shin EJ, Nam Y, Kim HC, Nagai T et al (2015) Nobiletin, a citrus flavonoid, improves cognitive impairment and reduces soluble Aβ levels in a triple transgenic mouse model of Alzheimer's disease (3XTg-AD). Behav Brain Res 289:69–77
259. Eghlidi D, Luna SL, Brown D, Garyfallou V, Kohama S, Urbanski HF (2018) Gene expression profiling of the SCN in young and old rhesus macaques. J Mol Endocrinol 61(2):57–67
260. Coria-Lucero CD, Golini RS, Ponce IT, Deyurka N, Anzulovich AC, Delgado SM et al (2016) Rhythmic Bdnf and TrkB expression patterns in the prefrontal cortex are lost in aged rats. Brain Res 1653:51–58
261. Duncan MJ, Herron JM, Hill SA (2001) Aging selectively suppresses vasoactive intestinal peptide messenger RNA expression in the suprachiasmatic nucleus of the Syrian hamster. Brain Res Mol Brain Res 87(2):196–203
262. Duncan MJ, Prochot JR, Cook DH, Tyler Smith J, Franklin KM (2013) Influence of aging on Bmal1 and Per2 expression in extra-SCN oscillators in hamster brain. Brain Res 1491:44–53
263. Chen CY, Logan RW, Ma T, Lewis DA, Tseng GC, Sibille E et al (2016) Effects of aging on circadian patterns of gene expression in the human prefrontal cortex. Proc Natl Acad Sci U S A 113(1):206–211
264. Benloucif S, Masana MI, Dubocovich ML (1997) Light-induced phase shifts of circadian activity rhythms and immediate early gene expression in the suprachiasmatic nucleus are attenuated in old C3H/HeN mice. Brain Res 747(1):34–42
265. Duffy JF, Zeitzer JM, Czeisler CA (2007) Decreased sensitivity to phase-delaying effects of moderate intensity light in older subjects. Neurobiol Aging 28(5):799–807
266. Zhang Y, Takahashi JS, Turek FW (1996) Critical period for cycloheximide blockade of light-induced phase advances of the circadian locomotor activity rhythm in golden hamsters. Brain Res 740(1–2):285–290
267. Yan SS, Wang W (2016) The effect of lens aging and cataract surgery on circadian rhythm. Int J Ophthalmol 9(7):1066–1074
268. Brøndsted AE, Sander B, Haargaard B, Lund-Andersen H, Jennum P, Gammeltoft S et al (2015) The effect of cataract surgery on circadian photoentrainment: a randomized trial of blue-blocking versus neutral intraocular lenses. Ophthalmology 122(10):2115–2124
269. Brøndsted AE, Haargaard B, Sander B, Lund-Andersen H, Jennum P, Kessel L (2017) The effect of blue-blocking and neutral intraocular lenses on circadian photoentrainment and sleep one year after cataract surgery. Acta Ophthalmol 95(4):344–351
270. Erichsen JH, Brøndsted AE, Kessel L (2015) Effect of cataract surgery on regulation of circadian rhythms. J Cataract Refract Surg 41(9):1997–2009
271. Ayaki M, Muramatsu M, Negishi K, Tsubota K (2013) Improvements in sleep quality and gait speed after cataract surgery. Rejuvenation Res 16(1):35–42
272. Ayaki M, Negishi K, Tsubota K (2014) Rejuvenation effects of cataract surgery with ultraviolet blocking intra-ocular lens on circadian rhythm and gait speed. Rejuvenation Res 17(4):359–365
273. Witting W, Mirmiran M, Bos NP, Swaab DF (1993) Effect of light intensity on diurnal sleep-wake distribution in young and old rats. Brain Res Bull 30(1–2):157–162

274. Gomez D, Barbosa A, Théry M, Aujard F, Perret M (2012) Age affects photoentrainment in a nocturnal primate. J Biol Rhythm 27(2):164–171
275. Duffy JF, Zitting KM, Chinoy ED (2015) Aging and circadian rhythms. Sleep Med Clin 10(4):423–434
276. Hood S, Amir S (2017) The aging clock: circadian rhythms and later life. J Clin Invest 127(2):437–446
277. Farajnia S, Michel S, Deboer T, van der Leest HT, Houben T, Rohling JH et al (2012) Evidence for neuronal desynchrony in the aged suprachiasmatic nucleus clock. J Neurosci 32(17):5891–5899
278. Allen CN, Nitabach MN, Colwell CS (2017) Membrane currents, gene expression, and circadian clocks. Cold Spring Harb Perspect Biol 9(5). pii: a027714. https://doi.org/10.1101/cshperspect.a027714
279. Kalló I, Kalamatianos T, Piggins HD, Coen CW (2004) Ageing and the diurnal expression of mRNAs for vasoactive intestinal peptide and for the VPAC2 and PAC1 receptors in the suprachiasmatic nucleus of male rats. J Neuroendocrinol 16(9):758–766
280. Kawakami F, Okamura H, Tamada Y, Maebayashi Y, Fukui K, Ibata Y (1997) Loss of day-night differences in VIP mRNA levels in the suprachiasmatic nucleus of aged rats. Neurosci Lett 222(2):99–102
281. Krajnak K, Kashon ML, Rosewell KL, Wise PM (1998) Aging alters the rhythmic expression of vasoactive intestinal polypeptide mRNA but not arginine vasopressin mRNA in the suprachiasmatic nuclei of female rats. J Neurosci 18(12):4767–4774
282. Sellix MT, Evans JA, Leise TL, Castanon-Cervantes O, Hill DD, DeLisser P et al (2012) Aging differentially affects the re-entrainment response of central and peripheral circadian oscillators. J Neurosci 32(46):16193–16202
283. Cai A, Scarbrough K, Hinkle DA, Wise PM (1997) Fetal grafts containing suprachiasmatic nuclei restore the diurnal rhythm of CRH and POMC mRNA in aging rats. Am J Phys 273(5):R1764–R1770
284. van Gool WA, Witting W, Mirmiran M (1987) Age-related changes in circadian sleep-wakefulness rhythms in male rats isolated from time cues. Brain Res 413(2):384–387
285. Viswanathan N, Davis FC (1995) Suprachiasmatic nucleus grafts restore circadian function in aged hamsters. Brain Res 686(1):10–16
286. Cayetanot F, Bentivoglio M, Aujard F (2005) Arginine-vasopressin polypeptide rhythms in the suprachiasmatic nucleus of the mouse lemur reveal aging-related alterations of circadian pacemaker neurons in a non-human primate. Eur J Neurosci 22 (4): 902-910
287. Umezaki Y, Yoshii T, Kawaguchi T, Helfrich-Förster C, Tomioka K (2012) Pigment-dispersing factor is involved in age-dependent rhythm changes in Drosophila melanogaster. J Biol Rhythm 27(6):423–432
288. Kofuji P, Mure LS, Massman LJ, Purrier N, Panda S, Engeland WC (2016) Intrinsically photosensitive retinal ganglion cells (ipRGCs) are necessary for light entrainment of peripheral clocks. PLoS One 11(12):e0168651. https://doi.org/10.1371/journal.pone.0168651
289. Schibler U, Gotic I, Saini C, Gos P, Curie T, Emmenegger Y et al (2015) Clock-talk: interactions between central and peripheral circadian oscillators in mammals. Cold Spring Harb Symp Quant Biol 80:223–232
290. Davidson AJ, Yamazaki S, Arble DM, Menaker M, Block GD (2008) Resetting of central and peripheral circadian oscillators in aged rats. Neurobiol Aging 29(3):471–477
291. Davidson AJ, Sellix MT, Daniel J, Yamazaki S, Menaker M, Block GD (2006) Chronic jet-lag increases mortality in aged mice. Curr Biol 16(21):R914–R916
292. Anisimov VN, Baturin DA, Popovich IG, Zabezhinski MA, Manton KG, Semenchenko AV et al (2004) Effect of exposure to light-at-night on life span and spontaneous carcinogenesis in female CBA mice. Int J Cancer 111(4):475–479
293. Vinogradova IA, Anisimov VN, Bukalev AV, Semenchenko AV, Zabezhinski MA (2009) Circadian disruption induced by light-at-night accelerates aging and promotes tumorigenesis in rats. Aging (Albany NY) 1(10):855–865

294. Bonaconsa M, Malpeli G, Montaruli A, Carandente F, Grassi-Zucconi G, Bentivoglio M (2014) Differential modulation of clock gene expression in the suprachiasmatic nucleus, liver and heart of aged mice. Exp Gerontol 55:70–79
295. Tahara Y, Takatsu Y, Shiraishi T, Kikuchi Y, Yamazaki M, Motohashi H et al (2017) Age-related circadian disorganization caused by sympathetic dysfunction in peripheral clock regulation. NPJ Aging Mech Dis 3:16030. https://doi.org/10.1038/npjamd.2016.30
296. Sutton GM, Ptitsyn AA, Floyd ZE, Yu G, Wu X, Hamel K et al (2013) Biological aging alters circadian mechanisms in murine adipose tissue depots. Age (Dordr) 35(3):533–547
297. Sandu C, Liu T, Malan A, Challet E, Pévet P, Felder-Schmittbuhl MP (2015) Circadian clocks in rat skin and dermal fibroblasts: differential effects of aging, temperature and melatonin. Cell Mol Life Sci 72(11):2237–2248
298. Ando H, Ushijima K, Kumazaki M, Takamura T, Yokota N, Saito T et al (2010) Influence of age on clock gene expression in peripheral blood cells of healthy women. J Gerontol A Biol Sci Med Sci 65(1):9–13
299. Luo W, Chen WF, Yue Z, Chen D, Sowcik M, Sehgal A et al (2012) Old flies have a robust central oscillator but weaker behavioral rhythms that can be improved by genetic and environmental manipulations. Aging Cell 11(3):428–438
300. Rakshit K, Krishnan N, Guzik EM, Pyza E, Giebultowicz JM (2012) Effects of aging on the molecular circadian oscillations in Drosophila. Chronobiol Int 29(1):5–14
301. Giebultowicz JM (2001) Peripheral clocks and their role in circadian timing: insights from insects. Philos Trans R Soc Lond Ser B Biol Sci 356(1415):1791–1799
302. Plautz JD, Kaneko M, Hall JC, Kay SA (1997) Independent photoreceptive circadian clocks throughout Drosophila. Science 278(5343):1632–1635
303. Ceriani MF, Hogenesch JB, Yanovsky M, Panda S, Straume M, Kay SA (2002) Genome-wide expression analysis in Drosophila reveals genes controlling circadian behavior. J Neurosci 22(21):9305–9319
304. Duffield GE, Best JD, Meurers BH, Bittner A, Loros JJ, Dunlap JC (2002) Circadian programs of transcriptional activation, signaling, and protein turnover revealed by microarray analysis of mammalian cells. Curr Biol 12(7):551–557
305. Panda S, Antoch MP, Miller BH, Su AI, Schook AB, Straume M et al (2002) Coordinated transcription of key pathways in the mouse by the circadian clock. Cell 109(3):307–320
306. Giebultowicz JM (2018) Circadian regulation of metabolism and healthspan in Drosophila. Free Radic Biol Med 119:62–68
307. Krishnan N, Davis AJ, Giebultowicz JM (2008) Circadian regulation of response to oxidative stress in Drosophila melanogaster. Biochem Biophys Res Commun 374(2):299–303
308. Neufeld-Cohen A, Robles MS, Aviram R, Manella G, Adamovich Y, Ladeuix B et al (2016) Circadian control of oscillations in mitochondrial rate-limiting enzymes and nutrient utilization by PERIOD proteins. Proc Natl Acad Sci U S A 113(12):E1673–E1682
309. Peek CB, Affinati AH, Ramsey KM, Kuo HY, Yu W, Sena LA et al (2013) Circadian clock NAD+ cycle drives mitochondrial oxidative metabolism in mice. Science 342(6158):1243417. https://doi.org/10.1126/science.1243417
310. Klichko VI, Chow ES, Kotwica-Rolinska J, Orr WC, Giebultowicz JM, Radyuk SN (2015) Aging alters circadian regulation of redox in Drosophila. Front Genet 6:83. https://doi.org/10.3389/fgene.2015.00083
311. Beaver LM, Klichko VI, Chow ES, Kotwica-Rolinska J, Williamson M, Orr WC et al (2012) Circadian regulation of glutathione levels and biosynthesis in Drosophila melanogaster. PLoS One 7(11):e50454. https://doi.org/10.1371/journal.pone.0050454
312. Gong C, Li C, Qi X, Song Z, Wu J, Hughes ME, Li X (2015) The daily rhythms of mitochondrial gene expression and oxidative stress regulation are altered by aging in the mouse liver. Chronobiol Int 32(9):1254–1263
313. Vinod C, Jagota A (2016) Daily NO rhythms in peripheral clocks in aging male Wistar rats: protective effects of exogenous melatonin. Biogerontology 17(5–6):859–871

314. Klarsfeld A, Rouyer F (1998) Effects of circadian mutations and LD periodicity on the life span of Drosophila melanogaster. J Biol Rhythm 13(6):471–478
315. Krishnan N, Kretzschmar D, Rakshit K, Chow E, Giebultowicz JM (2009) The circadian clock gene period extends healthspan in aging Drosophila melanogaster. Aging (Albany NY) 1(11):937–948
316. Fu L, Pelicano H, Liu J, Huang P, Lee C (2002) The circadian gene Period2 plays an important role in tumor suppression and DNA damage response in vivo. Cell 111(1):41–50
317. Lee CC (2005) The circadian clock and tumor suppression by mammalian period genes. Methods Enzymol 393:852–861
318. Krishnan N, Rakshit K, Chow ES, Wentzell JS, Kretzschmar D, Giebultowicz JM (2012) Loss of circadian clock accelerates aging in neurodegeneration-prone mutants. Neurobiol Dis 45(3):1129–1135
319. Dubrovsky YV, Samsa WE, Kondratov RV (2010) Deficiency of circadian protein CLOCK reduces lifespan and increases age-related cataract development in mice. Aging (Albany NY) 2(12):936–944
320. Kondratov RV, Kondratova AA, Gorbacheva VY, Vykhovanets OV, Antoch MP (2006) Early aging and age-related pathologies in mice deficient in BMAL1, the core componentof the circadian clock. Genes Dev 20(14):1868–1873
321. Ali AA, Schwarz-Herzke B, Stahr A, Prozorovski T, Aktas O, von Gall C (2015) Premature aging of the hippocampal neurogenic niche in adult Bmal1-deficient mice. Aging (Albany NY) 7(6):435–449
322. Musiek ES (2015) Circadian clock disruption in neurodegenerative diseases: cause and effect? Front Pharmacol 6:29. https://doi.org/10.3389/fphar.2015.00029
323. Musiek ES, Lim MM, Yang G, Bauer AQ, Qi L, Lee Y et al (2013) Circadian clock proteins regulate neuronal redox homeostasis and neurodegeneration. J Clin Invest 123(12):5389–5400
324. Banks G, Nolan PM, Peirson SN (2016) Reciprocal interactions between circadian clocks and aging. Mamm Genome 27(7–8):332–340
325. McDearmon EL, Patel KN, Ko CH, Walisser JA, Schook AC, Chong JL et al (2006) Dissecting the functions of the mammalian clock protein BMAL1 by tissue-specific rescue in mice. Science 314(5803):1304–1308
326. Yang G, Chen L, Grant GR, Paschos G, Song WL, Musiek ES et al (2016) Timing of expression of the core clock gene Bmal1 influences its effects on aging and survival. Sci Transl Med 8(324):324ra316. https://doi.org/10.1126/scitranslmed.aad3305
327. Li JC, Xu F (1997) Influences of light-dark shifting on the immune system, tumor growth and life span of rats, mice and fruit flies as well as on the counteraction of melatonin. Biol Signals 6(2):77–89
328. Cuesta M, Boudreau P, Dubeau-Laramée G, Cermakian N, Boivin DB (2016) Simulated night shift disrupts circadian rhythms of immune functions in humans. J Immunol 196(6):2466–2475
329. Li J, Terry EE, Fejer E, Gamba D, Hartmann N, Logsdon J et al (2017) Achilles is a circadian clock-controlled gene that regulates immune function in Drosophila. Brain Behav Immun 61:127–136
330. Li J, Yu RY, Emran F, Chen BE, Hughes ME (2019) Achilles-mediated and sex-specific regulation of circadian mRNA rhythms in drosophila. J Biol Rhythm 34(2):131–143
331. Libert S, Bonkowski MS, Pointer K, Pletcher SD, Guarente L (2012) Deviation of innate circadian period from 24 h reduces longevity in mice. Aging Cell 11(5):794–800
332. Park N, Cheon S, Son GH, Cho S, Kim K (2012) Chronic circadian disturbance by a shortened light-dark cycle increases mortality. Neurobiol Aging 33(6):1122.e1111–1122.e1122
333. Hurd MW, Ralph MR (1998) The significance of circadian organization for longevity in the golden hamster. J Biol Rhythm 13(5):430–436
334. Oklejewicz M, Daan S (2002) Enhanced longevity in tau mutant Syrian hamsters, Mesocricetus auratus. J Biol Rhythm 17(3):210–216

335. Aschoff J, Fatranská M, Giedke H, Doerr P, Stamm D, Wisser H (1971) Human circadian rhythms in continuous darkness: entrainment by social cues. Science 171(3967):213–215
336. Aschoff J, von Saint PU, Wever R (1971) Lifetime of flies under influence of time displacement. Naturwissenschaften 58(11):574. https://doi.org/10.1007/bf00598736
337. Boomgarden AC, Sagewalker GD, Shah AC, Haider SD, Patel P, Wheeler HE et al (2019) Chronic circadian misalignment results in reduced longevity and large-scale changes in gene expression in Drosophila. BMC Genomics 20(1):14. https://doi.org/10.1186/s12864-018-5401-7
338. Pittendrigh CS, Minis DH (1972) Circadian systems: longevity as a function of circadian resonance in Drosophila melanogaster. Proc Natl Acad Sci U S A 69(6):1537–1539
339. Noordam R, Jansen SW, Akintola AA, Oei NY, Maier AB, Pijl H et al (2012) Familial longevity is marked by lower diurnal salivary cortisol levels: the Leiden Longevity Study. PLoS One 7(2):e31166. https://doi.org/10.1371/journal.pone.0031166
340. van den Berg R, Noordam R, Kooijman S, Jansen SWM, Akintola AA, Slagboom PE et al (2017) Familial longevity is characterized by high circadian rhythmicity of serum cholesterol in healthy elderly individuals. Aging Cell 16(2):237–243
341. Paolisso G, Gambardella A, Ammendola S, D'Amore A, Balbi V, Varricchio M et al (1996) Glucose tolerance and insulin action in healthy centenarians. Am J Phys 270(5 Pt 1):E890–E894
342. Wijsman CA, van Opstal AM, Kan HE, Maier AB, Westendorp RG, Slagboom PE et al (2012) Proton magnetic resonance spectroscopy shows lower intramyocellular lipid accumulation in middle-aged subjects predisposed to familial longevity. Am J Physiol Endocrinol Metab 302(3):E344–E348
343. Gutman R, Genzer Y, Chapnik N, Miskin R, Froy O (2011) Long-lived mice exhibit 24 h locomotor circadian rhythms at young and old age. Exp Gerontol 46(7):606–609
344. Cho E, Lee KJ, Seo JW, Byun CJ, Chung SJ, Suh DC et al (2012) Neuroprotection by urokinase plasminogen activator in the hippocampus. Neurobiol Dis 46(1):215–224
345. Mondino A, Blasi F (2004) uPA and uPAR in fibrinolysis, immunity and pathology. Trends Immunol 25(8):450–455
346. Zhang Y, Pothakos K, Tsirka SA (2005) Extracellular proteases: biological and behavioral roles in the mammalian central nervous system. Curr Top Dev Biol 66:161–188
347. Kant GJ, Mougey EH, Meyerhoff JL (1986) Diurnal variation in neuroendocrine response to stress in rats: plasma ACTH, beta-endorphin, beta-LPH, corticosterone, prolactin and pituitary cyclic AMP responses. Neuroendocrinology 43(3):383–390
348. Torrellas A, Guaza C, Borrell J, Borrell S (1981) Adrenal hormones and brain catecholamines responses to morning and afternoon immobilization stress in rats. Physiol Behav 26(1):129–133
349. Cano P, Cardinali DP, Spinedi E, Esquifino AI (2008) Effect of aging on 24-hour pattern of stress hormones and leptin in rats. Life Sci 83(3–4):142–148
350. Cano P, Jiménez-Ortega V, Larrad A, Reyes Toso CF, Cardinali DP, Esquifino AI (2008) Effect of a high-fat diet on 24-h pattern of circulating levels of prolactin, luteinizing hormone, testosterone, corticosterone, thyroid-stimulating hormone and glucose, and pineal melatonin content, in rats. Endocrine 33(2):118–125
351. Korkushko OV, Lapin BA, Goncharova ND, Khavinson VK, Shatilo VB, Vengerin AA et al (2007) Normalizing effect of the pineal gland peptides on the daily melatonin rhythm in old monkeys and elderly people. Adv Gerontol 20(1):74–85
352. Strahler J, Berndt C, Kirschbaum C, Rohleder N (2010) Aging diurnal rhythms and chronic stress: distinct alteration of diurnal rhythmicity of salivary alpha-amylase and cortisol. Biol Psychol 84(2):248–256
353. Strahler J, Mueller A, Rosenloecher F, Kirschbaum C, Rohleder N (2010) Salivary alpha-amylase stress reactivity across different age groups. Psychophysiology 47(3):587–595
354. McDonald MJ, Rosbash M (2001) Microarray analysis and organization of circadian gene expression in Drosophila. Cell 107(5):567–578

355. McCarthy JJ, Andrews JL, McDearmon EL, Campbell KS, Barber BK, Miller BH et al (2007) Identification of the circadian transcriptome in adult mouse skeletal muscle. Physiol Genomics 31(1):86–95
356. Hardeland R, Coto-Montes A, Poeggeler B (2003) Circadian rhythms, oxidative stress, and antioxidative defense mechanisms. Chronobiol Int 20(6):921–962
357. Patel SA, Velingkaar NS, Kondratov RV (2014) Transcriptional control of antioxidant defense by the circadian clock. Antioxid Redox Signal 20(18):2997–3006
358. Xu YQ, Zhang D, Jin T, Cai DJ, Wu Q, Lu Y et al (2012) Diurnal variation of hepatic antioxidant gene expression in mice. PLoS One 7(8):e44237. https://doi.org/10.1371/journal.pone.0044237
359. Jacobi D, Liu S, Burkewitz K, Kory N, Knudsen NH, Alexander RK et al (2015) Hepatic Bmal1 regulates rhythmic mitochondrial dynamics and promotes metabolic fitness. Cell Metab 22(4):709–720
360. Yuan G, Hua B, Cai T, Xu L, Li E, Huang Y et al (2017) Clock mediates liver senescence by controlling ER stress. Aging (Albany NY) 9(12):2647–2665
361. Aguirre V, Werner ED, Giraud J, Lee YH, Shoelson SE, White MF (2002) Phosphorylation of Ser307 in insulin receptor substrate-1 blocks interactions with the insulin receptor and inhibits insulin action. J Biol Chem 277(2):1531–1537
362. Deng J, Lu PD, Zhang Y, Scheuner D, Kaufman RJ, Sonenberg N et al (2004) Translational repression mediates activation of nuclear factor kappa B by phosphorylated translation initiation factor 2. Mol Cell Biol 24(23):10161–10168
363. Hotamisligil GS (2006) Inflammation and metabolic disorders. Nature 444(7121):860–867
364. Zhang K, Kaufman RJ (2008) From endoplasmic-reticulum stress to the inflammatory response. Nature 454(7203):455–462
365. Stern AL, Naidoo N (2015) Wake-active neurons across aging and neurodegeneration: a potential role for sleep disturbances in promoting disease. Springerplus 4:25. https://doi.org/10.1186/s40064-014-0777-6
366. Santos CX, Tanaka LY, Wosniak J, Laurindo FR (2009) Mechanisms and implications of reactive oxygen species generation during the unfolded protein response: roles of endoplasmic reticulum oxidoreductases, mitochondrial electron transport, and NADPH oxidase. Antioxid Redox Signal 11(10):2409–2427
367. Butler R (2010) National Research Council (US) Center for Economic, Governance, and International Studies. Grand challenges of our aging society: workshop summary. Washington (DC): Enhancing Healthy Aging. National Academies Press (US) 3. https://www.ncbi.nlm.nih.gov/books/NBK220195/
368. Bélanger PM (1996) Circadian rhythms in hepatic biotransformation of drugs. Pathol Biol (Paris) 44(6):564–570
369. Mailloux A, Benstaali C, Bogdan A, Auzéby A, Touitou Y (1999) Body temperature and locomotor activity as marker rhythms of aging of the circadian system in rodents. Exp Gerontol 34(6):733–740
370. Touitou Y, Haus E (1994) Aging of the human endocrine and neuroendocrine time structure. Ann N Y Acad Sci 719:378–397
371. Carlsson A, Serin F (1950) Time of day as a factor influencing the toxicity of nikethamide. Acta Pharmacol Toxicol (Copenh) 6(2):181–186
372. Carlsson A, Serin F (1950) The toxicity of nikethamide at different times of the day. Acta Pharmacol Toxicol (Copenh) 6(2):187–193
373. Haus E, Halberg F (1959) 24-hour rhythm in susceptibility of C mice to a toxic dose of ethanol. J Appl Physiol 14:878–880
374. Scheving LE, Vedral DF, Pauly JE (1968) Daily circadian rhythm in rats to D-amphetamine sulphate: effect of blinding and continuous illumination on the rhythm. Nature 219(5154):621–622
375. Hirst M, Kavaliers M, Teskey GC (1984) Age and day-night changes in clonidine-induced analgesia in mice. Can J Physiol Pharmacol 62(9):1102–1105

376. Kavaliers M, Hirst M (1986) Aging and day-night rhythms in feeding in mice: effects of the putative sigma opiate agonist, N-allylnormetazocine (SKF-10,047). Neurobiol Aging 7(3):179–183
377. Kavaliers M, Hirst M, Teskey GC (1984) Aging and daily rhythms of analgesia in mice: effects of natural illumination and twilight. Neurobiol Aging 5(2):111–114
378. Yehuda S, Carasso RL (1983) Changes in circadian rhythms of thermoregulation and motor activity in rats as a function of aging: effects of d-amphetamine and alpha-MSH. Peptides 4(6):865–869
379. Okamoto M, Kita T, Okuda H, Tanaka T, Nakashima T (1994) Effects of aging on acute toxicity of nicotine in rats. Pharmacol Toxicol 75(1):1–6
380. Cherry KE, Morton MR (1989) Drug sensitivity in older adults: the role of physiologic and pharmacokinetic factors. Int J Aging Hum Dev 28(3):159–174
381. Dowling GJ, Weiss SR, Condon TP (2008) Drugs of abuse and the aging brain. Neuropsychopharmacology 33(2):209–218
382. De Nobrega AK, Lyons LC (2016) Circadian modulation of alcohol-induced sedation and recovery in male and female drosophila. J Biol Rhythm 31(2):142–160
383. De Nobrega AK, Mellers AP, Lyons LC (2017) Aging and circadian dysfunction increase alcohol sensitivity and exacerbate mortality in Drosophila melanogaster. Exp Gerontol 97:49–59
384. Van der Linde K, Lyons LC (2011) Circadian modulation of acute alcohol sensitivity but not acute tolerance in Drosophila. Chronobiol Int 28(5):397–406
385. Kendler KS, Ohlsson H, Sundquist J, Sundquist K (2016) Alcohol use disorder and mortality across the lifespan: a longitudinal cohort and co-relative analysis. JAMA Psychiat 73(6):575–581
386. Solanas G, Peixoto FO, Perdiguero E, Jardí M, Ruiz-Bonilla V, Datta D et al (2017) Aged stem cells reprogram their daily rhythmic functions to adapt to stress. Cell 170(4):678–692.e620
387. Kuintzle RC, Chow ES, Westby TN, Gvakharia BO, Giebultowicz JM, Hendrix DA (2017) Circadian deep sequencing reveals stress-response genes that adopt robust rhythmic expression during aging. Nat Commun 8:14529. https://doi.org/10.1038/ncomms14529
388. Hebert LE, Weuve J, Scherr PA, Evans DA (2013) Alzheimer disease in the United States (2010-2050) estimated using the 2010 census. Neurology 80(19):1778–1783
389. Marras C, Beck JC, Bower JH, Roberts E, Ritz B, Ross GW et al (2018) Prevalence of Parkinson's disease across North America. NPJ Parkinsons Dis 4:21. https://doi.org/10.1038/s41531-018-0058-0
390. Prince M, Wimo A, Guerchet M, Ali GC, Wu YT, Prina M (2015) World Alzheimer Report 2015-The Global Impact of Dementia, An analysis of prevalence, incidence, cost and trends. https://www.alz.co.uk/research/worldalzheimerreport2015summary.pdf
391. Satlin A, Volicer L, Stopa EG, Harper D (1995) Circadian locomotor activity and core-body temperature rhythms in Alzheimer's disease. Neurobiol Aging 16(5):765–771
392. Ancoli-Israel S, Klauber MR, Jones DW, Kripke DF, Martin J, Mason W et al (1997) Variations in circadian rhythms of activity, sleep, and light exposure related to dementia in nursing-home patients. Sleep 20(1):18–23
393. Harper DG, Volicer L, Stopa EG, McKee AC, Nitta M, Satlin A (2005) Disturbance of endogenous circadian rhythm in aging and Alzheimer disease. Am J Geriatr Psychiatry 13(5):359–368
394. Witting W, Kwa IH, Eikelenboom P, Mirmiran M, Swaab DF (1990) Alterations in the circadian rest-activity rhythm in aging and Alzheimer's disease. Biol Psychiatry 27(6):563–572
395. Canevelli M, Valletta M, Trebbastoni A, Sarli G, D'Antonio F, Tariciotti L et al (2016) Sundowning in dementia: clinical relevance, pathophysiological determinants, and therapeutic approaches. Front Med (Lausanne) 3:73. https://doi.org/10.3389/fmed.2016.00073
396. Volicer L, Harper DG, Manning BC, Goldstein R, Satlin A (2001) Sundowning and circadian rhythms in Alzheimer's disease. Am J Psychiatry 158(5):704–711

397. Uchida K, Okamoto N, Ohara K, Morita Y (1996) Daily rhythm of serum melatonin in patients with dementia of the degenerate type. Brain Res 717(1–2):154–159
398. Hatfield CF, Herbert J, van Someren EJ, Hodges JR, Hastings MH (2004) Disrupted daily activity/rest cycles in relation to daily cortisol rhythms of home-dwelling patients with early Alzheimer's dementia. Brain 127(Pt 5):1061–1074
399. Cermakian N, Lamont EW, Boudreau P, Boivin DB (2011) Circadian clock gene expression in brain regions of Alzheimer's disease patients and control subjects. J Biol Rhythm 26(2):160–170
400. Cronin P, McCarthy MJ, Lim ASP, Salmon DP, Galasko D, Masliah E et al (2017) Circadian alterations during early stages of Alzheimer's disease are associated with aberrant cycles of DNA methylation in BMAL1. Alzheimers Dement 13(6):689–700
401. Baker E, Sims R, Leonenko G, Frizzati A, Harwood JC, Grozeva D et al (2019) Gene-based analysis in HRC imputed genome wide association data identifies three novel genes for Alzheimer's disease. PLoS One 14(7):e0218111. https://doi.org/10.1371/journal.pone.0218111
402. Creese J, Bédard M, Brazil K, Chambers L (2008) Sleep disturbances in spousal caregivers of individuals with Alzheimer's disease. Int Psychogeriatr 20(1):149–161
403. Hood S, Cassidy P, Cossette MP, Weigl Y, Verwey M, Robinson B et al (2010) Endogenous dopamine regulates the rhythm of expression of the clock protein PER2 in the rat dorsal striatum via daily activation of D2 dopamine receptors. J Neurosci 30(42):14046–14058
404. Mattam U, Jagota A (2015) Daily rhythms of serotonin metabolism and the expression of clock genes in suprachiasmatic nucleus of rotenone-induced Parkinson's disease male Wistar rat model and effect of melatonin administration. Biogerontology 16(1):109–123
405. Gu Z, Wang B, Zhang YB, Ding H, Zhang Y, Yu J et al (2015) Association of ARNTL and PER1 genes with Parkinson's disease: a case-control study of Han Chinese. Sci Rep 5:15891. https://doi.org/10.1038/srep15891
406. Lou F, Li M, Luo X, Ren Y (2018) CLOCK 3111T/C variant correlates with motor fluctuation and sleep disorders in Chinese patients with Parkinson's disease. Parkinsons Dis 2018:4670380. https://doi.org/10.1155/2018/4670380
407. Adler P, Mayne J, Walker K, Ning Z, Figeys D (2019) Therapeutic targeting of casein kinase 1δ/ε in an Alzheimer's disease mouse model. J Proteome Res 18(9):3383–3393
408. Maiese K (2014) Driving neural regeneration through the mammalian target of rapamycin. Neural Regen Res 9(15):1413–1417
409. Maiese K (2014) Taking aim at Alzheimer's disease through the mammalian target of rapamycin. Ann Med 46(8):587–596
410. Maiese K (2017) Moving to the rhythm with clock (circadian) genes, autophagy, mTOR, and SIRT1 in degenerative disease and cancer. Curr Neurovasc Res 14(3):299–304
411. Musiek ES, Bhimasani M, Zangrilli MA, Morris JC, Holtzman DM, Ju YS (2018) Circadian rest-activity pattern changes in aging and preclinical Alzheimer disease. JAMA Neurol 75(5):582–590
412. Ju YS, Ooms SJ, Sutphen C, Macauley SL, Zangrilli MA, Jerome G et al (2017) Slow wave sleep disruption increases cerebrospinal fluid amyloid-β levels. Brain 140(8):2104–2111
413. Sprecher KE, Koscik RL, Carlsson CM, Zetterberg H, Blennow K, Okonkwo OC et al (2017) Poor sleep is associated with CSF biomarkers of amyloid pathology in cognitively normal adults. Neurology 89(5):445–453
414. Gao J, Huang X, Park Y, Hollenbeck A, Blair A, Schatzkin A et al (2011) Daytime napping, nighttime sleeping, and Parkinson disease. Am J Epidemiol 173(9):1032–1038
415. Tranah GJ, Blackwell T, Stone KL, Ancoli-Israel S, Paudel ML, Ensrud KE et al (2011) Circadian activity rhythms and risk of incident dementia and mild cognitive impairment in older women. Ann Neurol 70(5):722–732
416. Walsh CM, Blackwell T, Tranah GJ, Stone KL, Ancoli-Israel S, Redline S et al (2014) Weaker circadian activity rhythms are associated with poorer executive function in older women. Sleep 37(12):2009–2016

417. Rogers-Soeder TS, Blackwell T, Yaffe K, Ancoli-Israel S, Redline S, Cauley JA et al (2018) Rest-activity rhythms and cognitive decline in older men: the osteoporotic fractures in men sleep study. J Am Geriatr Soc 66(11):2136–2143
418. Bokenberger K, Ström P, Dahl Aslan AK, Åkerstedt T, Pedersen NL (2017) Shift work and cognitive aging: a longitudinal study. Scand J Work Environ Health 43(5):485–493
419. Bokenberger K, Ström P, Dahl Aslan AK, Johansson AL, Gatz M, Pedersen NL et al (2017) Association between sleep characteristics and incident dementia accounting for baseline cognitive status: a prospective population-based study. J Gerontol A Biol Sci Med Sci 72(1):134–139
420. Leng Y, Goldman SM, Cawthon PM, Stone KL, Ancoli-Israel S, Yaffe K (2018) Excessive daytime sleepiness, objective napping and 11-year risk of Parkinson's disease in older men. Int J Epidemiol 47(5):1679–1686
421. Bokenberger K, Sjölander A, Dahl Aslan AK, Karlsson IK, Åkerstedt T, Pedersen NL (2018) Shift work and risk of incident dementia: a study of two population-based cohorts. Eur J Epidemiol 33(10):977–987
422. Schernhammer ES, Lassen CF, Kenborg L, Ritz B, Olsen JH, Hansen J (2015) Occupational history of night shift work and Parkinson's disease in Denmark. Scand J Work Environ Health 41(4):377–383
423. Cedernaes J, Osorio RS, Varga AW, Kam K, Schiöth HB, Benedict C (2017) Candidate mechanisms underlying the association between sleep-wake disruptions and Alzheimer's disease. Sleep Med Rev 31:102–111
424. Di Meco A, Joshi YB, Praticò D (2014) Sleep deprivation impairs memory, tau metabolism, and synaptic integrity of a mouse model of Alzheimer's disease with plaques and tangles. Neurobiol Aging 35(8):1813–1820
425. Ju YE, McLeland JS, Toedebusch CD, Xiong C, Fagan AM, Duntley SP et al (2013) Sleep quality and preclinical Alzheimer disease. JAMA Neurol 70(5):587–593
426. Rothman SM, Herdener N, Frankola KA, Mughal MR, Mattson MP (2013) Chronic mild sleep restriction accentuates contextual memory impairments, and accumulations of cortical Aβ and pTau in a mouse model of Alzheimer's disease. Brain Res 1529:200–208
427. Lucey BP, Hicks TJ, McLeland JS, Toedebusch CD, Boyd J, Elbert DL et al (2018) Effect of sleep on overnight cerebrospinal fluid amyloid β kinetics. Ann Neurol 83(1):197–204
428. Zhu Y, Zhan G, Fenik P, Brandes M, Bell P, Francois N et al (2018) Chronic sleep disruption advances the temporal progression of tauopathy in P301S mutant mice. J Neurosci 38(48):10255–10270
429. de Vivo L, Bellesi M, Marshall W, Bushong EA, Ellisman MH, Tononi G et al (2017) Ultrastructural evidence for synaptic scaling across the wake/sleep cycle. Science 355(6324):507–510
430. Fahrenkrug J, Popovic N, Georg B, Brundin P, Hannibal J (2007) Decreased VIP and VPAC2 receptor expression in the biological clock of the R6/2 Huntington's disease mouse. J Mol Neurosci 31(2):139–148
431. Kudo T, Schroeder A, Loh DH, Kuljis D, Jordan MC, Roos KP (2011) Dysfunctions in circadian behavior and physiology in mouse models of Huntington's disease. Exp Neurol 228(1):80–90
432. Pallier PN, Maywood ES, Zheng Z, Chesham JE, Inyushkin AN, Dyball R et al (2007) Pharmacological imposition of sleep slows cognitive decline and reverses dysregulation of circadian gene expression in a transgenic mouse model of Huntington's disease. J Neurosci 27(29):7869–7878
433. Stopa EG, Volicer L, Kuo-Leblanc V, Harper D, Lathi D, Tate B et al (1999) Pathologic evaluation of the human suprachiasmatic nucleus in severe dementia. J Neuropathol Exp Neurol 58(1):29–39
434. 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(1):37–44

435. Levy-Lahad E, Wasco W, Poorkaj P, Romano DM, Oshima J, Pettingell WH et al (1995) Candidate gene for the chromosome 1 familial Alzheimer's disease locus. Science 269(5226):973–977
436. Bélanger V, Picard N, Cermakian N (2006) The circadian regulation of Presenilin-2 gene expression. Chronobiol Int 23(4):747–766
437. Chauhan R, Chen KF, Kent BA, Crowther DC (2017) Central and peripheral circadian clocks and their role in Alzheimer's disease. Dis Model Mech 10(10):1187–1199
438. Homolak J, Mudrovčić M, Vukić B, Toljan K (2018) Circadian rhythm and Alzheimer's disease. Med Sci (Basel) 6(3). pii: E52. https://doi.org/10.3390/medsci6030052
439. Hood S, Amir S (2017) Neurodegeneration and the circadian clock. Front Aging Neurosci 9:170. https://doi.org/10.3389/fnagi.2017.00170
440. Leng Y, Musiek ES, Hu K, Cappuccio FP, Yaffe K (2019) Association between circadian rhythms and neurodegenerative diseases. Lancet Neurol 18(3):307–318
441. Li S, Wang Y, Wang F, Hu LF, Liu CF (2017) A new perspective for Parkinson's disease: circadian rhythm. Neurosci Bull 33(1):62–72
442. Musiek ES (2017) Circadian rhythms in AD pathogenesis: a critical appraisal. Curr Sleep Med Rep 3(2):85–92
443. Castanon-Cervantes O, Wu M, Ehlen JC, Paul K, Gamble KL, Johnson RL et al (2010) Dysregulation of inflammatory responses by chronic circadian disruption. J Immunol 185(10):5796–5805
444. Potter GD, Skene DJ, Arendt J, Cade JE, Grant PJ, Hardie LJ (2016) Circadian rhythm and sleep disruption: causes, metabolic consequences, and countermeasures. Endocr Rev 37(6):584–608
445. Zee PC, Attarian H, Videnovic A (2013) Circadian rhythm abnormalities. Continuum (Minneap Minn) 19(1 Sleep Disorders):132–147
446. Ortiz-Tudela E, Mteyrek A, Ballesta A, Innominato PF, Lévi F (2013) Cancer chronotherapeutics: experimental, theoretical, and clinical aspects. Handb Exp Pharmacol 217:261–288
447. Antoch MP, Kondratov RV (2013) Pharmacological modulators of the circadian clock as potential therapeutic drugs: focus on genotoxic/anticancer therapy. Handb Exp Pharmacol 217:289–309
448. Chen Z, Yoo SH, Takahashi JS (2013) Small molecule modifiers of circadian clocks. Cell Mol Life Sci 70(16):2985–2998
449. Gloston GF, Yoo SH, Chen ZJ (2017) Clock-enhancing small molecules and potential applications in chronic diseases and aging. Front Neurol 8:100. https://doi.org/10.3389/fneur.2017.00100
450. Kojetin DJ, Burris TP (2014) REV-ERB and ROR nuclear receptors as drug targets. Nat Rev Drug Discov 13(3):197–216
451. Schroeder AM, Colwell CS (2013) How to fix a broken clock. Trends Pharmacol Sci 34(11):605–619
452. Wallach T, Kramer A (2015) Chemical chronobiology: toward drugs manipulating time. FEBS Lett 589(14):1530–1538
453. Froy O (2018) Circadian rhythms, nutrition and implications for longevity in urban environments. Proc Nutr Soc 77(3):216–222
454. Froy O, Miskin R (2007) The interrelations among feeding, circadian rhythms and ageing. Prog Neurobiol 82(3):142–150
455. Froy O, Miskin R (2010) Effect of feeding regimens on circadian rhythms: implications for aging and longevity. Aging (Albany NY) 2(1):7–27
456. Golbidi S, Daiber A, Korac B, Li H, Essop MF, Laher I (2017) Health benefits of fasting and caloric restriction. Curr Diab Rep 17(12):123. https://doi.org/10.1007/s11892-017-0951-7
457. Kessler K, Pivovarova-Ramich O (2019) Meal timing, aging, and metabolic health. Int J Mol Sci 20(8). pii: E1911. https://doi.org/10.3390/ijms20081911
458. Longo VD, Panda S (2016) Fasting, circadian rhythms, and time-restricted feeding in healthy lifespan. Cell Metab 23(6):1048–1059

459. Mattson MP, Allison DB, Fontana L, Harvie M, Longo VD, Malaisse WJ et al (2014) Meal frequency and timing in health and disease. Proc Natl Acad Sci U S A 111(47):16647–16653
460. Paoli A, Tinsley G, Bianco A, Moro T (2019) The influence of meal frequency and timing on health in humans: the role of fasting. Nutrients 11(4). pii: E719. https://doi.org/10.3390/nu11040719
461. Cunningham JEA, Stamp JA, Shapiro CM (2019) Sleep and major depressive disorder: a review of non-pharmacological chronotherapeutic treatments for unipolar depression. Sleep Med 61:6–18
462. Mitolo M, Tonon C, La Morgia C, Testa C, Carelli V, Lodi R (2018) Effects of light treatment on sleep, cognition, mood, and behavior in Alzheimer's disease: a systematic review. Dement Geriatr Cogn Disord 46(5–6):371–384
463. Rutten S, Vriend C, van den Heuvel OA, Smit JH, Berendse HW, van der Werf YD (2012) Bright light therapy in Parkinson's disease: an overview of the background and evidence. Parkinsons Dis 2012:767105. https://doi.org/10.1155/2012/767105
464. van Wamelen DJ, Roos RA, Aziz NA (2015) Therapeutic strategies for circadian rhythm and sleep disturbances in Huntington disease. Neurodegener Dis Manag 5(6):549–559
465. Wu YH, Swaab DF (2007) Disturbance and strategies for reactivation of the circadian rhythm system in aging and Alzheimer's disease. Sleep Med 8(6):623–636
466. Yamadera H, Ito T, Suzuki H, Asayama K, Ito R, Endo S (2000) Effects of bright light on cognitive and sleep-wake (circadian) rhythm disturbances in Alzheimer-type dementia. Psychiatry Clin Neurosci 54(3):352–353
467. Van Someren EJ, Kessler A, Mirmiran M, Swaab DF (1997) Indirect bright light improves circadian rest-activity rhythm disturbances in demented patients. Biol Psychiatry 41(9):955–963
468. Fetveit A, Bjorvatn B (2004) The effects of bright-light therapy on actigraphical measured sleep last for several weeks post-treatment. A study in a nursing home population. J Sleep Res 13(2):153–158
469. Lyketsos CG, Lindell Veiel L, Baker A, Steele C (1999) A randomized, controlled trial of bright light therapy for agitated behaviors in dementia patients residing in long-term care. Int J Geriatr Psychiatry 14(7):520–525
470. Okumoto Y, Koyama E, Matsubara H, Nakano T, Nakamura R (1998) Sleep improvement by light in a demented aged individual. Psychiatry Clin Neurosci 52(2):194–196
471. Mishima K, Okawa M, Hishikawa Y, Hozumi S, Hori H, Takahashi K (1994) Morning bright light therapy for sleep and behavior disorders in elderly patients with dementia. Acta Psychiatr Scand 89(1):1–7
472. Dowling GA, Graf CL, Hubbard EM, Luxenberg JS (2007) Light treatment for neuropsychiatric behaviors in Alzheimer's disease. West J Nurs Res 29(8):961–975
473. Fetveit A, Bjorvatn B (2005) Bright-light treatment reduces actigraphic-measured daytime sleep in nursing home patients with dementia: a pilot study. Am J Geriatr Psychiatry 13(5):420–423
474. Skjerve A, Holsten F, Aarsland D, Bjorvatn B, Nygaard HA, Johansen IM (2004) Improvement in behavioral symptoms and advance of activity acrophase after short-term bright light treatment in severe dementia. Psychiatry Clin Neurosci 58(4):343–347
475. Sekiguchi H, Iritani S, Fujita K (2017) Bright light therapy for sleep disturbance in dementia is most effective for mild to moderate Alzheimer's type dementia: a case series. Psychogeriatrics 17(5):275–281
476. Paus S, Schmitz-Hübsch T, Wüllner U, Vogel A, Klockgether T, Abele M (2007) Bright light therapy in Parkinson's disease: a pilot study. Mov Disord 22(10):1495–1498
477. Willis GL, Turner EJ (2007) Primary and secondary features of Parkinson's disease improve with strategic exposure to bright light: a case series study. Chronobiol Int 24(3):521–537
478. Videnovic A, Klerman EB, Wang W, Marconi A, Kuhta T, Zee PC (2017) Timed light therapy for sleep and daytime sleepiness associated with Parkinson disease: a randomized clinical trial. JAMA Neurol 74(4):411–418

479. Willis GL, Boda J, Freelance CB (2018) Polychromatic light exposure as a therapeutic in the treatment and management of Parkinson's disease: a controlled exploratory trial. Front Neurol 9:741. https://doi.org/10.3389/fneur.2018.00741
480. Rutten S, Vriend C, Smit JH, Berendse HW, van Someren EJW, Hoogendoorn AW (2019) Bright light therapy for depression in Parkinson disease: a randomized controlled trial. Neurology 92(11):e1145–e1156
481. Fetveit A, Skjerve A, Bjorvatn B (2003) Bright light treatment improves sleep in institutionalised elderly--an open trial. Int J Geriatr Psychiatry 18(6):520–526
482. Kohsaka M, Fukuda N, Honma H, Kobayashi R, Sakakibara S, Koyama E et al (1999) Effects of moderately bright light on subjective evaluations in healthy elderly women. Psychiatry Clin Neurosci 53(2):239–241
483. Royer M, Ballentine NH, Eslinger PJ, Houser K, Mistrick R, Behr R (2012) Light therapy for seniors in long term care. J Am Med Dir Assoc 13(2):100–102
484. Usui A, Ishizuka Y, Matsushita Y, Fukuzawa H, Kanba S (2000) Bright light treatment for night-time insomnia and daytime sleepiness in elderly people: comparison with a short-acting hypnotic. Psychiatry Clin Neurosci 54(3):374–376
485. Friedman L, Spira AP, Hernandez B, Mather C, Sheikh J, Ancoli-Israel S et al (2012) Brief morning light treatment for sleep/wake disturbances in older memory-impaired individuals and their caregivers. Sleep Med 13(5):546–549
486. Kobayashi R, Fukuda N, Kohsaka M, Sasamoto Y, Sakakibara S, Koyama E et al (2001) Effects of bright light at lunchtime on sleep of patients in a geriatric hospital I. Psychiatry Clin Neurosci 55(3):287–289
487. Lieverse R, Van Someren EJ, Nielen MM, Uitdehaag BM, Smit JH, Hoogendijk WJ (2011) Bright light treatment in elderly patients with nonseasonal major depressive disorder: a randomized placebo-controlled trial. Arch Gen Psychiatry 68(1):61–70
488. Loving RT, Kripke DF, Elliott JA, Knickerbocker NC, Grandner MA (2005) Bright light treatment of depression for older adults [ISRCTN55452501]. BMC Psychiatry 5:41. https://doi.org/10.1186/1471-244X-5-42
489. Tsai YF, Wong TK, Juang YY, Tsai HH (2004) The effects of light therapy on depressed elders. Int J Geriatr Psychiatry 19(6):545–548
490. Chang CH, Liu CY, Chen SJ, Tsai HC (2018) Efficacy of light therapy on nonseasonal depression among elderly adults: a systematic review and meta-analysis. Neuropsychiatr Dis Treat 14:3091–3102
491. Güzel Özdemir P, Boysan M, Smolensky MH, Selvi Y, Aydin A, Yilmaz E (2015) Comparison of venlafaxine alone versus venlafaxine plus bright light therapy combination for severe major depressive disorder. J Clin Psychiatry 76(5):e645–e654
492. Martiny K (2004) Adjunctive bright light in non-seasonal major depression. Acta Psychiatr Scand Suppl 425:7–28
493. Martiny K, Lunde M, Undén M, Dam H, Bech P (2006) The lack of sustained effect of bright light, after discontinuation, in non-seasonal major depression. Psychol Med 36(9):1247–1252
494. Canazei M, Pohl W, Bauernhofer K, Papousek I, Lackner HK, Bliem HR et al (2017) Psychophysiological effects of a single, short, and moderately bright room light exposure on mildly depressed geriatric inpatients: a pilot study. Gerontology 63(4):308–317
495. Mishima K, Okawa M, Hozumi S, Hishikawa Y (2000) Supplementary administration of artificial bright light and melatonin as potent treatment for disorganized circadian rest-activity and dysfunctional autonomic and neuroendocrine systems in institutionalized demented elderly persons. Chronobiol Int 17(3):419–432
496. Riemersma-van der Lek RF, Swaab DF, Twisk J, Hol EM, Hoogendijk WJ, Van Someren EJ (2008) Effect of bright light and melatonin on cognitive and noncognitive function in elderly residents of group care facilities: a randomized controlled trial. JAMA 299(22):2642–2655
497. Lucassen PJ, Chung WC, Vermeulen JP, Van Lookeren CM, Van Dierendonck JH, Swaab DF (1995) Microwave-enhanced in situ end-labeling of fragmented DNA: parametric studies in

relation to postmortem delay and fixation of rat and human brain. J Histochem Cytochem 43(11):1163–1171
498. Lucassen PJ, Goudsmit E, Pool CW, Mengod G, Palacios JM, Raadsheer FC et al (1995) In situ hybridization for vasopressin mRNA in the human supraoptic and paraventricular nucleus; quantitative aspects of formalin-fixed paraffin-embedded tissue sections as compared to cryostat sections. J Neurosci Methods 57(2):221–230
499. Maruani J, Geoffroy PA (2019) Bright light as a personalized precision treatment of mood disorders. Front Psych 10:85. https://doi.org/10.3389/fpsyt.2019.00085
500. Magnusson A, Boivin D (2003) Seasonal affective disorder: an overview. Chronobiol Int 20(2):189–207
501. Burgess HJ, Sharkey KM, Eastman CI (2002) Bright light, dark and melatonin can promote circadian adaptation in night shift workers. Sleep Med Rev 6(5):407–420
502. Campbell SS, Kripke DF, Gillin JC, Hrubovcak JC (1988) Exposure to light in healthy elderly subjects and Alzheimer's patients. Physiol Behav 42(2):141–144
503. Luijpen MW, Scherder EJ, Van Someren EJ, Swaab DF, Sergeant JA (2003) Non-pharmacological interventions in cognitively impaired and demented patients--a comparison with cholinesterase inhibitors. Rev Neurosci 14(4):343–368
504. Ancoli-Israel S, Gehrman P, Martin JL, Shochat T, Marler M, Corey-Bloom J et al (2003) Increased light exposure consolidates sleep and strengthens circadian rhythms in severe Alzheimer's disease patients. Behav Sleep Med 1(1):22–36
505. Dowling GA, Burr RL, Van Someren EJ, Hubbard EM, Luxenberg JS, Mastick J et al (2008) Melatonin and bright-light treatment for rest-activity disruption in institutionalized patients with Alzheimer's disease. J Am Geriatr Soc 56(2):239–246
506. Friedman L, Zeitzer JM, Kushida C, Zhdanova I, Noda A, Lee T et al (2009) Scheduled bright light for treatment of insomnia in older adults. J Am Geriatr Soc 57(3):441–452
507. Fukuda N, Kobayashi R, Kohsaka M, Honma H, Sasamoto Y, Sakakibara S (2001) Effects of bright light at lunchtime on sleep in patients in a geriatric hospital II. Psychiatry Clin Neurosci 55(3):291–293
508. Ohashi Y, Okamoto N, Uchida K, Iyo M, Mori N, Morita Y (1999) Daily rhythm of serum melatonin levels and effect of light exposure in patients with dementia of the Alzheimer's type. Biol Psychiatry 45(12):1646–1652
509. Fukuda N, Kohsaka M, Sasamoto Y, Koyama E, Kobayashi R, Honma H et al (1998) Effects of short duration morning bright light in healthy elderly subjects. I: subjective feeling and ophthalmological examinations. Psychiatry Clin Neurosci 52(2):250–251
510. Viola AU, James LM, Schlangen LJ, Dijk DJ (2008) Blue-enriched white light in the workplace improves self-reported alertness, performance and sleep quality. Scand J Work Environ Health 34(4):297–306
511. Wright KP, McHill AW, Birks BR, Griffin BR, Rusterholz T, Chinoy ED (2013) Entrainment of the human circadian clock to the natural light-dark cycle. Curr Biol 23(16):1554–1558
512. Murphy PJ, Campbell SS (1996) Enhanced performance in elderly subjects following bright light treatment of sleep maintenance insomnia. J Sleep Res 5(3):165–172
513. Steffens DC, Fisher GG, Langa KM, Potter GG, Plassman BL (2009) Prevalence of depression among older Americans: the aging, demographics and memory study. Int Psychogeriatr 21(5):879–888
514. Steinman LE, Frederick JT, Prohaska T, Satariano WA, Dornberg-Lee S, Fisher R et al (2007) Recommendations for treating depression in community-based older adults. Am J Prev Med 33(3):175–181
515. Sumaya IC, Rienzi BM, Deegan JF, Moss DE (2001) Bright light treatment decreases depression in institutionalized older adults: a placebo-controlled crossover study. J Gerontol A Biol Sci Med Sci 56(6):M356–M360
516. Benedetti F, Avery DH, Bauer M, Bunney WE, Çaliyurt O, Camardese G et al (2018) Evidence for the efficacy of bright light therapy for bipolar depression. Am J Psychiatry 175(9):905–906

517. Lam RW, Levitt AJ, Levitan RD, Michalak EE, Cheung AH, Morehouse R et al (2016) Efficacy of bright light treatment, fluoxetine, and the combination in patients with nonseasonal major depressive disorder: a randomized clinical trial. JAMA Psychiat 73(1):56–63
518. Levitt AJ, Joffe RT, Kennedy SH (1991) Bright light augmentation in antidepressant nonresponders. J Clin Psychiatry 52(8):336–337
519. Prasko J, Horacek J, Klaschka J, Kosova J, Ondrackova I, Sipek J (2002) Bright light therapy and/or imipramine for inpatients with recurrent non-seasonal depression. Neuro Endocrinol Lett 23(2):109–113
520. Müller MJ, Seifritz E, Hatzinger M, Hemmeter U, Holsboer-Trachsler E (1997) Side effects of adjunct light therapy in patients with major depression. Eur Arch Psychiatry Clin Neurosci 247(5):252–258
521. Fernandez DC, Fogerson PM, Lazzerini Ospri L, Thomsen MB, Layne RM, Severin D et al (2018) Light affects mood and learning through distinct retina-brain pathways. Cell 175(1):71–84.e18
522. LeGates TA, Altimus CM, Wang H, Lee HK, Yang S, Zhao H et al (2012) Aberrant light directly impairs mood and learning through melanopsin-expressing neurons. Nature 491(7425):594–598
523. Dowling GA, Mastick J, Hubbard EM, Luxenberg JS, Burr RL (2005) Effect of timed bright light treatment for rest-activity disruption in institutionalized patients with Alzheimer's disease. Int J Geriatr Psychiatry 20(8):738–743
524. Münch M, Schmieder M, Bieler K, Goldbach R, Fuhrmann T, Zumstein N et al (2017) Bright light delights: effects of daily light exposure on emotions, restactivity cycles, sleep and melatonin secretion in severely demented patients. Curr Alzheimer Res 14(10):1063–1075
525. Barrick AL, Sloane PD, Williams CS, Mitchell CM, Connell BR, Wood W et al (2010) Impact of ambient bright light on agitation in dementia. Int J Geriatr Psychiatry 25(10):1013–1021
526. Sloane PD, Williams CS, Mitchell CM, Preisser JS, Wood W, Barrick AL et al (2007) High-intensity environmental light in dementia: effect on sleep and activity. J Am Geriatr Soc 55(10):1524–1533
527. Onega LL, Pierce TW, Epperly L (2018) Bright light therapy to treat depression in individuals with mild/moderate or severe dementia. Issues Ment Health Nurs 39(5):370–373
528. Haffmans PM, Sival RC, Lucius SA, Cats Q, van Gelder L (2001) Bright light therapy and melatonin in motor restless behaviour in dementia: a placebo-controlled study. Int J Geriatr Psychiatry 16(1):106–110
529. Kirisoglu C, Guilleminault C (2004) Twenty minutes versus forty-five minutes morning bright light treatment on sleep onset insomnia in elderly subjects. J Psychosom Res 56(5):537–542
530. Loving RT, Kripke DF, Knickerbocker NC, Grandner MA (2005) Bright green light treatment of depression for older adults [ISRCTN69400161]. BMC Psychiatry 5:42. https://doi.org/10.1186/1471-244X-5-42
531. Genhart MJ, Kelly KA, Coursey RD, Datiles M, Rosenthal NE (1993) Effects of bright light on mood in normal elderly women. Psychiatry Res 47(1):87–97
532. Gill S, Le HD, Melkani GC, Panda S (2015) Time-restricted feeding attenuates age-related cardiac decline in Drosophila. Science 347(6227):1265–1269
533. Zhang S, Ratliff EP, Molina B, El-Mecharrafie N, Mastroianni J, Kotzebue RW et al (2018) Aging and intermittent fasting impact on transcriptional regulation and physiological responses of adult drosophila neuronal and muscle tissues. Int J Mol Sci 19(4). https://doi.org/10.3390/ijms19041140
534. Walcott EC, Tate BA (1996) Entrainment of aged, dysrhythmic rats to a restricted feeding schedule. Physiol Behav 60(5):1205–1208
535. Maywood ES, Fraenkel E, McAllister CJ, Wood N, Reddy AB, Hastings MH et al (2010) Disruption of peripheral circadian timekeeping in a mouse model of Huntington's disease and its restoration by temporally scheduled feeding. J Neurosci 30(30):10199–10204
536. Ehrnhoefer DE, Martin DDO, Schmidt ME, Qiu X, Ladha S, Caron NS et al (2018) Preventing mutant huntingtin proteolysis and intermittent fasting promote autophagy in

models of Huntington disease. Acta Neuropathol Commun 6(1):16. https://doi.org/10.1186/s40478-018-0518-0
537. Wang HB, Loh DH, Whittaker DS, Cutler T, Howland D, Colwell CS (2018) Time-restricted feeding improves circadian dysfunction as well as motor symptoms in the Q175 mouse model of Huntington's disease. eNeuro 5(1). pii: ENEURO.0431-17.2017. https://doi.org/10.1523/ENEURO.0431-17.2017
538. Delahaye LB, Bloomer RJ, Butawan MB, Wyman JM, Hill JL, Lee HW et al (2018) Time-restricted feeding of a high-fat diet in male C57BL/6 mice reduces adiposity but does not protect against increased systemic inflammation. Appl Physiol Nutr Metab 43(10):1033–1042
539. Filipski E, Innominato PF, Wu M, Li XM, Iacobelli S, Xian LJ et al (2005) Effects of light and food schedules on liver and tumor molecular clocks in mice. J Natl Cancer Inst 97(7):507–517
540. Halberg N, Henriksen M, Söderhamn N, Stallknecht B, Ploug T, Schjerling P et al (2005) Effect of intermittent fasting and refeeding on insulin action in healthy men. J Appl Physiol (1985) 99(6):2128–2136
541. Anton SD, Lee SA, Donahoo WT, McLaren C, Manini T, Leeuwenburgh C et al (2019) The effects of time restricted feeding on overweight, older adults: a pilot study. Nutrients 11(7). pii: E1500. https://doi.org/10.3390/nu11071500
542. Marinac CR, Godbole S, Kerr J, Natarajan L, Patterson RE, Hartman SJ (2015) Objectively measured physical activity and cognitive functioning in breast cancer survivors. J Cancer Surviv 9(2):230–238
543. Marinac CR, Natarajan L, Sears DD, Gallo LC, Hartman SJ, Arredondo E et al (2015) Prolonged nightly fasting and breast cancer risk: findings from NHANES (2009-2010). Cancer Epidemiol Biomark Prev 24(5):783–789
544. Salgado-Delgado R, Angeles-Castellanos M, Saderi N, Buijs RM, Escobar C (2010) Food intake during the normal activity phase prevents obesity and circadian desynchrony in a rat model of night work. Endocrinology 151(3):1019–1029
545. Salgado-Delgado R, Nadia S, Angeles-Castellanos M, Buijs RM, Escobar C (2010) In a rat model of night work, activity during the normal resting phase produces desynchrony in the hypothalamus. J Biol Rhythm 25(6):421–431
546. Archer SN, Laing EE, Möller-Levet CS, van der Veen DR, Bucca G, Lazar AS et al (2014) Mistimed sleep disrupts circadian regulation of the human transcriptome. Proc Natl Acad Sci U S A 111(6):E682–E691
547. Scheer FA, Hilton MF, Mantzoros CS, Shea SA (2009) Adverse metabolic and cardiovascular consequences of circadian misalignment. Proc Natl Acad Sci U S A 106(11):4453–4458
548. Wefers J, van Moorsel D, Hansen J, Connell NJ, Havekes B, Hoeks J et al (2018) Circadian misalignment induces fatty acid metabolism gene profiles and compromises insulin sensitivity in human skeletal muscle. Proc Natl Acad Sci U S A 115(30):7789–7794
549. Almoosawi S, Prynne CJ, Hardy R, Stephen AM (2013) Diurnal eating rhythms: association with long-term development of diabetes in the 1946 British birth cohort. Nutr Metab Cardiovasc Dis 23(10):1025–1030
550. Almoosawi S, Prynne CJ, Hardy R, Stephen AM (2013) Time-of-day of energy intake: association with hypertension and blood pressure 10 years later in the 1946 British Birth Cohort. J Hypertens 31(5):882–892
551. Almoosawi S, Prynne CJ, Hardy R, Stephen AM (2013) Time-of-day and nutrient composition of eating occasions: prospective association with the metabolic syndrome in the 1946 British birth cohort. Int J Obes 37(5):725–731
552. Antunes LC, Levandovski R, Dantas G, Caumo W, Hidalgo MP (2010) Obesity and shift work: chronobiological aspects. Nutr Res Rev 23(1):155–168
553. Bray MS, Ratcliffe WF, Grenett MH, Brewer RA, Gamble KL, Young ME (2013) Quantitative analysis of light-phase restricted feeding reveals metabolic dyssynchrony in mice. Int J Obes 37(6):843–852

554. Jiang P, Turek FW (2017) Timing of meals: when is as critical as what and how much. Am J Physiol Endocrinol Metab 312(5):E369–E380
555. Catterson JH, Khericha M, Dyson MC, Vincent AJ, Callard R, Haveron SM et al (2018) Short-term, intermittent fasting induces long-lasting gut health and TOR-independent lifespan extension. Curr Biol 28(11):1714–1724.e1714
556. Serra M, Marongiu F, Pisu MG, Laconi E (2019) Time-restricted feeding delays the emergence of the age-associated, neoplastic-prone tissue landscape. Aging (Albany NY) 11(11):3851–3863
557. Sherman H, Frumin I, Gutman R, Chapnik N, Lorentz A, Meylan J et al (2011) Long-term restricted feeding alters circadian expression and reduces the level of inflammatory and disease markers. J Cell Mol Med 15(12):2745–2759
558. Gabel K, Hoddy KK, Haggerty N, Song J, Kroeger CM, Trepanowski JF et al (2018) Effects of 8-hour time restricted feeding on body weight and metabolic disease risk factors in obese adults: a pilot study. Nutr Healthy Aging 4(4):345–353
559. Gabel K, Hoddy KK, Varady KA (2019) Safety of 8-h time restricted feeding in adults with obesity. Appl Physiol Nutr Metab 44(1):107–109
560. Jamshed H, Beyl RA, Della Manna DL, Yang ES, Ravussin E, Peterson CM (2019) Early time-restricted feeding improves 24-hour glucose levels and affects markers of the circadian clock, aging, and autophagy in humans. Nutrients 11(6). pii: E1234. https://doi.org/10.3390/nu11061234
561. Kaur S, Thankachan S, Begum S, Blanco-Centurion C, Sakurai T, Yanagisawa M et al (2008) Entrainment of temperature and activity rhythms to restricted feeding in orexin knock out mice. Brain Res 1205:47–54
562. Marinac CR, Sears DD, Natarajan L, Gallo LC, Breen CI, Patterson RE (2015) Frequency and circadian timing of eating may influence biomarkers of inflammation and insulin resistance associated with breast cancer risk. PLoS One 10(8):e0136240. https://doi.org/10.1371/journal.pone.0136240
563. Mihaylova MM, Cheng CW, Cao AQ, Tripathi S, Mana MD, Bauer-Rowe KE et al (2018) Fasting activates fatty acid oxidation to enhance intestinal stem cell function during homeostasis and aging. Cell Stem Cell 22(5):769–778.e764
564. Schafer MJ, Mazula DL, Brown AK, White TA, Atkinson E, Pearsall VM et al (2019) Late-life time-restricted feeding and exercise differentially alter healthspan in obesity. Aging Cell 18(4):e12966. https://doi.org/10.1111/acel.12966
565. Wang H, van Spyk E, Liu Q, Geyfman M, Salmans ML, Kumar V et al (2017) Time-restricted feeding shifts the skin circadian clock and alters UVB-induced DNA damage. Cell Rep 20(5):1061–1072
566. Wei M, Brandhorst S, Shelehchi M, Mirzaei H, Cheng CW, Budniak J et al (2017) Fasting-mimicking diet and markers/risk factors for aging, diabetes, cancer, and cardiovascular disease. Sci Transl Med 9(377). pii: eaai8700. https://doi.org/10.1126/scitranslmed.aai8700
567. Lettieri-Barbato D, Cannata SM, Casagrande V, Ciriolo MR, Aquilano K (2018) Time-controlled fasting prevents aging-like mitochondrial changes induced by persistent dietary fat overload in skeletal muscle. PLoS One 13(5):e0195912. https://doi.org/10.1371/journal.pone.0195912
568. Chaix A, Zarrinpar A, Miu P, Panda S (2014) Time-restricted feeding is a preventative and therapeutic intervention against diverse nutritional challenges. Cell Metab 20(6):991–1005
569. Duncan MJ, Smith JT, Narbaiza J, Mueez F, Bustle LB, Qureshi S et al (2016) Restricting feeding to the active phase in middle-aged mice attenuates adverse metabolic effects of a high-fat diet. Physiol Behav 167:1–9
570. Hatori M, Vollmers C, Zarrinpar A, DiTacchio L, Bushong EA, Gill S et al (2012) Time-restricted feeding without reducing caloric intake prevents metabolic diseases in mice fed a high-fat diet. Cell Metab 15(6):848–860
571. Zarrinpar A, Chaix A, Yooseph S, Panda S (2014) Diet and feeding pattern affect the diurnal dynamics of the gut microbiome. Cell Metab 20(6):1006–1017

572. Jakubowicz D, Barnea M, Wainstein J, Froy O (2013) High caloric intake at breakfast vs. dinner differentially influences weight loss of overweight and obese women. Obesity (Silver Spring) 21(12):2504–2512
573. Villanueva JE, Livelo C, Trujillo AS, Chandran S, Woodworth B, Andrade L et al (2019) Time-restricted feeding restores muscle function in Drosophila models of obesity and circadian-rhythm disruption. Nat Commun 10(1):2700. https://doi.org/10.1038/s41467-019-10563-9
574. Hartman SJ, Marinac CR, Natarajan L, Patterson RE (2015) Lifestyle factors associated with cognitive functioning in breast cancer survivors. Psychooncology 24(6):669–675
575. Patterson RE, Laughlin GA, LaCroix AZ, Hartman SJ, Natarajan L et al (2015) Intermittent fasting and human metabolic health. J Acad Nutr Diet 115(8):1203–1212
576. Lin JD, Liu C, Li S (2008) Integration of energy metabolism and the mammalian clock. Cell Cycle 7(4):453–457
577. Oishi K, Miyazaki K, Ishida N (2002) Functional CLOCK is not involved in the entrainment of peripheral clocks to the restricted feeding: entrainable expression of mPer2 and BMAL1 mRNAs in the heart of clock mutant mice on Jcl:ICR background. Biochem Biophys Res Commun 298(2):198–202
578. Adamovich Y, Rousso-Noori L, Zwighaft Z, Neufeld-Cohen A, Golik M, Kraut-Cohen J (2014) Circadian clocks and feeding time regulate the oscillations and levels of hepatic triglycerides. Cell Metab 19(2):319–330
579. Thaiss CA, Zeevi D, Levy M, Zilberman-Schapira G, Suez J, Tengeler AC et al (2014) Transkingdom control of microbiota diurnal oscillations promotes metabolic homeostasis. Cell 159(3):514–529
580. Vollmers C, Gill S, DiTacchio L, Pulivarthy SR, Le HD, Panda S (2009) Time of feeding and the intrinsic circadian clock drive rhythms in hepatic gene expression. Proc Natl Acad Sci U S A 106(50):21453–21458
581. Comperatore CA, Stephan FK (1987) Entrainment of duodenal activity to periodic feeding. J Biol Rhythm 2(3):227–242
582. Saito M, Murakami E, Nishida T, Fujisawa Y, Suda M (1976) Circadian rhythms of digestive enzymes in the small intestine of the rat. II. Effects of fasting and refeeding. J Biochem 80(3):563–568
583. Albrecht U (2017) The circadian clock, metabolism and obesity. Obes Rev 18(Suppl 1):25–33
584. Manoogian ENC, Panda S (2017) Circadian rhythms, time-restricted feeding, and healthy aging. Ageing Res Rev 39:59–67
585. Madeo F, Carmona-Gutierrez D, Hofer SJ, Kroemer G (2019) Caloric restriction mimetics against age-associated disease: targets, mechanisms, and therapeutic potential. Cell Metab 29(3):592–610
586. Hanjani NA, Vafa M (2018) Protein restriction, epigenetic diet, intermittent fasting as new approaches for preventing age-associated diseases. Int J Prev Med 9:58. https://doi.org/10.4103/ijpvm.IJPVM_397_16
587. Choi IY, Lee C, Longo VD (2017) Nutrition and fasting mimicking diets in the prevention and treatment of autoimmune diseases and immunosenescence. Mol Cell Endocrinol 455:4–12
588. Hoshino S, Kobayashi M, Higami Y (2018) Mechanisms of the anti-aging and prolongevity effects of caloric restriction: evidence from studies of genetically modified animals. Aging (Albany NY) 10(9):2243–2251
589. Hirota T, Lee JW, St John PC, Sawa M, Iwaisako K, Noguchi T et al (2012) Identification of small molecule activators of cryptochrome. Science 337(6098):1094–1097
590. Humphries PS, Bersot R, Kincaid J, Mabery E, McCluskie K, Park T et al (2016) Carbazole-containing sulfonamides and sulfamides: discovery of cryptochrome modulators as antidiabetic agents. Bioorg Med Chem Lett 26(3):757–760
591. Chun SK, Jang J, Chung S, Yun H, Kim NJ, Jung JW et al (2014) Identification and validation of cryptochrome inhibitors that modulate the molecular circadian clock. ACS Chem Biol 9(3):703–710

592. Jang J, Chung S, Choi Y, Lim HY, Son Y, Chun SK et al (2018) The cryptochrome inhibitor KS15 enhances E-box-mediated transcription by disrupting the feedback action of a circadian transcription-repressor complex. Life Sci 200:49–55
593. Chun SK, Chung S, Kim HD, Lee JH, Jang J, Kim J et al (2015) A synthetic cryptochrome inhibitor induces anti-proliferative effects and increases chemosensitivity in human breast cancer cells. Biochem Biophys Res Commun 467(2):441–446
594. Meng QJ, Maywood ES, Bechtold DA, Lu WQ, Li J, Gibbs JE et al (2010) Entrainment of disrupted circadian behavior through inhibition of casein kinase 1 (CK1) enzymes. Proc Natl Acad Sci U S A 107(34):15240–15245
595. Carrino M, Quotti Tubi L, Fregnani A, Canovas Nunes S, Barilà G, Trentin L et al (2019) Prosurvival autophagy is regulated by protein kinase CK1 alpha in multiple myeloma. Cell Death Discov 5:98. https://doi.org/10.1038/s41420-019-0179-1
596. Xiong Y, Zhou L, Su Z, Song J, Sun Q, Liu SS et al (2019) Longdaysin inhibits Wnt/β--catenin signaling and exhibits antitumor activity against breast cancer. Onco Targets Ther 12:993–1005
597. Tsakiri EN, Gaboriaud-Kolar N, Iliaki KK, Tchoumtchoua J, Papanagnou ED, Chatzigeorgiou S et al (2017) The indirubin derivative 6-bromoindirubin-3'-oxime activates proteostatic modules, reprograms cellular bioenergetic pathways, and exerts antiaging effects. Antioxid Redox Signal 27(14):1027–1047
598. Vougogiannopoulou K, Ferandin Y, Bettayeb K, Myrianthopoulos V, Lozach O, Fan Y et al (2008) Soluble 3′,6-substituted indirubins with enhanced selectivity toward glycogen synthase kinase −3 alter circadian period. J Med Chem 51(20):6421–6431
599. Zhao H, Meng W, Li Y, Liu W, Fu B, Yang Y et al (2016) The protective effects of CHIR99021 against oxidative injury in LO2 cells. Pharmazie 71(11):629–635
600. Naujok O, Lentes J, Diekmann U, Davenport C, Lenzen S (2014) Cytotoxicity and activation of the Wnt/beta-catenin pathway in mouse embryonic stem cells treated with four GSK3 inhibitors. BMC Res Notes 7:273. https://doi.org/10.1186/1756-0500-7-273
601. Oh J, Kim Y, Che L, Kim JB, Chang GE, Cheong E et al (2017) Regulation of cAMP and GSK3 signaling pathways contributes to the neuronal conversion of glioma. PLoS One 12(11):e0178881. https://doi.org/10.1371/journal.pone.0178881
602. Andrabi M, Andrabi MM, Kunjunni R, Sriwastva MK, Bose S, Sagar R et al (2019) Lithium acts to modulate abnormalities at behavioral, cellular and molecular levels in sleep deprivation induced mania-like behavior. Bipolar Disord. https://doi.org/10.1111/bdi.12838. [Epub ahead of print]
603. Iwahana E, Akiyama M, Miyakawa K, Uchida A, Kasahara J, Fukunaga K et al (2004) Effect of lithium on the circadian rhythms of locomotor activity and glycogen synthase kinase-3 protein expression in the mouse suprachiasmatic nuclei. Eur J Neurosci 19(8):2281–2287
604. Li J, Lu WQ, Beesley S, Loudon AS, Meng QJ (2012) Lithium impacts on the amplitude and period of the molecular circadian clockwork. PLoS One 7(3):e33292. https://doi.org/10.1371/journal.pone.0033292
605. Sawai Y, Okamoto T, Muranaka Y, Nakamura R, Matsumura R, Node K et al (2019) In vivo evaluation of the effect of lithium on peripheral circadian clocks by real-time monitoring of clock gene expression in near-freely moving mice. Sci Rep 9(1):10909. https://doi.org/10.1038/s41598-019-47053-3
606. He B, Nohara K, Park N, Park YS, Guillory B, Zhao Z et al (2016) The small molecule nobiletin targets the molecular oscillator to enhance circadian rhythms and protect against metabolic syndrome. Cell Metab 23(4):610–621
607. Lee YS, Cha BY, Choi SS, Choi BK, Yonezawa T, Teruya T et al (2013) Nobiletin improves obesity and insulin resistance in high-fat diet-induced obese mice. J Nutr Biochem 24(1):156–162
608. Matsuzaki K, Miyazaki K, Sakai S, Yawo H, Nakata N, Moriguchi S et al (2008) Nobiletin, a citrus flavonoid with neurotrophic action, augments protein kinase A-mediated phosphoryla-

tion of the AMPA receptor subunit, GluR1, and the postsynaptic receptor response to glutamate in murine hippocampus. Eur J Pharmacol 578(2–3):194–200
609. Goan YG, Wu WT, Liu CI, Neoh CA, Wu YJ (2019) Involvement of mitochondrial dysfunction, endoplasmic reticulum stress, and the PI3K/AKT/mTOR pathway in nobiletin-induced apoptosis of human bladder cancer cells. Molecules 24(16). pii: E2881 https://doi.org/10.3390/molecules24162881
610. Keshtkar S, Kaviani M, Jabbarpour Z, Geramizadeh B, Motevaseli E, Nikeghbalian S et al (2019) Protective effect of nobiletin on isolated human islets survival and function against hypoxia and oxidative stress-induced apoptosis. Sci Rep 9(1):11701. https://doi.org/10.1038/s41598-019-48262-6
611. Nohara K, Nemkov T, D'Alessandro A, Yoo SH, Chen Z (2019) Coordinate regulation of cholesterol and bile acid metabolism by the clock modifier nobiletin in metabolically challenged old mice. Int J Mol Sci 20(17). pii: E4281. https://doi.org/10.3390/ijms20174281
612. Nohara K, Mallampalli V, Nemkov T, Wirianto M, Yang J, Ye Y et al (2019) Nobiletin fortifies mitochondrial respiration in skeletal muscle to promote healthy aging against metabolic challenge. Nat Commun 10(1):3923. https://doi.org/10.1038/s41467-019-11926-y
613. Oyama Y, Bartman CM, Gile J, Sehrt D, Eckle T (2018) The circadian PER2 enhancer nobiletin reverses the deleterious effects of midazolam in myocardial ischemia and reperfusion injury. Curr Pharm Des 24(28):3376–3383
614. Mao Q, Liang X, Wu Y, Lu Y (2019) Nobiletin protects against myocardial injury and myocardial apoptosis following coronary microembolization via activating PI3K/Akt pathway in rats. Naunyn Schmiedeberg's Arch Pharmacol 392(9):1121–1130
615. Zhang BF, Jiang H, Chen J, Guo X, Li Y, Hu Q et al (2019) Nobiletin ameliorates myocardial ischemia and reperfusion injury by attenuating endoplasmic reticulum stress-associated apoptosis through regulation of the PI3K/AKT signal pathway. Int Immunopharmacol 73:98–107
616. Yabuki Y, Ohizumi Y, Yokosuka A, Mimaki Y, Fukunaga K (2014) Nobiletin treatment improves motor and cognitive deficits seen in MPTP-induced Parkinson model mice. Neuroscience 259:126–141
617. Byun JK, Choi YK, Kang YN, Jang BK, Kang KJ, Jeon YH et al (2015) Retinoic acid-related orphan receptor alpha reprograms glucose metabolism in glutamine-deficient hepatoma cells. Hepatology 61(3):953–964
618. Helleboid S, Haug C, Lamottke K, Zhou Y, Wei J, Daix S et al (2014) The identification of naturally occurring neoruscogenin as a bioavailable, potent, and high-affinity agonist of the nuclear receptor RORα (NR1F1). J Biomol Screen 19(3):399–406
619. Kong J, Shepel PN, Holden CP, Mackiewicz M, Pack AI, Geiger JD (2002) Brain glycogen decreases with increased periods of wakefulness: implications for homeostatic drive to sleep. J Neurosci 22(13):5581–5587
620. Sun Q, Chen L, Gao M, Jiang W, Shao F, Li J et al (2012) Ruscogenin inhibits lipopolysaccharide-induced acute lung injury in mice: involvement of tissue factor, inducible NO synthase and nuclear factor (NF)-κB. Int Immunopharmacol 12(1):88–93
621. Solt LA, Kumar N, Nuhant P, Wang Y, Lauer JL, Liu J et al (2011) Suppression of TH17 differentiation and autoimmunity by a synthetic ROR ligand. Nature 472(7344):491–494
622. Chen Z, Yoo SH, Park YS, Kim KH, Wei S, Buhr E et al (2012) Identification of diverse modulators of central and peripheral circadian clocks by high-throughput chemical screening. Proc Natl Acad Sci U S A 109(1):101–106
623. Zhang Y, Wang JH, Zhang YY, Wang YZ, Wang J, Zhao Y et al (2016) Deletion of interleukin-6 alleviated interstitial fibrosis in streptozotocin-induced diabetic cardiomyopathy of mice through affecting TGFβ1 and miR-29 pathways. Sci Rep 6:23010. https://doi.org/10.1038/srep23010
624. Kumar N, Kojetin DJ, Solt LA, Kumar KG, Nuhant P, Duckett DR et al (2011) Identification of SR3335 (ML-176): a synthetic RORα selective inverse agonist. ACS Chem Biol 6(3):218–222

625. Gibbs JE, Blaikley J, Beesley S, Matthews L, Simpson KD, Boyce SH et al (2012) The nuclear receptor REV-ERBα mediates circadian regulation of innate immunity through selective regulation of inflammatory cytokines. Proc Natl Acad Sci U S A 109(2):582–587
626. Grant D, Yin L, Collins JL, Parks DJ, Orband-Miller LA, Wisely GB et al (2010) GSK4112, a small molecule chemical probe for the cell biology of the nuclear heme receptor Rev-erbα. ACS Chem Biol 5(10):925–932
627. Banerjee S, Wang Y, Solt LA, Griffett K, Kazantzis M, Amador A et al (2014) Pharmacological targeting of the mammalian clock regulates sleep architecture and emotional behaviour. Nat Commun 5:5759. https://doi.org/10.1038/ncomms6759
628. De Mei C, Ercolani L, Parodi C, Veronesi M, Lo Vecchio C, Bottegoni G et al (2015) Dual inhibition of REV-ERBβ and autophagy as a novel pharmacological approach to induce cytotoxicity in cancer cells. Oncogene 34(20):2597–2608
629. Chang HC, Guarente L (2013) SIRT1 mediates central circadian control in the SCN by a mechanism that decays with aging. Cell 153(7):1448–1460
630. Pifferi F, Dal-Pan A, Languille S, Aujard F (2013) Effects of resveratrol on daily rhythms of locomotor activity and body temperature in young and aged grey mouse lemurs. Oxidative Med Cell Longev 2013:187301. https://doi.org/10.1155/2013/187301
631. Bellet MM, Nakahata Y, Boudjelal M, Watts E, Mossakowska DE, Edwards KA et al (2013) Pharmacological modulation of circadian rhythms by synthetic activators of the deacetylase SIRT1. Proc Natl Acad Sci U S A 110(9):3333–3338
632. Yao H, Sundar IK, Huang Y, Gerloff J, Sellix MT, Sime PJ et al (2015) Disruption of Sirtuin 1-Mediated control of circadian molecular clock and inflammation in chronic obstructive pulmonary disease. Am J Respir Cell Mol Biol 53(6):782–792
633. Ye T, Wei L, Shi J, Jiang K, Xu H, Hu L et al (2019) Sirtuin1 activator SRT2183 suppresses glioma cell growth involving activation of endoplasmic reticulum stress pathway. BMC Cancer 19(1):706. https://doi.org/10.1186/s12885-019-5852-5
634. Doi M, Murai I, Kunisue S, Setsu G, Uchio N, Tanaka R et al (2016) Gpr176 is a Gz-linked orphan G-protein-coupled receptor that sets the pace of circadian behaviour. Nat Commun 7:10583. https://doi.org/10.1038/ncomms10583
635. Jones KA, Hatori M, Mure LS, Bramley JR, Artymyshyn R, Hong SP et al (2013) Small-molecule antagonists of melanopsin-mediated phototransduction. Nat Chem Biol 9(10):630–635
636. Lee JW, Hirota T, Kumar A, Kim NJ, Irle S, Kay SA (2015) Development of small-molecule cryptochrome stabilizer derivatives as modulators of the circadian clock. ChemMedChem 10(9):1489–1497
637. Nangle S, Xing W, Zheng N (2013) Crystal structure of mammalian cryptochrome in complex with a small molecule competitor of its ubiquitin ligase. Cell Res 23(12):1417–1419
638. Hirota T, Okano T, Kokame K, Shirotani-Ikejima H, Miyata T, Fukada Y (2002) Glucose down-regulates Per1 and Per2 mRNA levels and induces circadian gene expression in cultured Rat-1 fibroblasts. J Biol Chem 277(46):44244–44251
639. Haus EL, Smolensky MH (2013) Shift work and cancer risk: potential mechanistic roles of circadian disruption, light at night, and sleep deprivation. Sleep Med Rev 17(4):273–284
640. Kochan DZ, Ilnytskyy Y, Golubov A, Deibel SH, McDonald RJ, Kovalchuk O (2015) Circadian disruption-induced microRNAome deregulation in rat mammary gland tissues. Oncoscience 2(4):428–442
641. Kochan DZ, Kovalchuk O (2015) Circadian disruption and breast cancer: an epigenetic link? Oncotarget 6(19):16866–16882
642. Hirota T, Lee JW, Lewis WG, Zhang EE, Breton G, Liu X et al (2010) High-throughput chemical screen identifies a novel potent modulator of cellular circadian rhythms and reveals CKIα as a clock regulatory kinase. PLoS Biol 8(12):e1000559. https://doi.org/10.1371/journal.pbio.1000559
643. Reischl S, Vanselow K, Westermark PO, Thierfelder N, Maier B, Herzel H et al (2007) Beta-TrCP1-mediated degradation of PERIOD2 is essential for circadian dynamics. J Biol Rhythm 22(5):375–386

644. Vanselow K, Vanselow JT, Westermark PO, Reischl S, Maier B, Korte T et al (2006) Differential effects of PER2 phosphorylation: molecular basis for the human familial advanced sleep phase syndrome (FASPS). Genes Dev 20(19):2660–2672
645. Lee JW, Hirota T, Peters EC, Garcia M, Gonzalez R, Cho CY et al (2011) A small molecule modulates circadian rhythms through phosphorylation of the period protein. Angew Chem Int Ed Engl 50(45):10608–10611
646. Isojima Y, Nakajima M, Ukai H, Fujishima H, Yamada RG, Masumoto KH et al (2009) CKIepsilon/delta-dependent phosphorylation is a temperature-insensitive, period-determining process in the mammalian circadian clock. Proc Natl Acad Sci U S A 106(37):15744–15749
647. Yagita K, Yamanaka I, Koinuma S, Shigeyoshi Y, Uchiyama Y (2009) Mini screening of kinase inhibitors affecting period-length of mammalian cellular circadian clock. Acta Histochem Cytochem 42(3):89–93
648. Takano A, Uchiyama M, Kajimura N, Mishima K, Inoue Y, Kamei Y et al (2004) A missense variation in human casein kinase I epsilon gene that induces functional alteration and shows an inverse association with circadian rhythm sleep disorders. Neuropsychopharmacology 29(10):1901–1909
649. Xu Y, Padiath QS, Shapiro RE, Jones CR, Wu SC, Saigoh N et al (2005) Functional consequences of a CKIdelta mutation causing familial advanced sleep phase syndrome. Nature 434(7033):640–644
650. Jope RS, Johnson GV (2004) The glamour and gloom of glycogen synthase kinase-3. Trends Biochem Sci 29(2):95–102
651. Mansour HA, Talkowski ME, Wood J, Pless L, Bamne M, Chowdari KV et al (2005) Serotonin gene polymorphisms and bipolar I disorder: focus on the serotonin transporter. Ann Med 37(8):590–602
652. Mansour HA, Wood J, Chowdari KV, Dayal M, Thase ME, Kupfer DJ et al (2005) Circadian phase variation in bipolar I disorder. Chronobiol Int 22(3):571–584
653. Sahar S, Zocchi L, Kinoshita C, Borrelli E, Sassone-Corsi P (2010) Regulation of BMAL1 protein stability and circadian function by GSK3beta-mediated phosphorylation. PLoS One 5(1):e8561. https://doi.org/10.1371/journal.pone.0008561
654. Spengler ML, Kuropatwinski KK, Comas M, Gasparian AV, Fedtsova N, Gleiberman AS et al (2012) Core circadian protein CLOCK is a positive regulator of NF-κB-mediated transcription. Proc Natl Acad Sci U S A 109(37):E2457–E2465
655. Yin L, Wang J, Klein PS, Lazar MA (2006) Nuclear receptor Rev-erbalpha is a critical lithium-sensitive component of the circadian clock. Science 311(5763):1002–1005
656. Iitaka C, Miyazaki K, Akaike T, Ishida N (2005) A role for glycogen synthase kinase-3beta in the mammalian circadian clock. J Biol Chem 280(33):29397–29402
657. Mancinelli R, Carpino G, Petrungaro S, Mammola CL, Tomaipitinca L, Filippini A et al (2017) Multifaceted roles of GSK-3 in cancer and autophagy-related diseases. Oxidative Med Cell Longev 2017:4629495. https://doi.org/10.1155/2017/4629495
658. Centers for Disease Control and Prevention (2017) QuickStats: death rates for chronic liver disease and cirrhosis, by sex and age group — national vital statistics system, United States, 2000 and 2015. Morb Mort Wkly Rep (MMWR). https://www.cdc.gov/mmwr/volumes/66/wr/mm6638a9.htm
659. Marciano DP, Chang MR, Corzo CA, Goswami D, Lam VQ, Pascal BD et al (2014) The therapeutic potential of nuclear receptor modulators for treatment of metabolic disorders: PPARγ, RORs, and Rev-erbs. Cell Metab 19(2):193–208
660. Zhao X, Hirota T, Han X, Cho H, Chong LW, Lamia K et al (2016) Circadian amplitude regulation via FBXW7-targeted REV-ERBα degradation. Cell 165(7):1644–1657
661. He B, Chen Z (2016) Molecular targets for small-molecule modulators of circadian clocks. Curr Drug Metab 17(5):503–512
662. Mulvihill EE, Burke AC, Huff MW (2016) Citrus flavonoids as regulators of lipoprotein metabolism and atherosclerosis. Annu Rev Nutr 36:275–299

663. Walle T (2007) Methoxylated flavones, a superior cancer chemopreventive flavonoid subclass? Semin Cancer Biol 17(5):354–362
664. Huang H, Li L, Shi W, Liu H, Yang J, Yuan X et al (2016) The multifunctional effects of nobiletin and its metabolites. Evid Based Complement Alternat Med 2016:2918796. https://doi.org/10.1155/2016/2918796
665. Evans M, Prathie SP, Guthrie N (2012) Bioavailability of citrus polymethoxylated flavones and their biological role in metabolic syndrome and hyperlipidemia. In: Noreddin A (ed) Readings in advanced pharmacokinetics - theory, methods and applications. IntechOpen, London. ISBN: 978-953-51-0533-6
666. Bass J, Lazar MA (2016) Circadian time signatures of fitness and disease. Science 354(6315):994–999
667. Nohara K, Shin Y, Park N, Jeong K, He B, Koike N et al (2015) Ammonia-lowering activities and carbamoyl phosphate synthetase 1 (Cps1) induction mechanism of a natural flavonoid. Nutr Metab (Lond) 12:23. https://doi.org/10.1186/s12986-015-0020-7
668. Shinozaki A, Misawa K, Ikeda Y, Haraguchi A, Kamagata M, Tahara Y et al (2017) Potent effects of flavonoid nobiletin on amplitude, period, and phase of the circadian clock rhythm in PER2::LUCIFERASE mouse embryonic fibroblasts. PLoS One 12(2):e0170904. https://doi.org/10.1371/journal.pone.0170904
669. Bonney S, Kominsky D, Brodsky K, Eltzschig H, Walker L, Eckle T (2013) Cardiac Per2 functions as novel link between fatty acid metabolism and myocardial inflammation during ischemia and reperfusion injury of the heart. PLoS One 8(8):e71493. https://doi.org/10.1371/journal.pone.0071493
670. Eckle T, Kewley EM, Brodsky KS, Tak E, Bonney S, Gobel M et al (2014) Identification of hypoxia-inducible factor HIF-1A as transcriptional regulator of the A2B adenosine receptor during acute lung injury. J Immunol 192(3):1249–1256
671. Shah MS, Brownlee M (2016) Molecular and cellular mechanisms of cardiovascular disorders in diabetes. Circ Res 118(11):1808–1829
672. Mamontova A, Séguret-Macé S, Esposito B, Chaniale C, Bouly M, Delhaye-Bouchaud N et al (1998) Severe atherosclerosis and hypoalphalipoproteinemia in the staggerer mouse, a mutant of the nuclear receptor RORalpha. Circulation 98(24):2738–2743
673. Kang HS, Okamoto K, Kim YS, Takeda Y, Bortner CD, Dang H et al (2011) Nuclear orphan receptor TAK1/TR4-deficient mice are protected against obesity-linked inflammation, hepatic steatosis, and insulin resistance. Diabetes 60(1):177–188
674. Kang HS, Okamoto K, Takeda Y, Beak JY, Gerrish K, Bortner CD et al (2011) Transcriptional profiling reveals a role for RORalpha in regulating gene expression in obesity-associated inflammation and hepatic steatosis. Physiol Genomics 43(13):818–828
675. Tesmer LA, Lundy SK, Sarkar S, Fox DA (2008) Th17 cells in human disease. Immunol Rev 223:87–113

Chapter 10
The Challenge of Antidepressant Therapeutics in Alzheimer's Disease

Madia Lozupone, Maddalena La Montagna, Francesca D'Urso, Carla Piccininni, Angelo Rinaldi, Massimiliano Beghi, Cesare Maria Cornaggia, Rodolfo Sardone, Vincenzo Solfrizzi, Antonio Daniele, Davide Seripa, Gianluigi Giannelli, Antonello Bellomo, and Francesco Panza

1 Introduction

The prominent symptomatic feature of Alzheimer's disease (AD) is memory dysfunction, caused by amyloid-β peptide (Aβ) deposition and neurofibrillary tangles (NFTs). However, neuropsychiatric symptoms (NPS) are often present in nearly all patients with AD, above all at the onset of the disease [1, 2]. The prevalence of depression in dementia ranges from 16% to 45%, depending on diagnostic definitions used, study designs, and the sample populations [3]. Recognition and treatment of depression is important considering the negative consequences, such as higher rates of disability, impaired quality of life, and greater mortality (for example, for suicide) [4]. A recent study found that the suicide rate among persons with

Madia Lozupone and Maddalena La Montagna contributed equally with all other contributors.

M. Lozupone
Neurodegenerative Disease Unit, Department of Basic Medical Sciences, Neuroscience and Sense Organs, University of Bari Aldo Moro, Bari, Italy

M. La Montagna · F. D'Urso · C. Piccininni · A. Rinaldi · A. Bellomo
Psychiatric Unit, Department of Clinical and Experimental Medicine, University of Foggia, Foggia, Italy

M. Beghi
Department of Mental Health, AUSL Romagna, Ravenna, Italy

C. M. Cornaggia
School of Medicine and Surgery, University of Milano-Bicocca, Milan, Italy

R. Sardone · G. Giannelli · F. Panza (✉)
Unit of Epidemiological Research on Aging, National Institute of Gastroenterology 'Saverio de Bellis', Research Hospital, Castellana Grotte, Bari, Italy
e-mail: geriat.dot@uniba.it

P. C. Guest (ed.), *Reviews on New Drug Targets in Age-Related Disorders*, Advances in Experimental Medicine and Biology 1260,
https://doi.org/10.1007/978-3-030-42667-5_10

dementia was 9.3 per 100,000 person-years and 424.5/100,000 person-years among those diagnosed in the past 12 months [5].

It is not clear if depression is a prodromal symptom that comes before cognitive and memory deficits or if the two factors are independent [6]. Depressive symptoms are prevalent among older adults without dementia and may be early manifestations of AD before the onset of mild cognitive impairment (MCI). Depression symptoms are multi-factorial and may work synergistically with Aβ (hallmark of AD pathology that can be measured *in vivo*) and related processes to affect cognition over time in older adults. It is probable that depression symptoms in cognitively healthy older individuals together with brain amyloid, the biological marker of AD, could trigger changes in memory and thinking over time, as recently shown [7]. Other risk factors could modify the relationship between depression and cognitive deficits, including brain metabolism dysfunction (e.g., due to mitochondrial dysregulation or diabetes mellitus) and volume changes in the hippocampus, the part of the brain associated with learning and formation of new memories [8]. The objective of the present review is to highlight the etiology of depression in AD patients, in comparison to that in depression alone, and to speculate on more appropriate and alternative treatments.

2 Common Mechanisms Underlying Depression and Alzheimer's Disease

Other mechanisms, including tau-mediated neurodegeneration, hypertension, hypercortisolemia and inflammation, may be involved and need to be investigated, including the presence of neuropathological markers such as the tau protein, Aβ, and vascular disease [9]. Clarifying the relationships between the AD-related pathology and NPS of AD patients may be useful for elucidating the underlying pathophysiological process. We believe that steady overproduction of Aβ in AD and in chronic central nervous system (CNS) diseases such as depression may represent an attempt of the brain to mitigate or repair the associated neuronal damage/insult [8]. To demonstrate this, when 270 cognitively normal older subjects were followed longitudinally for 1–5 years, early anxious-depressive symptoms were found to be

V. Solfrizzi
C. Frugoni' Internal and Geriatric Medicine and Memory Unit, University of Bari Aldo Moro, Bari, Italy

A. Daniele
Institute of Neurology, Catholic University of Sacred Heart, Rome, Italy

Institute of Neurology, Fondazione Policlinico Universitario A. Gemelli IRCCS, Rome, Italy

D. Seripa
Research Laboratory, Complex Structure of Geriatrics, Department of Medical Sciences, Fondazione IRCCS Casa Sollievo della Sofferenza, Foggia, Italy

associated with brain Aβ burden [10, 11]. To summarize, the sudden reductions of brain Aβ levels with potent anti-Aβ drugs may worsen cognition and exacerbate NPS, such as depressive symptoms and suicide ideation. Since anti-Aβ drugs have repeatedly been associated with worsening of cognition and NPS in both AD and MCI patients, and even in cognitively unimpaired subjects, we conclude that both AD-associated neuronal death and neuropsychiatric disturbances may cause an increase in brain Aβ levels and not *vice versa* [12].

In addition, the study of neurotrasmission may provide a key to understanding the pathogenesis of NPS and how neurotransmitters could interact with Aβ peptide cascade. In particular, glutamatergic transmission in late-life major depression and AD could represent an overlap in the signaling transduction mechanisms [13]. A cellular mechanism of Aβ-dependent neuronal dysfunction that can be active before plaque formation, specifically an impairment of glutamate homeostasis, might underlie the disturbed plasticity of hippocampal synapses [14]. Moreover, depression is associated with selective loss of noradrenergic cells in the locus coeruleus and possibly the serotonergic raphe nuclei regions of the brain, along with severe loss of serotonin receptors and serotonin transporter binding [15–17]. Furthermore, dysfunction of hypothalamic-pituitary-adrenal (HPA) axis and decrease of neurotrophic factors and chronic inflammation are crucial, and both AD and depressive disorders have these characteristics [18, 19]. Brain-derived neurotrophic factor (BDNF) and serotonin (5-HT) regulate synaptic plasticity, neurogenesis, and neuronal survival. The increased levels of glucocorticoids and pro-inflammatory cytokines, and the reduced levels of BDNF and 5-HT could increase Aβ toxicity and hippocampal atrophy, underlying the transition from depression to AD [20].

However, the profile of cognitive deficits, including depressive symptoms, differs according to the brain regions affected by neurodegeneration. The most profound BDNF deficits have been reported to occur in the hippocampus, parietal, entorhinal and frontal cortex for AD. The same regions are involved in the pathophysiology of depressive symptoms [21]. Given that synaptic loss is the major correlate of cognitive impairment over the presence of plaques or tangles, there is a recent view of AD as a "synaptic pathology" [22]. Aβ monomers are normally generated and secreted at firing synapses, and are not toxic but neuroprotective as they have an active role in synaptic regulation [23] and are crucial for neuronal function [24].

In contrast, the increase in the levels of soluble Aβ-oligomers can lead to an impairment in cAMP-response-element-binding protein (CREB) activation in the brains of patients with AD and in mouse models of AD. Furthermore, Aβ-oligomers are known to alter signal transduction pathways crucial for learning and memory processes [25, 26] and change the trafficking of N-methyl-D-aspartate (NMDA) type of glutamate receptors [27]. In summary, altered levels of BDNF in AD are downstream of Aβ-accumulation and could be related to Aβ–induced dysregulation of CREB transcription [25, 28]. This is important as BDNF appears to be an important underlying molecule behind the restitution of a normal cognitive phenotype in animal models of chronic stress [29].

The activation of the HPA axis and hypersecretion of glucocorticoids leading to a reduction of hippocampal volume, are known factors involved in the incidence of AD and cognitive impairment [30]. Structures involved in the control of the physiological status of an organism are susceptible to modulation by chronic stress. In particular, the hippocampus is altered by prolonged exposure to aversive situations [31]. Thus, alterations in those pathways could play an important role in the etiology of both depression and AD. Also late-life depression itself is linked to downregulation of neurotrophic factors such as BDNF [32], activation of neuroinflammatory pathways, and increased secretion of pro-inflammatory cytokines and C-reactive protein [33].

The term "gut-brain axis" refers to a crosstalk between the brain and the gut involving multiple overlapping pathways, including the autonomic, neuroendocrine, and immune systems as well as bacterial metabolites and neuromodulatory molecules [34]. Dysbiosis and impaired gut microbiota have been demonstrated to exert regulatory functions on inflammation and oxidative stress and represent a pathogenetic contributors shared by AD, depression, and type 2 diabetes mellitus [35–37], three disorders characterized by a prooxidative and proinflammatory condition. Finally, a resting-state functional magnetic resonance imaging (fMRI) study revealed decreased functional connectivity in the right middle frontal gyrus, and precentral and postcentral gyri, which fits a network dysfunction model in AD with depression that is distinct from major depressive disorder and AD separately [38]. However, the link between depression and AD is difficult to elucidate completely due to the presence of disease heterogeneity, multifactorial elements and secondary mechanisms, as well as the presence of systemic diseases.

3 Antidepressant Drug Mechanisms in Alzheimer's Disease

Several studies using animal models of AD have led to the possibility that selective serotonin reuptake inhibitors (SSRIs) may reduce Aβ plaque burden and cognitive impairment, presumably by shifting the balance from pro-amyloidogenic toward non-amyloidogenic processing of the amyloid precursor protein (APP) [39–41]. A dose-dependent relationship on such an effect was demonstrated in animal studies using the antidepressants citalopram and fluoxetine [42, 43]. Recently, some studies have highlighted the possibility that fluoxetine is neuroprotective against Aβ induced neurodegeneration also via a paracrine signaling mechanism mediated by transforming-growth-factor-β1 (TGF-β1), that does not depend on the serotonin transporter blockade. Deficits of TGF-β1 are thought to contribute to cognitive deficits and depressive disorder treatment resistance in AD patients, by increasing Aβ accumulation and promoting 'amyloid-related depression' [44].

In particular, the activation of serotonin receptors (5-HT-Rs) by SSRIs leads to the activation of extracellular signal-regulated kinases that increase α-secretase activity (the non-amyloidogenic pathway) and reduce β- and

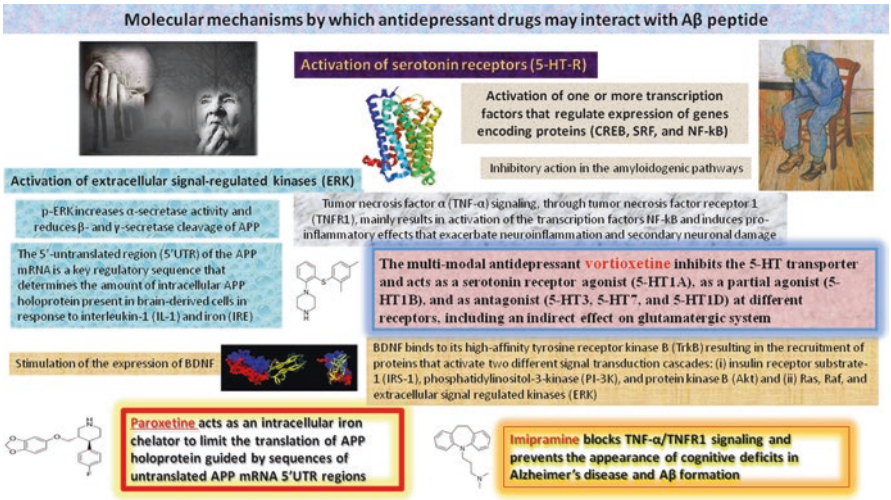


Fig. 10.1 Molecular mechanisms by which antidepressant drugs may interact with amyloid-β (Aβ) peptide

γ-secretase cleavage of APP which lead to production of the amyloidogenic form of Aβ (Fig. 10.1). The 5-HT signaling pathway activates one or more transcription factors that regulate expression of genes encoding proteins such as CREB, serum response factor (SRF), and nuclear factor kappa B (NF-kB), leading to an inhibitory action in the amyloidogenic pathways. The 5′-untranslated region (5′UTR) of the APP mRNA contains a key regulatory sequence that determines the amount of intracellular APP holoprotein present in brain-derived cells in response to interleukin-1 (IL-1) and iron. The antidepressant paroxetine acts to limit the translation of the APP holoprotein by chelating iron [20]. However, among all SSRIs, paroxetine is the most anticholinergic with deleterious cognitive effects in the elderly population and it failed to mitigate Aβ pathology in two other AD animal model studies [45, 46].

SSRIs can modulate other key inflammatory factors, such as tumor necrosis factor alpha (TNF-α), IL-1β and IL-6, and oxidative stress, as well as prevent microglia activation in the brain [47, 48]. TNFα, through the tumor necrosis factor receptor 1 (TNFR1), mainly results in activation of the transcription factor NF-kB and induces pro-inflammatory effects that exacerbate neuroinflammation and secondary neuronal damage. The antidepressant imipramine was found to block TNF-α/TNFR1 signaling and prevented the appearance of cognitive deficits and Aβ formation in an AD mouse model [20].

In addition, the anti-inflammatory effects of SSRIs are reflected in the brain by preventing elevated serotonin reuptake from the synapse as a result of elevated cytokine signaling or by direct action on reducing cytokine production [49, 50].

This highlights a possible therapeutic mechanism of action in slowing the global inflammatory response seen as a result of AD progression [51]. Furthermore, SSRIs may also be effective in lowering oxidative stress. This may be due to either increased endogenous antioxidant capacity or activity, or through possible antioxidant properties of the drugs themselves suggesting an alternative protective action [52, 53].

SSRI-mediated modulation of neuroinflammation and neurodegeneration could therefore explain the favorable outcomes of patients with AD under long-term SSRI treatment, although their potential role as AD therapeutics had not yet been determined [54]. In fact, according to another point of view, the antidepressants sertraline and paroxetine may increase calcium influx and induce mitochondrial damage-mediated apoptosis, causing astrocyte dysfunction. This impairment may be involved in the pathogenesis of neurodegenerative diseases [55].

In addition, there is evidence for beneficial effects of certain antiglutamatergic drugs, such as memantine, against depression AD [56, 57]. Memantine exhibited antidepressant-like effects in some, but not all, animal models of depression [58–60]. In addition to memantine, ketamine is a non-competitive NMDA receptor antagonist with a similar, although non-identical, pharmacologic profile (Fig. 10.2). The antidepressant and precognitive effects of Ketamine are not entirely shared with memantine. It has been demonstrated that the major antidepressant effect of ketamine is mediated by activation of the mechanistic target of rapamycin (mTOR) pathway, which is an atypical Ser/Thr kinase and a central controller of protein synthesis required for formation of new synaptic connec-

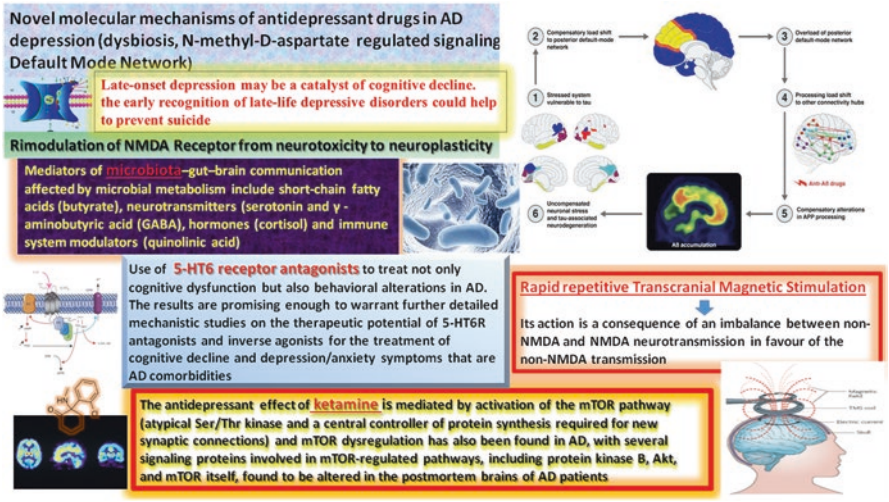


Fig. 10.2 Molecular mechanisms by which antidepressant drugs may act in Alzheimer's disease (AD) depression other than classical ones (dysbiosis, N-methyl-D-aspartate regulated signaling, Default Mode Network)

tions [61]. mTOR dysregulation has also been found in AD, with effects on several signaling proteins involved in mTOR-regulated pathways, including protein kinase B, Akt, and mTOR itself, as found in the postmortem brains of AD patients [62, 63]. Therefore, the elucidation of further molecular mechanisms underlying the rapid antidepressant effect of the potent NMDA antagonist ketamine has offered a reasonably strong scientific rationale to encourage testing to determine whether or not ketamine or any of its metabolites have procognitive effects in AD patients [64].

4 The Classical Treatment and the Antidepressant Challenge in Alzheimer's Disease

Therefore, there are no clear pharmacological treatment algorithms for depression in AD and there are no drugs approved by the Food and Drug Administration (FDA) for treatment the NPS in this disorder. SSRIs are considered the first line of treatment for late-life depressive disorders without dementia, but the evidence in support of the use of antidepressants to treat depression in dementia is not clear [65]. In particular, systematic reviews and meta-analyses in people with depression and AD have been discussed elsewhere with conflicting data on the effect of antidepressants compared to placebo [66]. Regarding classical antidepressant medications, sertraline was the one most frequently studied in several randomized controlled trials (RCTs). Unfortunately, such clinical studies are difficult to interpret to different study methods, heterogeneous patient populations, variability in outcome measures, and complicating factor of multi-treatment approaches [66].

Emerging evidence on the neurobiological substrates of depression in AD has led to the study of repositioned and novel antidepressant drugs in dementia as an alternative to classical antidepressant treatment (Fig. 10.2) [67]. A growing number of preclinical and clinical studies have supported the use of 5-HT6 receptor antagonists to treat not only the cognitive dysfunctions but also the behavioral alterations in AD [17, 68]. The results are promising enough to warrant further detailed mechanistic studies on the therapeutic potential of 5-HT6 receptor antagonists and inverse agonists for the treatment of the cognitive decline and the depression or anxiety symptoms that are AD co-morbidities [69–71]. Other studies with S47445, a novel positive allosteric modulator of alpha-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid (AMPA) receptors have been conducted in healthy elderly subjects. S47445 had a good safety profile and was well-tolerated, enhanced functional connectivity between brain networks involved in cognition (working memory, attention and Default Mode Networks), and increased glutamate concentrations in the posterior cingulate cortex. Although treatment with S47445 was also found to be safe and well-tolerated by patients with mild to moderate AD, the compound did not show significant benefits over placebo on measurements of cognition or depressive symptoms [72, 73].

The multi-modal antidepressant vortioxetine has a unique pharmacologic profile by inhibiting the 5-HT transporter and acting as a 5-HT1A receptor agonist, a partial agonist of 5-HT1B receptors, and as an antagonist of 5-HT3, 5-HT7 and 5-HT1D receptors, as well as an indirect effect on the glutamatergic system. An interesting observation was the potential utility of vortioxetine in the treatment of older depressed patients, as these individuals showed significant improvements in cognitive functions such as verbal memory and executive functioning [74, 75]. In depressed patients diagnosed with mild AD, vortioxetine was shown to significantly improve cognition when it was compared to other conventional antidepressants [76]. However, despite a favorable recommendation of the Psychopharmacologic Drug Advisory Committee, the FDA rejected a label expansion for vortioxetine that included claims of its specific effectiveness in the treatment of cognitive dysfunction in patients with major depressive disorder.

Few studies have tested the use of mood stabilizers as a treatment for depressive symptoms in dementia. Lithium has been shown to inhibit the activity of glycogen synthase kinase-3 (GSK-3), which is considered as a mediator of AD-related hyperphosphorylation of tau, which leads to the formation of paired helical filaments and neurofibrillary tangles. Therefore, lithium has been suggested to have potential therapeutic effects in AD [77, 78]. There are also limited data available for the role of carbamazepine in treating NPS in AD in smaller doses [79]. Aripiprazole is the first antipsychotic agent developed that is a partial dopamine D2 receptor agonist, as well as a serotonin 5HT2A receptor antagonist and a 5HT1A receptor partial agonist. However, this has not approved to treat dementia-related NPS and has received a FDA “black box” warning [80]. Thus further work at the preclinical and clinical levels should be performed to test the safety and efficacy of these compounds for the treatment of depressed patients with AD.

Finally, several studies have reported the usefulness of microbiota manipulation in the treatment of both AD and depressive disorders [81, 82]. In recent years, special attention has been given by researchers to probiotic supplementation with promising results [83, 84]. A recent study discovered that the medial prefrontal cortex may be involved in the differences between the antibiotic-treated and untreated mice. Within this region, it was the excitatory neurons, which are involved in learning and memory, which appeared to be involved in this response. However, in the absence of gut microbes, these neurons failed to form appropriate dendritic spines that are required in forming and maintaining synaptic connectivity [85].

5 Conclusions

Initiation of pharmacological interventions in AD should occur after non-pharmacological approaches, cognitive enhancers, and comprehensive assessment of medical and environmental factors has been completed [65]. The findings of the present review have raised the possibility that the etiology of the symptoms of

depression in individuals with dementia may be fundamentally different than the etiology of symptoms of depression in individuals without dementia. This may be a consequence of the damage and neuronal loss involved in the dementia process, although some mechanisms could be shared such as dysbiosis, neuroinflammation, NMDA-regulated signaling.

The impact of SSRIs on Aβ plaque formation rather than plaque clearance highlights the importance of potentially prescribing such antidepressants as early as possible in cognitively intact individuals, before the beginning of plaque deposition [39]. This is due to the fact that early-onset depression is a risk factor and late-onset depression may be a catalyst of cognitive decline. Furthermore the early recognition of late-life depressive disorders could help to prevent suicide.

Nevertheless, robust large RCTs are still needed to better account for the effect of SSRIs and their optimal doses and duration of use. A substantial body of evidence has suggested the involvement of common NMDA-regulated signaling pathways in depression and AD and a possible overlap of disease neurobiology [14]. Specifically, although the study ESKETINTRD3005 of ketamine treatment in patients aged 65 years and older showed no significant effect in this age-stratified population, this was only a short term study and the FDA concluded that there was no evidence of a waning treatment effect with increasing age [86]. However, in the absence of relevant data to late-life depression, it is not clear whether older patients would benefit similarly to younger ones. Instead, the new multimodal antidepressant vortioxetine appeared to be more promising, particularly in depression with MCI and in mild AD patients with depressive symptoms [74, 76].

Interestingly, in a recent study, older age was associated with increased odds of rapid repetitive transcranial magnetic stimulation (rTMS) response trajectory, although the sample only included adults under the age of 65 years. This finding is consistent with previous work which showed that rTMS was more effective for older adults and for late-life depression when rTMS coils at higher stimulus intensities were used (i.e., at 120% resting motor threshold) [87–89]. The effect of rTMS in AD patients appears to be similar to that seen in normal subjects after ketamine treatment, as a consequence of greater non-NMDA compared to NMDA neurotransmission [90]. Future studies should also take into account that mediators of microbiota–gut–brain communication affected by microbial metabolism include short-chain fatty acids (e.g., butyrate), neurotransmitters [e.g., serotonin and γ -aminobutyric acid (GABA)], hormones (e.g., cortisol), and immune system modulators (e.g., quinolinic acid) [91].

In conclusion, future research needs to explore the impact of earlier treatment on AD prevention or onset delay in depressed versus non-depressed cognitively intact individuals and further well-powered RCTs are needed before significant changes in current clinical practice can be introduced [92]. The greatest impact will most likely come through the use of personalized medicine approaches, with diagnosis and therapy based on an individual's unique genotype, phenotype and environmental exposure history [93]. The evaluation of genetic background may be also important as recent studies have shown the importance of genotyping for antidepressant treat-

ment success in AD. Gene polymorphisms may lead to modulation of antidepressant action [94] as well as the molecular processes underlying the pathology and trajectory of AD, highlighting the potential importance of taking such factors into account in RCTs designed for the treatment of depression in dementia and AD [95]. Depression symptoms themselves may be among the early changes in the preclinical stages of dementia syndromes and, just as importantly, these stages represent a clinical window of opportunity for closely monitoring at-risk individuals and potentially introducing interventions to prevent or slow cognitive decline [7].

References

1. Boccardi V, Conestabile Della Staffa M, Baroni M, Ercolani S, Croce MF, Ruggiero C et al (2017) Prevalence and correlates of behavioral disorders in old age subjects with cognitive impairment: results from the ReGAl project. J Alzheimers Dis 60(4):1275–1283
2. Lauriola M, Mangiacotti A, D'Onofrio G, Cascavilla L, Paris F, Ciccone F et al (2018) Late-life depression versus amnestic mild cognitive impairment: Alzheimer's disease incidence in 4 years of follow-up. Dement Geriatr Cogn Disord 46(3–4):140–153
3. Panza F, Frisardi V, Capurso C, D'Introno A, Colacicco AM, Imbimbo BP et al (2010) Late-life depression, mild cognitive impairment, and dementia: possible continuum? Am J Geriatr Psychiatry 18(2):98–116
4. Rehm J, Shield KD (2019) Global burden of disease and the impact of mental and addictive disorders. Curr Psychiatry Rep 21(2):10. https://doi.org/10.1007/s11920-019-0997-0
5. Annor FB, Bayakly RA, Morrison RA, Bryan MJ, Gilbert LK, Ivey-Stephenson AZ et al (2019) Suicide among persons with dementia, Georgia, 2013 to 2016. J Geriatr Psychiatry Neurol 32(1):31–39
6. Dekker AD, Strydom A, Coppus AM, Nizetic D, Vermeiren Y, Naudé PJ et al (2015) Behavioural and psychological symptoms of dementia in down syndrome: early indicators of clinical Alzheimer's disease? Cortex 73:36–61
7. Gatchel JR, Rabin JS, Buckley RF, Locascio JJ, Quiroz YT, Harvard Aging Brain Study et al (2019) Longitudinal association of depression symptoms with cognition and cortical amyloid among community-dwelling older adults. JAMA Netw Open 2(8):e198964. https://doi.org/10.1001/jamanetworkopen.2019.8964
8. Panza F, Lozupone M, Logroscino G, Imbimbo BP (2019) A critical appraisal of amyloid-β-targeting therapies for Alzheimer disease. Nat Rev Neurol 15(2):73–88
9. Sierksma AS, Van Den Hove DL, Steinbusch HW, Prickaerts J (2010) Major depression, cognitive dysfunction and Alzheimer's disease: is there a link? Eur J Pharmacol 626(1):72–82
10. Donovan NJ, Locascio JJ, Marshall GA, Gatchel J, Hanseeuw BJ, Harvard Aging Brain Study et al (2018) Longitudinal association of amyloid β and anxious depressive symptoms in cognitively normal older adults. Am J Psychiatry 175(6):530–537
11. Chung JK, Plitman E, Nakajima S, Chow TW, Chakravarty MM, Alzheimer's Disease Neuroimaging Initiative et al (2015) Lifetime history of depression predicts increased amyloid-beta accumulation in patients with mild cognitive impairment. J Alzheimers Dis 45(3):907–919
12. Panza F, Lozupone M, Bellomo A, Imbimbo BP (2019) Do anti-amyloid-β drugs affect neuropsychiatric status in Alzheimer's disease patients? Ageing Res Rev 55:100948. https://doi.org/10.1016/j.arr.2019.100948
13. Khundakar AA, Aj T (2015) Neuropathology of depression in Alzheimer's disease: current knowledge and the potential for new treatments. J Alzheimers Dis 44(1):27–41

14. Zott B, Simon MM, Hong W, Unger F, Chen-Engerer HJ, Frosch MP et al (2019) A vicious cycle of β amyloid-dependent neuronal hyperactivation. Science 365(6453):559–565
15. Zweig RM, Ross CA, Hedreen JC (1988) The neuropathology of aminergic nuclei in alzheimer's disease. Ann Neurol 24(2):233–242
16. Forstl H, Burns A, Luthert P (1992) Clinical and neuropathological correlates of depression in Alzheimer's disease. Psychol Med 22(4):877–884
17. Ferrero H, Solas M, Francis PT, Ramirez MJ (2017) Serotonin 5-HT6 receptor antagonists in Alzheimer's disease: therapeutic rationale and current development status. CNS Drugs 31(1):19–32
18. Swaab DF, Bao AM, Lucassen PJ (2005) The stress system in the human brain in depression and neurodegeneration. Ageing Res Rev 4(2):141–194
19. Wuwongse S, Chang RC, Law AC (2010) The putative neurodegenerative links between depression and Alzheimer's disease. Prog Neurobiol 91:362–375
20. Cassano T, Calcagnini S, Carbone A, Bukke VN, Orkisz S, Villani R et al (2019) Pharmacological treatment of depression in Alzheimer's disease: a challenging task. Front Pharmacol 10:1067. https://doi.org/10.3389/fphar.2019.01067
21. Hock C, Heese K, Hulette C, Rosenberg C, Otten U (2000) Region specific neurotrophin imbalances in Alzheimer disease: decreased levels of brain-derived neurotrophic factor and increased levels of nerve growth factor in hippocampus and cortical areas. Arch Neurol 57(6):846–851
22. Terry RD, Masliah E, Salmon DP, Butter N, Deteresa R, Hill R et al (1991) Physical basis of cognitive alterations in Alzheimer's disease: synapse loss is the major correlate of cognitive impairment. Ann Neurol 30(4):572–580
23. Giuffrida ML, Caraci F, Pignataro B, Cataldo S, De Bona P, Bruno V et al (2009) Beta-amyloid monomers are neuroprotective. J Neurosci 29(34):10582–10587
24. Abramov E, Dolev I, Fogel H, Ciccotosto GD, Ruff E, Slutsky I (2009) Amyloid-beta as a positive endogenous regulator of release probability at hippocampal synapses. Nat Neurosci 12(12):1567–1576
25. Caccamo A, Maldonado MA, Bokov AF, Majumder S, Oddo S (2010) CBP gene transfer increases BDNF levels and ameliorates learning and memory deficits in a mouse model of Alzheimer's disease. Proc Natl Acad Sci U S A 107(52):22687–22692
26. Bartolotti N, Segura L, Lazarov O (2016) Diminished CRE-induced plasticity is linked to memory deficits in familial Alzheimer's disease mice. J Alzheimers Dis 50(2):477–489
27. Snyder EM, Nong Y, Almeida CG, Paul S, Moran T, Choi E et al (2005) Regulation of NMDA receptor trafficking by amyloid-beta. Nat Neurosci 8(8):1051–1058
28. Pugazhenthi S, Wang M, Pham S, Sze CI, Eckman CB (2011) Downregulation of CREB expression in Alzheimer's brain and in abetatreated rat hippocampal neurons. Mol Neurodegener 6:60. https://doi.org/10.1186/1750-1326-6-60
29. Miranda M, Morici JF, Zanoni MB, Bekinschtein P (2019) Brain-derived neurotrophic factor: a key molecule for memory in the healthy and the pathological brain. Front Cell Neurosci 13:363. https://doi.org/10.3389/fncel.2019.00363
30. Wilson RS, Schneider JA, Boyle PA, Arnold SE, Tang Y, Bennett DA (2007) Chronic distress and incidence of mild cognitive impairment. Neurology 68(24):2085–2092
31. Kim EJ, Pellman B, Kim JJ (2015) Stress effects on the hippocampus: a critical review. Learn Mem 22:411–416
32. Neto FL, Borges G, Torres-Sanchez S, Mico JA, Berrocoso E (2011) Neurotrophins role in depression neurobiology: a review of basic and clinical evidence. Curr Neuropharmacol 9(4):530–552
33. Herbert J, Lucassen PJ (2016) Depression as a risk factor for Alzheimer's disease: genes, steroids, cytokines and neurogenesis - what do we need to know? Front Neuroendocrinol 41:153–171
34. Quigley EMM (2017) Microbiota-brain-gut axis and neurodegenerative diseases. Curr Neurol Neurosci Rep 17(12):94. https://doi.org/10.1007/s11910-017-0802-6

35. Kelly JR, Kennedy PJ, Cryan JF, Dinan TG, Clarke G, Hyland NP (2015) Breaking down the barriers: the gut microbiome, intestinal permeability and stress-related psychiatric disorders. Front Cell Neurosci 9:392. https://doi.org/10.3389/fncel.2015.00392
36. Tilg H, Moschen AR (2014) Microbiota and diabetes: an evolving relationship. Gut 63(9):1513–1521
37. Lozupone M, Seripa D, Stella E, La Montagna M, Solfrizzi V, Quaranta N et al (2017) Innovative biomarkers in psychiatric disorders: a major clinical challenge in psychiatry. Expert Rev Proteomics 14(9):809–824
38. Guo Z, Liu X, Hou H, Wei F, Liu J, Chen X (2016) Abnormal degree centrality in Alzheimer's disease patients with depression: a resting-state functional magnetic resonance imaging study. Exp Gerontol 79:61–66
39. Mdawar B, Ghossoub E, Khoury R (2020) Selective serotonin reuptake inhibitors and Alzheimer's disease. Neural Regen Res 15(1):41–46
40. Shen F, Smith JA, Chang R, Bourdet DL, Tsuruda PR, Obedencio GP et al (2011) 5-HT(4) receptor agonist mediated enhancement of cognitive function in vivo and amyloid precursor protein processing in vitro: a pharmacodynamic and pharmacokinetic assessment. Neuropharmacology 61(1–2):69–79
41. Fisher JR, Wallace CE, Tripoli DL, Sheline YI, Cirrito JR (2016) Redundant Gs-coupled serotonin receptors regulate amyloid-β metabolism in vivo. Mol Neurodegener 11:45. https://doi.org/10.1186/s13024-016-0112-5
42. Cirrito JR, Disabato BM, Restivo JL, Verges DK, Goebel WD, Sathyan A et al (2011) Serotonin signaling is associated with lower amyloid-b levels and plaques in transgenic mice and humans. Proc Natl Acad Sci U S A 108(36):14968–14973
43. Wang J, Zhang Y, Xu H, Zhu S, Wang H, He J et al (2014) Fluoxetine improves behavioral performance by suppressing the production of soluble beta-amyloid in APP/PS1 mice. Curr Alzheimer Res 11(7):672–680
44. Caraci F, Spampinato SF, Morgese MG, Tascedda F, Salluzzo MG, Giambirtone MC et al (2018) Neurobiological links between depression and AD: the role of TGF-β1 signaling as a new pharmacological target. Pharmacol Res 130:374–384
45. Olesen LO, Bouzinova EV, Severino M, Sivasaravanaparan M, Hasselstrom JB, Finsen B et al (2016) Behavioural phenotyping of APPswe/PS1δE9 mice: age-rrelated changes and effect of long-term paroxetine treatment. PLoS One 11:e0165144. https://doi.org/10.1371/journal.pone.0165144
46. Severino M, Sivasaravanaparan M, Olesen LO, von Linstow CU, Metaxas A, Bouzinova EV et al (2018) Established amyloid-β pathology is unaffected by chronic treatment with the selective serotonin reuptake inhibitor paroxetine. Alzheimers Dement (N Y) 4:215–223
47. Walker FR (2013) A critical review of the mechanism of action for the selective serotonin reuptake inhibitors: do these drugs possess anti-inflammatory properties and how relevant is this in the treatment of depression? Neuropharmacology 67:304–317
48. Alboni S, Poggini S, Garofalo S, Milior G, El Hajj H, Lecours C et al (2016) Fluoxetine treatment affects the inflammatory response and microglial function according to the quality of the living environment. Brain Behav Immun 58:261–271
49. Kempuraj D, Thangavel R, Selvakumar GP, Zaheer S, Ahmed ME, Raikwar SP et al (2017) Brain and peripheral atypical inflammatory mediators potentiate neuroinflammation and neurodegeneration. Front Cell Neurosci 11:216. https://doi.org/10.3389/fncel.2017.00216
50. Jeon SW, Kim YK (2016) Neuroinflammation and cytokine abnormality in major depression: cause or consequence in that illness? World J Psychiatry 6(3):283–293
51. Galecki P, Mossakowska-Wojcik J, Talarowska M (2018) The anti-inflammatory mechanism of antidepressants - SSRIs, SNRIs. Prog Neuropsychopharmacol Biol Psychiatry 80(Pt C):291–294
52. Lee SY, Lee SJ, Han C, Patkar AA, Masand PS, Pae CU (2013) Oxidative/nitrosative stress and antidepressants: targets for novel antidepressants. Prog Neuro-Psychopharmacol Biol Psychiatry 46:224–235

53. Chang CC, Lee CT, Lan TH, Ju PC, Hsieh YH, Lai TJ (2015) Effects of antidepressant treatment on total antioxidant capacity and free radical levels in patients with major depressive disorder. Psychiatry Res 230(2):575–580
54. Elsworthy RJ, Aldred S (2019) Depression in Alzheimer's disease: an alternative role for selective serotonin reuptake inhibitors? J Alzheimers Dis 69(3):651–661
55. Then CK, Liu KH, Liao MH, Chung KH, Wang JY, Shen SC et al (2017) Antidepressants, sertraline and paroxetine, increase calcium influx and induce mitochondrial damage mediated apoptosis of astrocytes. Oncotarget 8(70):115490–115502
56. Takahashi K, Kong Q, Lin Y, Stouffer N, Schulte DA, Lai L et al (2015) Restored glial glutamate transporter EAAT2 function as a potential therapeutic approach for Alzheimer's disease. J Exp Med 212(3):319–332
57. Danysz W, Parsons CG, Mobius HJ, Stoffler A, Quack G (2000) Neuroprotective and symptomatological action of memantine relevant for Alzheimer's disease – a unified glutamatergic hypothesis on the mechanism of action. Neurotox Res 2(2–3):85–97
58. Amidfar M, Kim YK, Wiborg O (2018) Effectiveness of memantine on depression-like behavior, memory deficits and brain mRNA levels of BDNF and TrkB in rats subjected to repeated unpredictable stress. Pharmacol Rep 70(3):600–606
59. Zhang K, Yamaki VN, Wei Z, Zheng Y, Cai X (2017) Differential regulation of GluA1 expression by ketamine and memantine. Behav Brain Res 316:152–159
60. Takahashi K, Nakagawasa O, Nemoto W, Kadota S, Isono J, Odaira T et al (2018) Memantine ameliorates depressive-like behaviors by regulating hippocampal cell proliferation and neuroprotection in olfactory bulbectomized mice. Neuropharmacology 137:141–155
61. Gong R, Park CS, Abbassi NR, Tang SJ (2006) Roles of glutamate receptors and the mammalian target of rapamycin (mTOR) signaling pathway in activity-dependent dendritic protein synthesis in hippocampal neurons. J Biol Chem 281(27):18802–18815
62. Li N, Lee B, Liu RJ, Banasr M, Dwyer JM, Iwata M et al (2010) mTOR-dependent synapse formation underlies the rapid antidepressant effects of NMDA antagonists. Science 329(5994):959–964
63. Hoeffer CA, Klann E (2010) mTOR signaling: at the crossroads of plasticity, memory and disease. Trends Neurosci 33(2):67–75
64. Smalheiser NR (2019) Ketamine: a neglected therapy for Alzheimer disease. Front Aging Neurosci 11:186. https://doi.org/10.3389/fnagi.2019.00186
65. Cummings J, Ritter A, Rothenberg K (2019) Advances in management of neuropsychiatric syndromes in neurodegenerative diseases. Curr Psychiatry Rep 21(8):79. https://doi.org/10.1007/s11920-019-1058-4
66. Lozupone M, La Montagna M, D'Urso F, Piccininni C, Sardone R, Dibello V et al (2018) Pharmacotherapy for the treatment of depression in patients with Alzheimer's disease: a treatment-resistant depressive disorder. Expert Opin Pharmacother 19(8):823–842
67. Geda YE, Schneider LS, Gitlin LN, Miller DS, Smith GS, Neuropsychiatric Syndromes Professional Interest Area of ISTAART et al (2013) Neuropsychiatric syndromes professional interest area of istaart. Neuropsychiatric symptoms in alzheimer's disease: past progress and anticipation of the future. Alzheimers Dement 9(5):602–608
68. Li X, Wang Q, Hu T, Wang Y, Zhao J, Lu J et al (2017) A tricyclic antidepressant, amoxapine, reduces amyloid-β generation through multiple serotonin receptor 6-mediated targets. Sci Rep 7(1):4983. https://doi.org/10.1038/s41598-017-04144-3
69. Grychowska K, Satała G, Kos T, Partyka A, Colacino E, Chaumont-Dubel S et al (2016) Novel 1H-Pyrrolo[3,2-c]quinoline based 5-HT6 receptor antagonists with potential application for the treatment of cognitive disorders associated with Alzheimer's disease. ACS Chem Neurosci 7(7):972–983
70. Yun HM, Park KR, Kim EC, Kim S, Hong JT (2015) Serotonin 6 receptor controls Alzheimer's disease and depression. Oncotarget 6(29):26716–26728
71. Upton N, Chuang TT, Hunter AJ, Virley DJ (2008) 5-HT6 receptor antagonists as novel cognitive enhancing agents for Alzheimer's disease. Neurotherapeutics 5(3):458–469

72. Lee K, Goodman L, Fourie C, Shenk S, Leitch B, Montgomery JM (2016) AMPA receptors as therapeutic targets for neurological disorders. Adv Protein Chem Struct Biol 103:203–261
73. Bernard K, Gouttefangeas S, Bretin S, Galtier S, Robert P, Holthoff-Detto V et al (2019) A 24-week double-blind placebo-controlled study of the efficacy and safety of the AMPA modulator S47445 in patients with mild to moderate Alzheimer's disease and depressive symptoms. Alzheimers Dement (N Y) 5:231–240
74. Katona C, Hansen T, Olsen CK (2012) A randomized, double-blind, placebo controlled, duloxetine referenced, fixed-dose study comparing the efficacy and safety of Lu AA21004 in elderly patients with major depressive disorder. Int Clin Psychopharmacol 27(4):215–223
75. McIntyre RS, Harrison J, Loft H, Jacobson W, Olsen CK (2016) The effects of vortioxetine on cognitive function in patients with major depressive disorder: a meta-analysis of three randomized controlled trials. Int J Neuropsychopharmacol. pii: pyw055. https://doi.org/10.1093/ijnp/pyw055. [Epub ahead of print]
76. Cumbo E, Cumbo S, Torregrossa S, Migliore D (2019) Treatment effects of vortioxetine on cognitive functions in mild Alzheimer's disease patients with depressive symptoms: a 12 month, open-label, observational study. J Prev Alzheimers Dis 6(3):192–197
77. Noble W, Planel E, Zehr C, Olm V, Meyerson J, Suleman F et al (2005) Inhibition of glycogen synthase kinase-3 by lithium correlates with reduced tauopathy and degeneration in vivo. Proc Natl Acad Sci U S A 102(19):6990–6995
78. Engel T, Goñi-Oliver P, Lucas JJ, Avila J, Hernández F (2006) Chronic lithium administration to FTDP-17 tau and GSK-3beta overexpressing mice prevents tau hyperphosphorylation and neurofibrillary tangle formation, but pre-formed neurofibrillary tangles do not revert. J Neurochem 99(6):1445–1455
79. Tariot PN, Erb R, Podgorski CA, Cox C, Patel S, Jakimovich L et al (1998) Efficacy and tolerability of carbamazepine for agitation and aggression in dementia. Am J Psychiatry 155(1):54–61
80. Streim JE, Porsteinsson AP, Breder CD, Swanink R, Marcus R, McQuade R et al (2008) A randomized, double blind, placebo-controlled study of aripiprazole for the treatment of psychosis in nursing home patients with Alzheimer disease. Am J Geriatr Psychiatry 16(7):537–550
81. Bambling M, Edwards SC, Hall S, Vitetta L (2017) A combination of probiotics and magnesium orotate attenuate depression in a small SSRI resistant cohort: an intestinal anti-inflammatory response is suggested. Inflammopharmacology 25(2):271–274
82. Panza F, Lozupone M, Solfrizzi V, Watling M, Imbimbo BP (2019) Time to test antibacterial therapy in Alzheimer's disease. Brain 142(10):2905–2929
83. Leblhuber F, Steiner K, Schuetz B, Fuchs D, Gostner JM (2018) Probiotic supplementation in patients with Alzheimer's dementia-an explorative intervention study. Curr Alzheimer Res 15(12):1106–1113
84. Pirbaglou M, Katz J, de Souza RJ, Stearns JC, Motamed M, Ritvo P (2016) Probiotic supplementation can positively affect anxiety and depressive symptoms: a systematic review of randomized controlled trials. Nutr Res 36(9):889–898
85. Chu C, Murdock MH, Jing D, Won TH, Chung H, Kressel AM et al (2019) The microbiota regulate neuronal function and fear extinction learning. Nature 574(7779):543–548
86. Kim J, Farchione T, Potter A, Chen Q, Temple R (2019) Esketamine for treatment-resistant depression - first FDA-approved antidepressant in a new class. N Engl J Med 381(1):1–4
87. Kaster TS, Downar J, Vila-Rodriguez F, Thorpe KE, Feffer K, Noda Y et al (2019) Trajectories of response to dorsolateral prefrontal rTMS in major depression: a THREE-D study. Am J Psychiatry 176(5):367–375
88. Levkovitz Y, Harel EV, Roth Y, Braw Y, Most D, Katz LN et al (2009) Deep transcranial magnetic stimulation over the prefrontal cortex: evaluation of antidepressant and cognitive effects in depressive patients. Brain Stimul 2(4):188–200
89. Kaster TS, Daskalakis ZJ, Noda Y, Knyahnytska Y, Downar J, Rajji TK et al (2018) Efficacy, tolerability, and cognitive effects of deep transcranial magnetic stimulation for late-life depression: a prospective randomized controlled trial. Neuropsychopharmacology 43(11):2231–2238

90. Di Lazzaro V, Oliviero A, Pilato F, Saturno E, Dileone M, Marra C et al (2004) Motor cortex hyperexcitability to transcranial magnetic stimulation in Alzheimer's disease. J Neurol Neurosurg Psychiatry 75(4):555–559
91. Valles-Colomer M, Falony G, Darzi Y, Tigchelaar EF, Wang J, Tito RY et al (2019) The neuroactive potential of the human gut microbiota in quality of life and depression. Nat Microbiol 4(4):623–632
92. Zissimopoulos J, Crimmins E, St Clair P (2014) The value of delaying Alzheimer's disease onset. Forum Health Econ Policy 18(1):25–39
93. Willard HW (2009) Organization, variation and expression of the human genome as a foundation of genomic and personalized medicine. In: Willard HW, Ginsburg GS (eds) Genomic and personalized medicine. Academic Press, London, UK, pp 4–21. ISBN: 9780123822277
94. Paroni G, Seripa D, Fontana A, D'Onofrio G, Gravina C, Urbano M et al (2017) Klotho gene and selective serotonin reuptake inhibitors: response to treatment in late-life major depressive disorder. Mol Neurobiol 54(2):1340–1351
95. Lozupone M, Panza F, Stella E, La Montagna M, Bisceglia P, Miscio G et al (2017) Pharmacogenetics of neurological and psychiatric diseases at older age: has the time come? Expert Opin Drug Metab Toxicol 13(3):259–277

Chapter 11
Anxiolytic Terpenoids and Aromatherapy for Anxiety and Depression

S. Agatonovic-Kustrin, E. Kustrin, V. Gegechkori, and D. W. Morton

1 Introduction

Although advances in medicine have immensely improved our health and life expectancy and scientific developments have given us a better quality of life and living conditions, constantly developing technologies, increased psychological pressures, and high expectations are increasingly affecting mental and emotional health, especially anxiety. Anxiety disorders are the most common mental health disorders and can have debilitating consequences for affected individuals. Existing drug therapies for anxiety disorders are limited by their potential for abuse, delay of therapeutic effect, dependence, and tolerance. Therefore, safe and evidence-based complementary or alternative therapies may offer significant benefits in the care of patients with anxiety disorders.

Recent studies have shown that aromatherapy with essential oils can reduce stress, anxiety, depression and promote physical and mental well-being. Essential oils have been used for centuries as traditional medicines. The long history of the therapeutic use of essential oils suggests that they may indeed be effective. Various

S. Agatonovic-Kustrin (✉) · D. W. Morton
Department of Pharmaceutical and Toxicological Chemistry named after Arzamastsev of the Institute of Pharmacy, I.M. Sechenov First Moscow State Medical University (Sechenov University), Moscow, Russia

School of Pharmacy and Biomedical Sciences, La Trobe Institute for Molecular Science, La Trobe University, Bendigo, VIC, Australia

E. Kustrin
Department of Creative Arts and English, La Trobe University, Bendigo, VIC, Australia

V. Gegechkori
Department of Pharmaceutical and Toxicological Chemistry named after Arzamastsev of the Institute of Pharmacy, I.M. Sechenov First Moscow State Medical University (Sechenov University), Moscow, Russia

P. C. Guest (ed.), *Reviews on New Drug Targets in Age-Related Disorders*,
Advances in Experimental Medicine and Biology 1260,
https://doi.org/10.1007/978-3-030-42667-5_11

plant-derived essential oils have traditionally been used to treat a variety of mental disorders. Although the use of aromatherapy for mental health is not a new discovery, recently there has been increasing academic interest in aromatherapy as an alternative to normal drug therapy as it has proven to have high efficacy in reducing stress and improving mood disorders [1]. Evidence that inhaled or topically applied essential oils enter the bloodstream and exhibit measurable psychological effects, indicates that the effects of essential oils are pharmacological and not just psychological [2]. This review focusses on the use of aromatherapy in mental health, for the treatment of stress, anxiety, depression, sleep disorders, and cognitive disorders.

Essential oils have very specific pharmacologic properties that can be used to produce specific physiological responses in the prevention and treatment of several diseases including cardiovascular disease, diabetes, Alzheimer's disease, and cancer, in addition to their bioactivity as antibacterial, antiviral and antioxidant agents [3]. However, aromatherapy appears to be most effective in the treatment of medical conditions that involve emotional and cognitive information processing [4], and the activity of the autonomous nervous system [5].

Essential oils can enter the body through inhalation, ingestion, or absorption through the mucous membranes and skin. The fastest direct route into the body and into the bloodstream is by inhalation [6]. Essential oils delivered via the inhalation route may exert psychological effects, because the olfactory bulb has limbic inputs in the amygdala and hippocampus that are associated with emotion and memory [7]. The olfactory system allows the sense of smell, which derives from a chemical reaction between the receptors in the brain and compounds from the essential oil. Once an aroma is perceived, the mind and body analyze the scent. This is accomplished by the reticular system of the brain, which integrates emotions with memories. The hypothalamus, the brain's basic centre for drives and emotions may be activated, which stimulates the pituitary gland. The pituitary gland produces hormones that affect other glands in the body. These hormones trigger physiological, psychological, and emotional reactions that influence feelings and behaviour. In this way, certain aromas will activate particular emotional reactions. Once an aroma is stored in the brain, each subsequent time it is inhaled, the brain and the body will evoke the same response. By directly inhaling an essential oil, the brain can analyze and store the scent, as well as the responses in the body the scent invokes. In this way, essential oils are therapeutic by inhalation.

Since the components of essential oils are small lipid soluble molecules, they can also be absorbed directly into the bloodstream through the skin. They can traverse cell membranes and exhibit pharmacologic effects at nanomolar concentrations, making them suitable candidates for potential pharmaceutical applications. Moreover, their lipophilic nature allows them to pass the blood brain barrier. The effects of aromatherapy are almost instantaneous, and some evidence suggests that aromatherapy can work beyond the level of conscious awareness. Exposure to aromatherapy below the detection threshold on unsuspecting subjects can affect emotions, cognition, daytime behaviors, and sleep [8]. In fact, the most effective application for essential oils in relieving emotional distress is by inhalation.

2 Anxiolytic Effects

Since ancient times, essential oils of many plants, such as jasmine, lavender, lemon balm and orange blossom have been used in aromatherapy for their calming effects. The use of essential oils for their psychoactive effects has been supported scientifically in recent years by several studies on animals and humans [2]. Lavender essential oil is the most studied to improve mood and to reduce stress and anxiety. Lavandula (commonly known as lavender) is a genus of 47 known species of flowering plants in the mint family (*Lamiaceae*). Different species of lavandula grown in different locations and altitudes, provide a wide range of aromatic strengths and slightly different essential oil compositions. The chemical composition of lavender essential oils has been correlated to their anxiolytic activity. All lavender species are highly aromatic plants that produce essential oils. However, only four species, true lavender or English lavender, (*Lavandula angustifolia*, formerly known as *L. officinalis*), spike lavender (*Lavandula latifolia*), lavandin (*Lavandula intermedia*) and Spanish lavender (*Lavandula stoechas*) are commercially used to produce lavender essential oils. Different essential oil chemotypes produce essential oils with different chemical compositions and they exhibit different therapeutic properties. Chemically, essential oil from all lavender species contains linalyl acetate, linalool and 1,8-cineole, along with a number of other compounds. The essential oil from true lavender has high levels of linalyl acetate (25–45%) and linalool (25–38%) and low amounts of camphor and 1,8-cineole [9]. Due to low amounts of camphor and 1,8-cineole and high ester and alcohol content, true lavender essential oil is gentle with no known contraindications, when compared to the other species. Due to its mild nature, it is generally the preferred choice for use in aromatherapy. It can alleviate cortisol and serotonin levels [10] and lower salivary stress markers such as chromogranin A (CgA) and cortisol in stress states [11]. Saliva CgA has been shown to be a biomarker of the acute stress response by the sympatho–adreno–medullary system, while cortisol is considered as a biomarker of the chronic stress response associated with the activation of the hypothalamic–pituitary–adrenal (HPA) axis [12].

A standardized essential oil extract of *Lavandula angustifolia,* Silexan, has been specially prepared and patented in Germany (W. Spitzner Arzneimittelfabrik GmbH, Ettlingen, Germany) and approved for oral use in subsyndromal (“mixed”) anxiety [13]. Silexan lavandula oil preparation contains linalool and linalyl acetate, two major constituents of lavender oil, at concentrations of 36.8% and 34.2%, respectively. Silexan is available as immediate release soft capsules containing 80 mg of lavender oil [14]. Rosewood, also known as Brazilian rosewood (*Aniba rosaeodora* Ducke) is an evergreen tree from the Amazon rainforest and a natural source of the purest linalool [15]. Its essential oil contains 80–90% linalool [16, 17]. The leaves, volatile oil and various extracts, are used in the traditional medicine of the Brazilian Amazon for their sedative, antidepressant and anticonvulsive effects [18].

The relaxing effects of linalool were observed on certain physiological parameters related to stress conditions. Studies in animals have shown that inhaled linalool

has an anxiolytic effect and decreases aggressive behaviour [19]. Inhalation of linalool chiral isomers by human subjects exposed to experimental stress has also led to different physiological responses. This shows that chirality influences the physiological effects [20]. Various physiological parameters of the autonomous nervous system (heart rate, blood pressure, electrodermal activity) and the endocrine system (salivary cortisol), were monitored. It was found that both linalool enantiomers can modulate salivary cortisol levels, a stress biomarker [21], and have relaxing effects. However, S-(+)-linalool acted as an activating agent for blood pressure and heart rate, while R-(−)-linalool proved to be stress-relieving, observed by a decrease in heart rate. Anxiolytic activity of monoterpenes, such as carvone, 1,4-cineole, carvacrol, and isopulegol was also demonstrated. Carvone, one of the constituents of *Lippia alba,* an aromatic, flowering shrub in the verbena family (*Verbenaceae*), is believed to be responsible for its action as a tranquilizer. *Lippia* species are used for their anxiolytic effect and as a tranquilizer in folk medicine [22]. Many pharmacological studies have assessed the tranquilizing effects of the leaves of *Lippia alba* and *Lippia multiflora* [23–25]. Analysis using gas chromatography–mass spectrometry (GC-MS) of the essential oil identified the major components as monoterpenes citral (59%), carvone (7%) and limonene (7%) [26].

3 Anxiety and Depression

The two most common mental health problems worldwide are anxiety and depression. While depression is a psychiatric disorder, affecting more than 300 million people worldwide [27], anxiety is a part of everyone's experience. However, persistent anxiety can cause real emotional distress and lead to anxiety disorders such as panic attacks, phobias and obsessional behaviours [28, 29].

Several studies have shown that there is a correlation between the use of aromatherapy and improved mood in patients that are suffering from anxiety and depression attacks. Bergamot essential oil is frequently used in aromatherapy and has recently gained popularity in improving mood, mild symptoms of stress-related disorders [30], and to facilitate sleep [31]. In the last decade, clinical studies have supported the therapeutic use of Bergamot essential oil suggesting that it may help to reduce anxiety and stress, and promote relaxation [32, 33]. Aromatherapy massage with bergamot oil has been shown to relieve symptoms of anxiety in patients with cancer [34]. Bergamot is a plant from the *Rutaceae* family, endemic to the Calabria region in Italy. It is a hybrid of bitter orange and lemon, originating from the *Citrus aurantium L.* and *Citrus limon L.* or *Citrus aurantifolia* Swing. [32]. Bergamot essential oil is extracted from the peel of a citrus fruit known as bergamot orange (*Citrus bergamia*). The chemical composition of bergamot essential oil is well known [35]. Its volatile fraction contains monoterpene limonene (25–53%) and high quantities of oxygenated compounds, such as two major components of lavender, linalool (2–22%) and linalyl acetate (16–40%), γ-terpinene, and β-pinene. Limonene, γ-terpinene and β-pinene, together with linalool, and linalyl acetate

constitute >90% of the whole oil [36]. It has been shown that the application of bergamot oil decreases levels of CgA [37]. Salivary CgA is an endocrinologic stress marker correlated to sympathetic nervous system activity and noradrenaline release rate [38]. It is a soluble protein that is co-stored and co-released with catecholamines from the adrenal medulla and sympathetic nerve endings. Thus, it has been proposed as a surrogate marker of sympathetic nervous system activity [39]. A series of studies have shown that the level of CgA is more sensitive to psychological stressors and increases more rapidly compared to salivary cortisol.

Humans are capable of learning during sleep. However, humans are also capable of changing the neural representation of a feared stimulus and unlearn during sleep. A sleep study has shown that exposure to odour cues during slow-wave sleep helps to eliminate an aversive visual association (fear) learned in that odour context [40]. Thus, conditioning of the human mind could enable aromatherapy to be administered with the aim of alleviating conditioned fear. This finding supported the hypothesis that aromatherapy can reduce anxiety levels significantly.

The positive effects of lavender have been investigated in many studies. It has been used to reduce anxiety before open-heart and abdominal surgeries [41], decrease anxiety among the patients undergoing coronary artery angiography [42], and relieve symptoms of anxiety for patients with cancer [34]. When effects of aromatherapy with lavender essential oil on anxiety and depression were analysed in haemodialysis patients, the results showed that the lavender essential oil lowered levels of depression, but did not affect anxiety levels [43]. Similarly, when aromatherapy with lavender oil was used to reduce anxiety prior to a scheduled colonoscopy or esophagogastroduodenoscopy, there was no statistical difference in the state of anxiety levels between pre- and post lavender inhalation in the experimental group [44].

A number of citrus oils have demonstrated anxiolytic effects. Neroli oil (*Citrus aurantium var. amara* L. floral essential oil), for example, has been found to reduce anxiety in post-cardiac surgery patients [45]. It has also shown reduced activity in gerbils using a forced swimming test [46]. Neroli oil is composed mainly of limonene (25%), β-pinene (20%), linalool (16%), and linalyl acetate (10%) [46]. Bitter orange (*Citrus aurantium*) oil was found to relieve anxiety in patients after oral administration or inhalation [47, 48]. For example inhalation of orange oil (*Citrus sinensis*) was used to reduce the anxiety level and improve mood in dental patients [49].

Numerous studies reported that rose oil (the floral essential oil of *Rosa damascene* Mill.) has physiological and psychological relaxation, and anti-anxiety effects [50]. A controlled study using transdermal absorption showed that rose oil decreased breathing rate, blood pressure, and blood oxygen saturation, consistent with a relaxing effect [51]. Rose oil is mainly composed of citronellol (13–53%), geraniol (7–27%), nerol (0–16%), 2-phenylethanol (1–10%), nonadecane (2–25%), and heneicosane (1–9%) [52]. A number of reports suggest that citronellol and geraniol are able to reduce anxiety, stress, and depression in patients [53, 54]. Geraniol, linalool and citronellol essential oil constituents, are all acyclic monoterpenol compounds.

Rose oil has shown anticonflict effects in mice, with the effects not mediated by the benzodiazepine binding site of the $GABA_A$ receptor complex [55]. The compounds responsible for this bioactivity have been identified as 2-phenylethanol and citronellol. Inhalation of rose oil can relieve anxiety in pregnant women [56] and decrease salivary cortisol and testosterone levels in healthy participants [57]. A near-infrared time-resolved spectroscopic method has shown that there is a significant decrease in the oxyhemoglobin concentration in the right prefrontal cortex of subjects during rose oil inhalation, indicating that olfactory stimulation by rose oil induces both physiological and psychological relaxation. The dorsal lateral (left and right) prefrontal cortex is thought to be responsible for cognitive control and goal-directed behavior. It is highly active during memory retrieval and in response to mentally demanding tasks [58]. The most sensitive measure of cerebral oxygenation is the oxyhemoglobin difference (Hbdiff) due to the high correlation with cerebral blood flow and mean arterial pressure changes. Thus, the oxyhemoglobin difference can be used to evaluate brain blood flow and oxygenation during a cognitive task [59].

Rose geranium oil has demonstrated a significant reduction in anxiety after inhalation [60]. Geranium oil is distilled from the leaves and sometimes also from the fragrant flowers of various species of *Pelargonium*, mostly from *Pelargonium graveolens.* Commercial rose geranium oil is predominantly composed of isomenthone (5–7%, often reported as menthone), linalool (3–11%), citronellol (15–44%), geraniol (2–39%) and citronellyl formate (6–20%) [61]. Rose, geranium, and citronella are the oils with the highest levels of citronellol. Geraniol, nerol, and citronellol, together with 2-phenylethanol, are known as the rose alcohols because they are the key materials responsible for the rose odor character.

The chemical constituents can vary greatly as a result of the species, origin location, and extraction method. For instance, limonene content varied wildly (between 20% and 97.99%) in two studies of *Citrus aurantium* oil. The chemical components of *Cananga odorata* essential oil also varied substantially in different studies, from being mostly comprised of benzyl acetate (25.1%), p-cresyl ether (16.5%), and linalool (13.6%) [62], to another study where it mainly consisted of methyl benzoate (34.00%), 4-methylanisole (19.82%), and benzyl benzoate (18.97%) [63]. Several Citrus essential oils contain high proportions of limonene as its major component. Orange peels are used as a sedative in several countries, and essential oils obtained from *Citrus aurantium* L. (Rutaceae) fruit peels can contain as much as 97.8% of limonene [64]. *Citrus aurantium* L. (Rutaceae), commonly known as sour orange, is used in traditional Brazilian medicine and other countries to treat anxiety, insomnia, and as an anticonvulsant, suggesting depressive action upon the central nervous system (CNS) [65]. The anxiolytic and sedative properties of citrus essential oil suggested by traditional uses have been assessed in mice [65, 66] and have also been shown in a clinical (dental) setting by Lehrner et al. [67]. The relaxant effects observed in female patients in a dental office were produced with a *Citrus sinensis* (L.) Osbeck (Rutaceae) essential oil, composed of 88.1% limonene and 3.77% myrcene [67]. A mixture of citrus oils was also capable of reducing the necessary treatment doses of antidepressants, normalizing neuroendocrine hormone levels and

immune function in depressive patients [68]. The ambient lemon odor was also found to decrease the number of health symptoms in young healthy subjects [68].

Aromatherapy with two essential oils from two plants in the *Satureja* genus, *Satureja brevicalyx* and *Satureja boliviana*, showed reductions of anxiety ranging between 20% and 47% in a randomized experimental trial with 108 participants [69]. *Satureja* is a genus of aromatic plants from the *Lamiaceae* family, related to rosemary and thyme, with *Satureja brevicalyx* and *Satureja boliviana* originating in the South American Andes. These plants are found to grow from southern Peru to Bolivia and northeast Argentina. Both have been used medicinally since ancient times by the Andean people. In the study of *Satureja* oil, essential oils from two species were used. An analysis of *S. brevicalyx* oil identified 39 constituents by GC-MS [69]. They represent 97.6% of the total oil, with linalool (21.1%), menthone (12.3%), geranyl acetate (11.2%), pulegone (10.4%), isomenthone (8.1%), bicyclogermacrene (7.3%), β-caryophyllene (6.5%) and p-cimene (5.3%) being the major components. In *S. boliviana* oil, 37 constituents were identified, representing 97.2% of total oil content. Major components were linalool (12.8%), menthone (10.7%), pulegone (9.7%), bicyclogermacrene (8.7%), geranyl acetate (8.6%), germacrene D (7.8%), p-cimene (6.4%) and carvacryl acetate (5.2%). The relatively higher reduction of anxiety scores using *Satureja brevicalyx* compared to the *Satureja boliviana* essential oil is likely attributed to their chemical differences. Anxiolytic effects of essential oils were attributed to linalool, a component that has dose-dependent effects on the central nervous system, including sedation, hypnotic, and anxiolytic effects [19, 70]. This means that differences in anxiety scores could be due to linalool content, although further studies are needed to prove this hypothesis. Besides the differences in linalool content (21.1 and 12.8% respectively), the *Satureja brevicalyx* essential oil contained a much higher content of isomenthone (8.1%) and β-caryophyllene (6.5%), while *Satureja boliviana* contained much more geranyl acetate (8.6%), germacrene D (7.8%), and carvacryl acetate (5.2%).

There are many other essential oils used in aromatherapy reported to exert an anxiolytic effect in clinical trials, such as sandalwood (*Santalum album*) oil [71], Roman chamomile (*Chamaemelum nobile*) oil [72], rosemary (*Rosmarinus officinalis*) oil [73], lemon balm (*Melissa officinalis*) oil [74], and pelargonium oil [75]. However, only a few studies have examined the constituents of these essential oils in relation to their anxiolytic activity.

4 Monoterpenols

Linalool, an acyclic tertiary monoterpene alcohol, is an odorous component found in many essential oils (Table 11.1). It is also an important fragrance component that is widely used in many perfume, soap, and shampoo formulations [76]. As lavender essential oil has been reported to have anxiolytic/sedative effects it may be that linalool, a major component of lavender essential oil, could be an important contributor to its pharmacological activity. This hypothesis has been confirmed in a

Table 11.1 The major components of plant essential oils used for the treatment of anxiety and depression

Plant(s)	Essential oil major components	References
Lavandula angustifolia (English lavender)	Linalyl acetate (25–45%) and linalool (25–38%)	[9]
Aniba rosaeodora (Brazilian rosewood)	Linalool (80–90%)	[16, 17]
Lippia alba (Bushy matgrass) and *Lippia multiflora* (bush tea)	Citral (59%), carvone (7%) and limonene (7%)	[22]
Citrus bergamia (bergamot)	Limonene (25–53%), linalyl acetate (16–40%), and linalool (2–22%).	[35, 36]
Citrus aurantium var. amara L. (neroli)	Limonene (25%), β-pinene (20%), linalool (16%), and linalyl acetate (10%)	[46]
Rosa damascene Mill. (rose)	Citronellol (13–53%), geraniol (7–27%), nonadecane (2–25%), nerol (0–16%), 2-phenylethanol (1–10%), and heneicosane (1–9%)	[52]
Pelargonium graveolens (rose geranium)	Citronellol (15–44%), geraniol (2–39%) and citronellyl formate (6–20%), linalool (3–11%), isomenthone (5–7%, often reported as menthone)	[61]
Satureja brevicalyx	Linalool (21.1%), menthone (12.3%), geranyl acetate (11.2%), pulegone (10.4%), isomenthone (8.1%), bicyclogermacrene (7.3%), β-caryophyllene (6.5%) and p-cimene (5.3%)	[69]
Satureja boliviana	Linalool (12.8%), menthone (10.7%), pulegone (9.7%), bicyclogermacrene (8.7%), geranyl acetate (8.6%), germacrene D (7.8%), p-cimene (6.4%) and carvacryl acetate (5.2%)	[69]

number of reported studies with mice. These have shown that inhalation of linalool vapor was able to improve social interactions, reduce anxiety, and reduce aggressive behaviour [1, 19, 77]. The molecular mechanisms behind the actions of linalool as an anxiolytic agent however are still unclear. Odorants bind to odorant receptors at the olfactory epithelium [78], but we don't know how they reach the brain. The axons of the olfactory sensory neurons may send information to second-order neurons in the olfactory bulb or these compounds may simply diffuse through lipid membranes due to their small size and high hydrophobicity [79, 80]. An *in vivo* study demonstrated that inhalation of *Abies sachalinensis* essential oil resulted in much higher concentrations of odorant compounds in the brain when compared to injection into the peritoneum [81]. Nevertheless, more detailed studies are required to work out how these compounds are transported in the body. Previous studies have shown that terpene substances may undergo major biotransformation processes *in vivo* so that the pharmacologically active compounds may actually be metabolites of essential oil components [82]. The effects of such secondary metabolites on the human central nervous system might be due to molecular and biochemical similarities. It has been observed that substances that exhibit modulatory effects at $GABA_A$ receptors shared structural similarities to these essential oil

components. A structural comparison of terpenoid structures that showed a strong modulatory effect on $GABA_A$ receptors of the α1β2 subtype, revealed that almost all substances that lack modulatory potential on $GABA_A$ receptors, did not contain a cyclic structure or they lacked a hydroxyl group. Thus, terpenes with distinct chemical structures (i.e. the presence of hydroxyl groups and a cyclic character (mono- or bicyclic)), may mediate sedative or anxiolytic mechanisms involving $GABA_A$ receptors [83]. This is an allosteric modulation, independent from the γ2 subunit, and similar to the action of alcohols and anesthetics.

The most common monoterpenols in plants are linalool, nerol, and geraniol, the latter two being *cis* and *trans* isomers, respectively (Fig. 11.1). Monoterpenols differ from other aliphatic monoterpenes in their chemical properties. The presence of an alcohol functional group makes them not only more polar and, therefore, soluble in water [84], but also more chemically reactive. Geraniol is easily converted to linalool under acidic conditions [85]. The acid-catalyzed rearrangements of nerol and geraniol and solvolysis of their derivatives are considered as models for terpenoid biosynthesis. In these reactions, geraniol or its derivatives give predominantly linalool and acyclic alkenes, whereas nerol and its derivatives generate largely cyclic products such as α-terpineol or limonene.

5 Conclusions

A number of essential oils are currently used for anxiety relief and relaxing effects. *In-vivo* studies on animal models have verified the anxiolytic effects of these essential oils and the interactions of their major components with central nervous system receptors. Therefore, it seems reasonable to argue that the modulation of glutamate and GABA neurotransmitter systems are likely to be the critical mechanisms responsible for the sedative, anxiolytic, and anticonvulsant proprieties of linalool

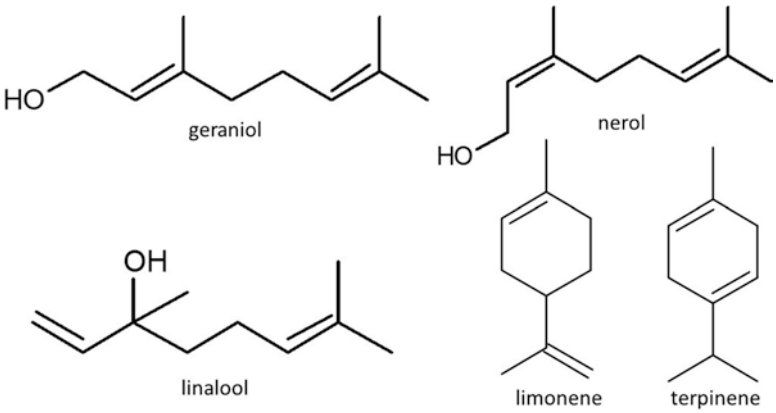


Fig. 11.1 Monoterpenols in essential oils with anxiolytic properties

and essential oils containing linalool in significant proportions. Popular anxiolytic essential oils are generally rich in terpenoid alcohols like linalool, geraniol and citronellol, and the monoterpene limonene (or citral). Therefore, other essential oils or formulations that contain these terpenoids as major components may serve as important aromatherapeutics for relief of anxiety.

References

1. Souto-Maior FN, de Carvalho FL, de Morais LC, Netto SM, de Sousa DP, de Almeida RN (2011) Anxiolytic-like effects of inhaled linalool oxide in experimental mouse anxiety models. Pharmacol Biochem Behav 100(2):259–263
2. Agatonovic-Kustrin S, Morton DW (2018) Essential oils and cognitive performance. In: Atta-ur-Rahman (ed) Frontiers in natural product chemistry (Book 4). Bentham Science Publishers, Sharjah, pp 91–118. ISBN-10: 168108726X
3. Edris AE (2007) Pharmaceutical and therapeutic potentials of essential oils and their individual volatile constituents: a review. Phytother Res 21(4):308–323
4. Villemure C, Bushnell MC (2009) Mood influences supraspinal pain processing separately from attention. J Neurosci 29(3):705–715
5. Haze S, Sakai K, Gozu Y (2002) Effects of fragrance inhalation on sympathetic activity in normal adults. Jpn J Pharmacol 90(3):247–253
6. Gatti G, Cajola RL (1923) L'azione delle essenze sul sistema nervoso. Riv Ital Delle Essenze Profumi 5(12):133–135
7. Gottfried JA (2011) Neurobiology of sensation and reward, 1st edn. CRC Press, Boca Raton. ISBN-10: 1420067265
8. Moss M, Cook J, Wesnes K, Duckett P (2003) Aromas of rosemary and lavender essential oils differentially affect cognition and mood in healthy adults. Int J Neurosci 113(1):15–38
9. Beale DJ, Morrison PD, Karpe AV, Dunn MS (2017) Chemometric analysis of lavender essential oils using targeted and untargeted GC-MS acquired data for the rapid identification and characterization of oil quality. Molecules 22(8). pii: E1339. https://doi.org/10.3390/molecules22081339
10. Mirzaei F, Keshtgar S, Kaviani M, Rajaeefard A (2008) The effect of lavender essence smelling during labor on cortisol and serotonin plasma levels and anxiety reduction in nulliparous women. J Kerman Univ Med Sci 16(3):245–254
11. Toda M, Morimoto K (2008) Effect of lavender aroma on salivary endocrinological stress markers. Arch Oral Biol 53(10):964–968
12. Soo-Quee Koh D, Choon-Huat Koh G (2007) The use of salivary biomarkers in occupational and environmental medicine. Occup Environ Med 64(3):202–210
13. Kasper S (2013) An orally administered lavandula oil preparation (Silexan) for anxiety disorder and related conditions: an evidence based review. Int J Psychiatry Clin Pract 17 Suppl 1:15–22
14. Kasper S, Müller WE, Volz HP, Möller HJ, Koch E, Dienel A (2018) Silexan in anxiety disorders: clinical data and pharmacological background. World J Biol Psychiatry 19(6):412–420
15. Castelo AVM, Del Menezzi CHS, Resck IS (2012) Seasonal variation in the yield and the chemical composition of essential oils from two Brazilian native arbustive species. J Appl Sci 12:753–760
16. Maia JGS, Andrade E, Couto HAR, da Silva ACM, Marx F, Henke C (2006) Plant sources of Amazon rosewood oil. Química Nova 30(8):1906–1910
17. Sampaio Lde F, Maia JG, de Parijós AM, de Souza RZ, Barata LE (2012) Linalool from rosewood (Aniba rosaeodora Ducke) oil inhibits adenylate cyclase in the retina, contributing to understanding its biological activity. Phytother Res 26(1):73–77

18. Dos Santos ÉRQ, Maia CSF, Fontes Junior EA, Melo AS, Pinheiro BG, Maia JGS (2018) Linalool-rich essential oils from the Amazon display antidepressant-type effect in rodents. J Ethnopharmacol 212:43–49
19. Linck VM, da Silva AL, Figueiró M, Caramão EB, Moreno PR, Elisabetsky E (2010) Effects of inhaled Linalool in anxiety, social interaction and aggressive behavior in mice. Phytomedicine 17(8–9):679–683
20. Hoferl M, Krist S, Buchbauer G (2006) Chirality influences the effects of linalool on physiological parameters of stress. Planta Med 72(13):1188–1192
21. Agatonovic-Kustrin S, Morton DW, Smirnov V, Petukhov A, Gegechkori V, Kuzina V et al (2019) Analytical strategies in lipidomics for discovery of functional biomarkers from human saliva. Dis Markers 2019:6741518. https://doi.org/10.1155/2019/6741518
22. Hatano VY, Torricelli AS, Giassi AC, Coslope LA, Viana MB (2012) Anxiolytic effects of repeated treatment with an essential oil from Lippia alba and (R)-(−)-carvone in the elevated T-maze. Braz J Med Biol Res 45:238–243
23. Vale TG, Matos FJ, de Lima TC, Viana GS (1999) Behavioral effects of essential oils from Lippia alba (Mill.) N.E. Brown chemotypes. J Ethnopharmacol 67(2):127–133
24. Abena AA, Atipo-Ebata JK, Hondi Assah T, Diatewa M (2001) Psychopharmacological properties of crude extract and essential oil of Lippia multiflora. Encéphale 27(4):360–364
25. Zétola M, De Lima TC, Sonaglio D, González-Ortega G, Limberger RP, Petrovick PR et al (2002) CNS activities of liquid and spray-dried extracts from Lippia alba—Verbenaceae (Brazilian false melissa). J Ethnopharmacol 82(2–3):207–215
26. da Silva LVF, Veras Mourão RH, Manimala J, Lnenicka GA (2018) The essential oil of Lippia alba and its components affect Drosophila behavior and synaptic physiology. J Exp Biol 221(Pt 14). pii: jeb176909. https://doi.org/10.1242/jeb.176909
27. WHO (2017) Depression and other common mental disorders: global health estimates. 2017, World Health Organization: Geneva. https://apps.who.int/iris/bitstream/handle/10665/254610/WHO-MSD-MER-2017.2-eng.pdf;sequence=1
28. Wahed WYA, Hassan SK (2017) Prevalence and associated factors of stress, anxiety and depression among medical Fayoum University students. Alexandria J Med 53(1):77–84
29. Craske MG, Stein MB (2016) Anxiety. Lancet 388(10063):3048–3059
30. Halcon LL (2002) Aromatherapy: therapeutic applications of plant essential oils. Minn Med 85(11):42–46
31. Wiebe E (2000) A randomized trial of aromatherapy to reduce anxiety before abortion. Eff Clin Pract 3(4):166–169
32. Navarra M, Mannucci C, Delbò M, Calapai G (2015) Citrus bergamia essential oil: from basic research to clinical application. Front Pharmacol 6:36. https://doi.org/10.3389/fphar.2015.00036
33. Watanabe E, Kuchta K, Kimura M, Rauwald HW, Kamei T, Imanishi J (2015) Effects of bergamot (Citrus bergamia (Risso) Wright & Arn.) essential oil aromatherapy on mood states, parasympathetic nervous system activity, and salivary cortisol levels in 41 healthy females. Forsch Komplementmed 22(1):43–49
34. Wilkinson SM, Love SB, Westcombe AM, Gambles MA, Burgess CC, Cargill A et al (2007) Effectiveness of aromatherapy massage in the management of anxiety and depression in patients with cancer: a multicenter randomized controlled trial. J Clin Oncol 25(5):532–539
35. Costa R, Dugo P, Navarra M, Raymo V, Dugo G, Mondello L (2010) Study on the chemical composition variability of some processed bergamot (Citrus bergamia) essential oils. Flavour Fragr J 25(1):4–12
36. Mondello L, Dugo P, Bartle KD, Dugo G, Cotroneo A (1995) Automated HPLC-HRGC: a powerful method for essential oils analysis. Part V. identification of terpene hydrocarbons of bergamot, lemon, mandarin, sweet orange, bitter orange, grapefruit, clementine and mexican lime oils by coupled HPLC-HRGC-MS (ITD). Flavour Fragr J 10(1):33–42
37. Seo JY (2009) The effects of aromatherapy on stress and stress responses in adolescents. J Korean Acad Nurs 39:357–365

38. Dimsdale JE, O'Connor DT, Ziegler M, Mills P (1992) Chromogranin A correlates with norepinephrine release rate. Life Sci 51(7):519–525
39. Nakane H, Asami O, Yamada Y, Harada T, Matsui N, Kanno T et al (1998) Salivary chromogranin A as an index of psychosomatic stress response. Biomed Res 19(6):401–406
40. Wixted JT (2013) Sleep aromatherapy curbs conditioned fear. Nature Neurosci 16(11):1510–1512
41. Hosseini S, Heydari A, Vakili M, Moghadam S, Tazyky S (2016) Effect of lavender essence inhalation on the level of anxiety and blood cortisol in candidates for open-heart surgery. Iran J Nurs Midwifery Res 21(4):397–401
42. Tahmasbi H, Mahmoodi G, Mokhberi V, Hassani S (2012) The impact of aromatherapy on the anxiety of patients experiencing coronary angiography. Zahedan J Res Med Sci 14(3):51–55
43. Bagheri-Nesami M, Shorofi SA, Nikkhah A, Espahbodi F (2017) The effects of lavender essential oil aromatherapy on anxiety and depression in haemodialysis patients. Pharm Biomed Res 3(1):8–13
44. Muzzarelli L, Force M, Sebold M (2006) Aromatherapy and reducing preprocedural anxiety: a controlled prospective study. Gastroenterol Nurs 29(6):466–471
45. Stevensen C (1994) The psychophysiological effects of aromatherapy massage following cardiac surgery. Complement Ther Med 2(1):27–35
46. Chen, YJ, Cheng F, Shih Y, Chang TM, Wang MF, Lan SS (2008) Inhalation of neroli essential oil and its anxiolytic effects. J Altern Complement Med 5(1): ISSN (Online) 1553–3840. https://doi.org/10.2202/1553-3840.1143
47. Farshbaf-Khalili A, Kamalifard M, Namadian M (2018) Comparison of the effect of lavender and bitter orange on anxiety in postmenopausal women: a triple-blind, randomized, controlled clinical trial. Complement Ther Clin Pract 31:132–138
48. Chaves Neto G, Braga JEF, Alves MF, de Morais Pordeus LC, Dos Santos SG, Scotti MT et al (2017) Anxiolytic effect of Citrus aurantium L. in crack users. Evid Based Complement Alternat Med 2017:7217619. https://doi.org/10.1155/2017/7217619
49. Lehrner J, Marwinski G, Lehr S, Johren P, Deecke L (2005) Ambient odors of orange and lavender reduce anxiety and improve mood in a dental office. Physiol Behav 86(1–2):92–95
50. Mohebitabar S, Shirazi M, Bioos S, Rahimi R, Malekshahi F, Nejatbakhsh F (2017) Therapeutic efficacy of rose oil: a comprehensive review of clinical evidence. Avicenna J Phytomed 7(3):206–213
51. Hongratanaworakit T (2009) Relaxing effect of rose oil on humans. Nat Prod Commun 4(2):291–296
52. Gochev V, Wlcek K, Buchbauer G, Stoyanova A, Dobreva A, Schmidt E (2008) Comparative evaluation of antimicrobial activity and composition of rose oils from various geographic origins, in particular Bulgarian rose oil. Nat Prod Commun 3(7):1063–1068
53. Qneibi M, Jaradat N, Emwas N (2019) Effect of geraniol and citronellol essential oils on the biophysical gating properties of AMPA receptors. Appl Sci 9:4693
54. Heghes SC, Vostinaru O, Rus LM, Mogosan C, Iuga CA, Filip L (2019) Antispasmodic effect of essential oils and their constituents: a review. Molecules 24(9). pii: E1675. https://doi.org/10.3390/molecules24091675
55. Umezu T (2000) Behavioral effects of plant-derived essential oils in the Geller type conflict test in mice. Jpn J Pharmacol 83(2):150–153
56. Kheirkhah M et al (2014) Comparing the effects of aromatherapy with rose oils and warm foot bath on anxiety in the first stage of labor in nulliparous women. Iran Red Crescent Med J 16(9):e14455. https://doi.org/10.5812/ircmj.14455
57. Fukui H, Komaki R, Okui M, Toyoshima K, Kuda K (2007) The effects of odor on cortisol and testosterone in healthy adults. Neuroendocrinol Lett 28(4):433–437
58. Causse M, Chua Z, Peysakhovich V, Del Campo N, Matton N (2017) Mental workload and neural efficiency quantified in the prefrontal cortex using fNIRS. Sci Rep 7(1):5222. https://doi.org/10.1038/s41598-017-05378-x

59. Moriarty T, Bourbeau K, Bellovary B, Zuhl MN (2019) Exercise intensity influences prefrontal cortex oxygenation during cognitive testing. Behav Sci 9(8). pii: E83. https://doi.org/10.3390/bs9080083
60. Morris N, Birtwistle S, Toms M (1995) Anxiety reduction by aromatherapy: anxiolytic effects of inhalation of geranium and rosemary. Int J Aromather 7(2):33–39
61. Peterson A, Machmudah S, Roy BC, Goto M, Sasaki M, Hirose T (2006) Extraction of essential oil from geranium (Pelargonium graveolens) with supercritical carbon dioxide. J Chem Technol Biotechnol 81(2):167–172
62. Jung DJ, Cha JY, Kim SE, Ko IG, Jee YS (2013) Effects of Ylang-Ylang aroma on blood pressure and heart rate in healthy men. J Exerc Rehabil 9(2):250–255
63. Hongratanaworakit T, Buchbauer G (2006) Relaxing effect of ylang ylang oil on humans after transdermal absorption. Phytother Res 20(9):758–763
64. González-Mas MC, Rambla JL, López-Gresa MP, Blázquez MA, Granell A (2019) Volatile compounds in citrus essential oils: a comprehensive review. Front Plant Sci 10(12). https://doi.org/10.3389/fpls.2019.00012
65. Carvalho-Freitas MIR, Costa M (2002) Anxiolytic and sedative effects of extracts and essential oil from Citrus aurantium L. Biol Pharm Bull 25(12):1629–1633
66. de Moraes PA, Galindo LA, Costa M (2006) Effects of the essential oil from Citrus aurantium L. in experimental anxiety models in mice. Life Sci 78(15):1720–1725
67. Lehrner J, Eckersberger C, Walla P, Pötsch G, Deecke L (2000) Ambient odor of orange in a dental office reduces anxiety and improves mood in female patients. Physiol Behav 71(1–2):83–86
68. Komori T, Fujiwara R, Tanida M, Nomura J, Yokoyama MM (1995) Effects of citrus fragrance on immune function and depressive states. Neuroimmunomodulation 2(3):174–180
69. Soto-Vásquez MR, Alvarado-García PAA (2017) Aromatherapy with two essential oils from Satureja genre and mindfulness meditation to reduce anxiety in humans. J Tradit Complement Med 7(1):121–125
70. Cheng BH, Sheen LY, Chang ST (2015) Evaluation of anxiolytic potency of essential oil and S-(+)-linalool from Cinnamomum osmophloeum ct. linalool leaves in mice. J Tradit Complement Med 5(1):27–34
71. Kyle G (2006) Evaluating the effectiveness of aromatherapy in reducing levels of anxiety in palliative care patients: results of a pilot study. Complement Ther Clin Pract 12(2):148–155
72. Wilkinson S, Aldridge J, Salmon I, Cain E, Wilson B (1999) An evaluation of aromatherapy massage in palliative care. Palliat Med 13(5):409–417
73. McCaffrey R, Thomas DJ, Kinzelman AO (2009) The effects of lavender and rosemary essential oils on test-taking anxiety among graduate nursing students. Holist Nurs Pract 23(2):88–93
74. Ballard CG, O'Brien JT, Reichelt K, Perry EK (2002) Aromatherapy as a safe and effective treatment for the management of agitation in severe dementia: the results of a double-blind. Placebo-controlled trial with Melissa. J Clin Psychiatry 63(7):553–558
75. Shirzadegan R, Gholami M, Hasanvand S, Birjandi M, Beiranvand A (2017) Effects of geranium aroma on anxiety among patients with acute myocardial infarction: a triple-blind randomized clinical trial. Complement Ther Clin Pract 29:201–206
76. Kamatou GPP, Viljoen A (2008) Linalool – a review of a biologically active compound of commercial importance. Nat Prod Commun 3(7):1183–1192
77. Buchbauer G, Jirovetz L, Jäger W, Plank C, Dietrich H (1993) Fragrance compounds and essential oils with sedative effects upon inhalation. J Pharm Sci 82:660–664
78. Buck LB (1993) Receptor diversity and spatial patterning in the mammalian olfactory system. Ciba Found Symp 179:51–64; discussion 64–7, 88–96
79. Neuhaus W, Trauner G, Gruber D, Oelzant S, Klepal W, Kopp B et al (2008) Transport of a GABAA receptor modulator and its derivatives from Valeriana officinalis L. s. l. across an in vitro cell culture model of the blood-brain barrier. Planta Med 74(11):1338–1344
80. Menini A, Lagostena L, Boccaccio A (2004) Olfaction: from odorant molecules to the olfactory cortex. Physiology 19(3):101–104

81. Satou T, Matsuura M, Takahashi M, Umezu T, Hayashi S, Sadamoto K (2011) Anxiolytic-like effect of essential oil extracted from Abies sachalinensis. Flavour Fragr J 26(6):416–420
82. Heinlein A, Buettner A (2012) Monitoring of biotransformation of hop aroma compounds in an in vitro digestion model. Food Funct 3(10):1059–1067
83. Kessler A, Sahin-Nadeem H, Lummis SC, Weigel I, Pischetsrieder M, Buettner A et al (2014) GABAA receptor modulation by terpenoids from Sideritis extracts. Mol Nutr Food Res 58(4):851–862
84. Weidenhamer JD, Macias FA, Fischer NH, Williamson GB (1993) Just how insoluble are monoterpenes? J Chem Ecol 19(8):1799–1807
85. Cori O, Chayet L, Maria-Perez L, Bunton CA, Hachey D (1986) Rearrangement of linalool, geraniol, nerol and their derivatives. J Org Chem 51(8):1310–1316

Chapter 12
The Role of Nutrition in Attenuating Age-Related Skeletal Muscle Atrophy

Behnaz Abiri and Mohammadreza Vafa

1 Introduction

Skeletal muscle contractions power human body movements and are important for maintaining stability. Skeletal muscle tissue accounts for about half of the human body mass and, in addition to its power-generating role, is a crucial factor in maintaining homeostasis. Given its central role in human mobility and metabolic function, any impairment in the contractile, material, and metabolic properties of skeletal muscle has an adverse effect on human health. Aging is related to a progressive loss of muscle mass, quality, and strength, a condition known as sarcopenia. Although this term is applied clinically to denote loss of muscle mass, it is often used to explain both some cellular processes (denervation, mitochondrial dysfunction, inflammatory and hormonal alterations) and some outcomes, such as reduced muscle strength, mobility, and function, a higher risk of falls, and decreased energy requirements (Fig. 12.1). Von Haeling et al. [1] have estimated its prevalence at 5–13% for the elderly population aged 60–70 years and at 11–50% for those aged 80 years or above. Lean muscle mass generally contributes up to 50% of total body weight in young adults, but decreases with aging to approximately 25% around 75–80 years of age. At the muscle fiber level, sarcopenia is described by specific type II muscle fiber atrophy, fiber necrosis, and fiber-type grouping. Several

B. Abiri
Department of Nutrition, Faculty of Paramedicine, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran

M. Vafa (✉)
Department of Nutrition, School of Public Health, Iran University of Medical Sciences, Tehran, Iran

Pediatric Growth and Development Research Center, Institute of Endocrinology and Metabolism, Iran University of Medical Sciences, Tehran, Iran
e-mail: vafa.m@iums.ac.ir

P. C. Guest (ed.), *Reviews on New Drug Targets in Age-Related Disorders*,
Advances in Experimental Medicine and Biology 1260,
https://doi.org/10.1007/978-3-030-42667-5_12

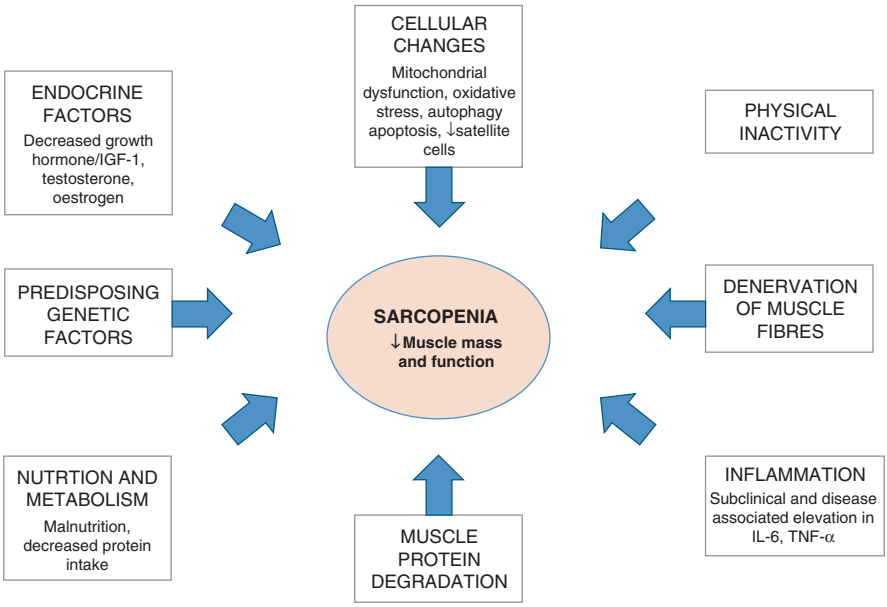


Fig. 12.1 Contributory factors of age-related muscle atrophy (sarcopenia)

probable mechanisms of age-related muscle atrophy have been reported. Age-related muscle loss is due to losses in the size and number of muscle fibers, possibly resulted from a multi-factorial process that includes physical activity level, nutrient intake, hormonal changes, metabolic homeostasis, oxidative stress, and lifespan. The specific contribution of each of these factors is unknown but there is growing evidence that the disruption of several positive regulators of muscle hypertrophy such as Akt and serum response factor (SRF) are an important feature in the progression of sarcopenia [2, 3]. Some studies demonstrated a functional defect in autophagy- and myostatin-dependent signaling in sarcopenic muscle [4–6]. In contrast, many researchers have failed to identify age-related increases in the levels of common negative regulators, such as atrophy gene-1 (atrogin-1), nuclear factor-kappaB (NF-κB) and calpain, in senescent mammalian muscles [2, 3, 7].

Poor diet is one of the most common problems practitioners encounter when treating elderly people. Many individuals in this population have low nutrient intakes, for different reasons that range from physical deficits to economic problems. Dental problems in the elderly may make them more likely to choose softer foods that often lack protein and delayed gastric emptying can decrease appetite and hormonal alterations may lead to longer-lasting feelings of satiety. Physical disability may also make packaged, processed foods more appealing [8]. Such dietary problems are not impossible to overcome. If we can understand the lifestyle factors that affect the rate of decrease in muscle mass and strength in older age, we can develop strategies that will help to prevent or slow sarcopenia, and allow people to have a higher quality of life in old age. This chapter aims to address several recent strategies for inhibiting this phenomenon.

2 The Role of Nutrition

The diets of elderly people affect to a large extent their health, and particularly the potential for counteracting the possible physiological etiological factors of sarcopenia. In the following sections, the effects of dietary factors on muscle metabolism will be discussed.

2.1 Amino Acid Supplementation

Many reviews demonstrate that certain nutritional interventions such as a high protein intake or an elevated intake of essential amino acids and the branched chain amino acid (BCAA) leucine with resistance training may help to slow fiber atrophy in sarcopenic muscle by modulation of both anabolic and catabolic pathways [9]. In particular, leucine can be considered as a regulatory amino acid with unique features. It has several effects on muscle metabolism regulation, such as control of protein synthesis and glucose homeostasis. In addition, leucine has been shown to be a nitrogen donor for the synthesis of muscle alanine and glutamine. Considering these findings, the administration of leucine as an anti-atrophic agent is biologically justified [9].

It has been reported that amino acid supplementation has a synergistic impact on the contraction-induced escalation of muscle protein synthesis following acute resistance exercises [10]. Treatment with amino acids has been found to induce additional hypertrophy in response to continuous resistance training [11]. Recent human studies have shown that amino acids have an effect in the phosphorylation of translational initiation factors, especially eIF4F and p70S6K, through an mTOR-mediated mechanism [12]. On the other hand, several other studies have not reported advantages from protein supplementation [13, 14]. These studies administered a single bout or short-term (10 day) ingestion to evaluate the rate of myofibrillar synthesis or protein synthesis [13]. In contrast, Godard et al. [14] aimed to evaluate the long-term supplementation of several amino acids and carbohydrate combined with resistance training. Unfortunately, they performed the evaluation of total muscle cross-sectional areas only using magnetic resonance imaging (MRI), and did not conduct a detailed morphological analysis. Since the examination of muscle cross-sectional area by MRI seems to be affected by the inner amount of adipose tissue, connective tissue, or water, it is unknown whether or not the protein supplementation showed positive effects on the morphometry of muscle fibers. In another study, the administration of many essential amino acids has been shown to have a positive effect on muscle mass and protein synthesis under both normal states [2, 3, 15], and with resistance training [12]. Although a positive slowing impact on sarcopenia has been reported in almost all studies utilizing many essential amino acids and comprising high levels of leucine, supplementation with essential amino acids not enriched with leucine may fail to increase muscle protein synthesis in the elderly.

Moreover, a greater amount of leucine should be supplemented along with large amounts of isoleucine and valine in order to avoid an imbalance of branched-chain amino acid levels [15].

2.2 Protein

Proteins are continuously broken down and resynthesized, and skeletal muscle may account for about one-quarter of the total body protein turnover [16]. When protein intake is inadequate, turnover of tissue protein is decreased while the opposite may occur with elevated intake. However, in the elderly, the amount of protein turned over reduces compared to young adults [17]. Net protein balance in the skeletal muscle is the result of protein synthesis and protein breakdown. When muscle protein breakdown is greater than the rate of muscle protein synthesis, the net protein balance is negative, while the opposite correlates with positive balance. Balance is achieved when muscle protein breakdown equals muscle protein synthesis [18]. The occurrence of sarcopenia may be the result of an elevated basal-fasted rate of muscle protein breakdown and/or decreased basal muscle protein synthesis [19]. Nevertheless, muscle protein breakdown may also lead to restore the functionality of proteins by allowing impaired proteins to be removed and recycled into new muscle proteins [20]. Muscle protein synthesis is more responsive than protein breakdown to diet-associated alterations in healthy subjects, making it the main target to stimulate muscle protein balance and eventual protein accumulation [20]. In our study of women aged 40–60 years old, protein intake, adjusted for physical activity and weight, was positively and significantly associated with fat free mass percentage [21]. Considerable discussion exists about the amount of protein intake required for optimal health in older adults, particularly when evaluating it in the light of energy needs [22]. Gersovitz et al. [23] provided older adults with diets containing 0.8 g egg protein/kg/day, and concluded that this amount was not adequate for most of the participants. Campbell et al. [24] also proposed that 0.8 g protein/kg/day may not be sufficient to completely meet the needs of all elderly people. In a study to evaluate dietary protein intake and alterations in lean mass in community-dwelling older adults, subjects in the highest quintile of protein intake (1.2 ± 0.4 g protein/kg body weight/day) lost about 40% less lean mass than did those in the lowest quintile of protein intake (0.8 ± 0.3 g protein/kg/day) [25]. According to some researchers, the recommend intake for the prevention of sarcopenia is 0.8–1.2 g of high-quality protein/kg/day [26] or higher amounts, such as 1.6 g protein/kg/day [27].

Moreover, Paddon-Jones and Rasmussen [28] revealed that muscle protein synthesis was decreased in old people when the ingested protein was less than about 20 g per meal, and a value of 25–30 g of high-quality protein per meal was recommended to maximize the anabolic response. Hence, elevating the distribution of protein intake in approximately equal parts through breakfast, lunch and dinner may be also an important factor of protein effectiveness [29].

2.3 Beta-Hydroxy-Beta-Metylbutyrate (HMB)

HMB is a product of leucine metabolism that has been demonstrated to slow protein breakdown in muscle tissue [30]. HMB may be effective at limiting the demands placed on elderly people by acute stresses, such as sudden increases in physical activity, an immunologic challenge, or acute malnutrition [30, 31]. Daily supplementation of HMB (2 g/day), arginine and lysine for 12 weeks positively changed measurements of functionality, strength, fat-free mass and protein synthesis, proposing that the strategy of targeted nutrition has the ability to influence muscle health in elderly women [32]. Therefore, an adequate intake of proteins (1.2/g/kg/day) is essential to prevent sarcopenia and amino acid supplementation, especially branched chain amino acids (leucine 2.5 g/day) as well as the intake of beta-hydroxy butyrate (2 g/day), is a well-established intervention for treating sarcopenia.

2.4 Creatine

Creatine is known as a non-protein nitrogenous tri-peptide, composed of glycine, arginine and methionine. In the human body, creatine is synthesized in the liver and pancreas from these amino acids. In addition, creatine is present in foods (meat and fish) and is taken with the diet in the amount of 1–2 g per day. Approximately 95% of the creatine in the body is stored in skeletal muscle, with about two-thirds of this is stored as phosphocreatine (PCr) and the remainder as free creatine. The energy provided for the phosphorylation of adenosinediphosphate (ADP) to adenosine triphosphate (ATP) during and after intense exercise depends on the amount of PCr stored in the muscle. With depletion of PCr during intense exercise, the availability of energy reduces due to the inability to resynthesize ATP in the amounts needed to maintain the high-intensity exercise [33]. Age-related reductions of creatine/PCr in skeletal muscle have been indicated in some studies [34, 35], although not all studies agree [36, 37]. The reduction of muscle creatine is biologically plausible, due to aging and, possibly, to certain co-morbidities, such as sarcopenia, and/or alterations of behavior with age, including decreased physical activity and/or changes in dietary intake, such as reduced consumption of meat products due to denture issues. Type II muscle fibers have a higher content of PCr compared to type I fibers [38], and sarcopenia is characterized by a preferential atrophy of the former fiber type [39]. The progressive atrophy of type II fibers may therefore partly account for the decreased muscle creatine in the elderly. In addition, the reduction of creatine in the muscles of the elderly is in line with previous evidence that documents an increase in oxidative processes in aged skeletal muscles, such as a reduction of lactate dehydrogenase [40] and reduced dependence on glycolysis [41]. Smith et al. first demonstrated an elevation in muscle PCr in middle-aged adults (58 years-old) as a result of short-term intake of high doses of creatine (0.3 g/kg/day for 5 days) [34]. In a similar study, Rawson et al. showed a smaller elevation in muscle PCr (7 versus 35%) in 70 year-olds compared with 24 year-olds, in response to ingestion of

creatine (20 g/day for 5 days) [37]. Brose et al. found an increase in total muscle creatine (30% men, 17% women) in 70 year-old participants who underwent 14 weeks of resistance training and intake of 5 g/day creatine [42], a result that is in line with the increases shown in younger adults [43, 44]. Eijnde et al. reported increases of 5% and 21% in total muscle creatine and free creatine, respectively, following 6 months of an exercise program for muscular endurance combined with 5 g/day creatine supplementation [45]. Hence, it appears that the muscle creatine in the elderly can be elevated with oral creatine supplementation at a dose of 5 g/day but the magnitude of the response can be significantly influenced by initial muscle creatine levels. Wyss et al. have proposed that the increase in extracellular creatine may reduce the absorption of creatine in muscle [46]. One of the most important findings was an improvement in fatigue resistance, which has been shown in several studies using different exercise tests [47–51]. Some researchers have indicated an increase in strength [49, 50] but this has not always been reported [47, 48]. Some researchers have shown that creatine supplementation may help to increase the performance of tasks identified in the activities of daily living (activity daily living; ADL) [50, 52, 53]. The is an important finding because of the relationship between the performance of ADL, fall risk and mortality. Among the studies that have investigated muscle mass, the majority found a greater increase in lean mass accretion after ingesting creatine in combination with resistance training [42, 54, 55] and Dalbo et al. mentioned that creatine is an effective intervention to counteract sarcopenia [56]. The timing of creatine ingestion (before and after resistance training sessions) can be more relevant than the amount of creatine [33]. In conclusion, an adequate creatine supplementation could be a useful intervention to combat sarcopenia, in particular fatigue associated with sarcopenia, although clinical studies are required to support this.

2.5 Long-Chain Polyunsaturated Fatty Acids (LCPUFAs)

Sarcopenia is recognised as an inflammatory status driven by cytokines and oxidative stress [57]. Since eicosanoids derived from 20-carbon polyunsaturated fatty acids are among the regulators of inflammation [58], this raises the probability that variations in intake of n-3 and n-6 LCPUFAs, and their balance in the diet, could be of importance. In particular, n-3 LPUFAs have the potential to be potent anti-inflammatory agents [58]. Although biochemical processes underlying the impacts of pro-inflammatory cytokines on skeletal muscle remain to be established [59], elevated circulation levels of cytokines including interleukin (IL)-6, C-reactive protein (CRP) and tumor necrosis factor (TNF)-α receptor II, may have harmful impacts on protein synthetic rates [60, 61]. However, these inflammatory processes may be decreased by n-3 LPUFAs, such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which are found in foods such as fatty fish [62, 63]. There is some observational evidence to support the effect of n-3 LCPUFA status on muscle function, as higher grip strength was reported in elderly men and women who had greater oily fish consumption [64]. In line with this finding, several studies of patients with

rheumatoid arthritis have indicated that supplementation with fish oil led to improvement in grip strength [58].

In a randomized controlled trial study to investigate the effects of n-3 LCPUA on the rate of muscle protein synthesis in older adults, 8 weeks of daily supplementation with 1.86 g EPA plus 1.50 g DHA had no effect on the basal rate of muscle protein synthesis, but amplified the hyper-aminoacidaemia–hyperinsulinaemia-stimulated elevation in the rate of muscle protein synthesis, which may be important to counteract the anabolic resistance related to aging [65]. Moreover, the intake of oily fish was related to an increase in grip strength in community-dwelling older adults, which raised the hypothesis of an anti-inflammatory impact of n-3 LCPUFA and a possible effect of these nutrients in the prevention of sarcopenia [66].

α-Linolenic acid (ALA) is the major plant-based n-3 LCPUFA and its effects may also occur via its conversion to EPA and DHA, when dietary intake of marine PUFAs is low [67, 68]. Although the precise efficacy of metabolic conversion of ALA to EPA and/or to DHA is an unresolved question, it has been established that desired tissue levels of EPA and DHA could be better achieved by consumption of these two nutrients [69]. Since LCPUFA synthesis occurs mainly in the liver, it is possible that natural alterations in physiological condition occurring with aging, or any additional pathologic states that may exist, influence the availability of these nutrients in different tissues [67]. However, considering the antithrombotic effects of n-3 LCPUFA, special attention should be given to the risks of potential severe adverse events after high doses ingestion, such as bleeding [62] or a slight rise in LDL cholesterol [70], particularly in older adults. In a review of Calder [63] evaluating the consumption of fish oil supplements by healthy adults and its effect on inflammatory processes, it was demonstrated that EPA and DHA intake higher than 2 g/day appeared to be needed to produce anti-inflammatory effects. The existence of Dietary Reference Intakes for EPA and DHA is still a matter for discussion, but consumption levels for an adult of up to 500 mg/day do not seem to raise safety concerns [69]. In addition, Villani et al. [62] conducted a systematic review on fish oil administration in elderly people, and concluded that the potential for adverse events associated with omega-3 supplementation appeared mild to moderate at worst. However, data are limited to establish definitive conclusions about the safety of these nutrients. In another randomized controlled clinical trial, supplementation of older adults with EPA and DHA led to an elevated anabolic response to amino acid and insulin infusion. While these novel data propose that the stimulation of muscle protein synthesis by n-3 LCPUFA supplementation could be beneficial for the prevention and treatment of sarcopenia [71], further evidence is required to establish the therapeutic potential of n-3 LCPUFAs in inflammatory states.

2.6 Antioxidant Supplementation

Free radicals are highly reactive molecular species with a single unpaired electron in the outer orbit seeking to pair with another free electron. In particular, reactive oxygen species (ROS), deriving from oxidative metabolism, have higher reactivity

than O_2. ROS are constantly generated in cells of aerobic organisms, in particular skeletal muscle, by the addition of a single electron to the oxygen molecule with subsequently injury of biological macromolecules, such as lipids and DNA. The interaction of ROS with normal cellular structures results in potentially nonreversible changes, with subsequent cellular loss of function and death. ROS generation has been found to be elevated in skeletal muscle during aging. During the aging process, it is probable that elevated levels of ROS lead to the alterations of mitochondrial DNA and to increases in myonuclear apoptosis. Hiona and Christiaan Leeuwenburgh [72] reviewed the potential mechanisms by which mitochondrial DNA mutations related to aging that favor mitochondrial dysfunction may influence the skeletal muscle, and concluded that mitochondrial DNA mutations may contribute to sarcopenia. Based on the mitochondrial "vicious cycle" hypothesis related to the free radical theory of aging, chronic ROS production and oxidative stress can favor mitochondrial DNA mutations, which in turn may result in an elevated mitochondrial ROS production, promoting a cycle of oxidative damage that may lead to muscle cell death [72], which in turn may contribute to sarcopenia [73]. The presence of elevated levels of pro-inflammatory cytokines that may occur with aging also contribute to an elevation of oxidative stress in skeletal muscle [74]. Hence, counteracting oxidative stress by exposure to anti-oxidants may be an important strategy to prevent sarcopenia [75].

The primary and auxiliary extra- and intracellular anti-oxidant protection systems include nutritive anti-oxidants (e.g. vitamin C, vitamin E, carotenoids, conjugated dienoic isomers of linoleic acid, carnosine, anserine and histidine), non-nutritive anti-oxidants (e.g. natural and synthetic phenols, and furanones/furfurals), enzymes (e.g. glutathione peroxidase/transferase or glutathione disulphide reductase that catalyze anti-oxidants regeneration), transition metals (e.g. iron, copper) binders and exporters (e.g. the glutathione conjugate transporter) [76].

Although sufficient intake of anti-oxidants may be considered as an important strategy to prevent sarcopenia [77], Chaput et al. [78] found no significant differences in anti-oxidant intake between the elderly participants with sarcopenia and the nonsarcopenic group. However, it should be noted that the intake of anti-oxidant nutrients in older adults with sarcopenia did not reach the Dietary Reference Intakes (DRIs) in the group of participants without sarcopenia. Nutritional approaches that have been suggested to prevent oxidative stress or benefit muscle protein metabolism via anti-oxidant approaches include resveratrol [79], vitamin E, vitamin C [80], carotenoids [81], vitamin A [82], dehydroepiandrosterone, ornithine, cysteine, N-acetylcysteine, carnitine, epigallocatechin gallate [83] zinc and selenium [82]. Considering that oxidative stress may favor the initiation of sarcopenia [73, 75, 84], future research should clarify specific protein targets for oxidative damage [85] and the mechanistic pathways by which anti-oxidants in foods or supplements may reduce oxidative stress.

In diabetes, antioxidant supplementation appears to prevent muscle atrophy [86]. The impact on cancer cachexia is partial although significant. In contrast, the data on antioxidant supplementation for mammalian sarcopenia are limited and controversial, despite the clinical relevance and large interest. A number of studies have

evaluated the possibility of delaying the aging process by elevating anti-oxidative capacity. For example, resveratrol, a natural polyphenol found in grapes, peanuts, and berries, has demonstrated a protective impact against oxidative stress in skeletal muscle. Although most human studies analyze the association between dietary antioxidant supplementation and physical performance or muscle strength measures, the effect is still debatable. As suggested by Bonetto et al. [86], oxidative stress probably would behave as an additional factor that would certainly amplify wasting stimuli but may not play a leading role in other cases for which the effectiveness of antioxidant therapy was not indicated. A recent statement from the Society on Sarcopenia, cachexia and wasting disease does not mention antioxidant supplementation as a possible tool to manage sarcopenia in older persons [87].

Future randomized controlled trials using single or several anti-oxidants, in supplements or food preparations, should also be investigated for efficacy to decrease oxidative stress in the muscle, and increase net protein balance in older adults.

2.7 Vitamin D

Vitamin D has been traditionally considered as a key regulator of bone metabolism, and calcium and phosphorus homeostasis through negative feedback with parathyroid hormone. Currently, approximately one billion people worldwide have vitamin D deficiency and most of these are elderly. The prevalence of low vitamin D concentrations in people older than 65 years of age has been estimated to be about 50%, but this statistic is variable because it is influenced by sociodemographic, clinical, therapeutic and environmental factors. Similarly, there is an age-dependent decrease found in vitamin D receptor expression in skeletal muscle. Prolonged vitamin D deficiency has been related to severe muscle weakness, which is found to be ameliorated with vitamin D supplementation. A large body of evidence indicates that low vitamin D levels represent an independent risk factor for falls in the elderly [88]. Supplementation with vitamin D in a clinical trial was found to elevate muscle strength and performance and decrease the risk of falling for community-living, elderly and nursing home residents with low vitamin D levels [89]. In contrast, several groups reported no positive impact of vitamin D supplementation on fall event outcomes [90]. Cesari et al. [91] attributed these discrepant findings to the selection criteria adopted to enroll study participants, compliance with the intervention, or the extreme heterogeneity of cut-off points defining the condition of deficiency. A more comprehensive knowledge of vitamin D-related mechanisms may provide a useful tool for preventing muscle atrophy in older persons.

The genomic impact of vitamin D on muscle includes changes in mRNA that will cause de novo protein synthesis involved in controlling cell proliferation and induction of terminal differentiation. In addition, the non-genomic impact of vitamin D on muscle includes the activation of protein kinase C (PKC) and Ca^{2+} in the cytosol. This effect leads to the active transportation of Ca^{2+} into the sarcoplasmic reticulum by Ca^{2+}-ATPase, elevating the calcium pool which is necessary for

muscle contraction [92]. Moreover, the activation of PKC has an impact on protein synthesis in the muscle cells. On other hand, because inflammation is a potential risk factor for sarcopenia, the anti-inflammatory impacts of vitamin D could lead to the improvements in skeletal muscle composition [92].

Vitamin D metabolites may influence muscle mass and function through indirect mechanisms such as hypophosphataemia [93] or secondary hyperparathyroidism of vitamin D deficiency [94]. Direct impacts may also occur through the 1,25(OH)2D3 receptor in muscle tissue [95]. In a systematic review investigating the impacts of exposure to vitamin D on muscle function, Rejnmark [94] identified 16 randomized controlled trials, and all except one of the studies were conducted in individuals above 50 years of age. Over seven studies, vitamin D supplementation resulted in positive impacts on muscle strength [94, 96]. Another systematic review and meta-analysis by Muir and Montero-Odasso [97], which evaluated the efficacy of vitamin D supplementation on muscle strength in elderly population aged over 60 years, found that all studies with ingested doses of 800–1000 IU per day reported useful impacts on muscle strength. In our study, vitamin D supplementation (1000 IU, daily, for 3 months) in vitamin D deficient middle-aged women (40–55 years-old) resulted in improvements in muscle function in the intervention compared to the placebo group. In addition, fat mass percentage was significantly reduced in vitamin D group at the end of intervention but the changes did not reach statistical significance compared with the placebo group. In both groups muscle strength did not differ significantly at the end of the intervention [98]. This might be explained by the possibilities of an insufficient dose of vitamin D supplementation in the vitamin D-deficient women, the period of vitamin D supplementation was not long enough, or the combination of both factors. In addition, baseline vitamin D status or baseline muscle strength or mass might have impacts on the response to vitamin D supplementation [98]. Furthermore, low vitamin D levels are a risk factor for falls in the elderly [99, 100], and its supplementation was demonstrated as an important strategy to decrease the risk of falls among ambulatory or institutionalized older individuals [101]. However, evidence on whether vitamin D supplementation influences muscle mass is scarce [102]. Although vitamin D functions include an important role for muscle health [103], an insufficient vitamin D nutritional status is frequently observed in older adults. In a study with older adults from 11 European countries, 36% of men and 47% of women had circulating concentrations of less than 12 ng/mL in wintertime, this being the lowest mean level found in Southern European countries [104]. Serum vitamin D concentrations may vary widely between participants from different countries [105] and variations in vitamin D status appear to be associated with contrasts in nutritional intake, sunlight exposure and clinical, therapeutic, sociodemographic and environmental factors [106].

2.8 Ursolic Acid

Ursolic acid, a water-insoluble pentacylic triterpenoid, is the major waxy component in apple peels. It is also found in many edible plants. Kunkel et al. [107] reported that ursolic acid decreased skeletal muscle atrophy in the setting of two

distinct atrophy-inducing stresses (fasting and muscle denervation). Ursolic acid might elevate muscle mass by inhibiting atrophy-related skeletal muscle gene expression. The above study found that acute ursolic acid treatment of fasted mice decreased atrogin-1 and MuRF1 mRNA levels in association with decreased muscle atrophy. Similarly, chronic ursolic acid treatment of unstressed mice decreased atrogin-1 and MuRF1 expression and induced muscle hypertrophy. Although ursolic acid elevated skeletal muscle Akt phosphorylation in vivo, the study could not determine if it acted directly on skeletal muscle, how quickly it acted, or if the effect needed insulin-like growth factor (IGF)-I or insulin, which are always present in healthy animals, even during fasting. To investigate these issues, Kunkel et al. [107] evaluated serum-starved skeletal myotubes and found that ursolic acid rapidly stimulated IGF-I receptor and insulin receptor activity, but only if IGF-I or insulin was also present. Altogether, their data suggests that ursolic acid first elevates the capacity of pre-existing IGF-I and insulin to activate skeletal muscle IGF-I receptors and insulin receptors, respectively. However, ursolic acid alone was not sufficient to increase phosphorylation of the IGF-I or insulin receptors, and its impacts also needed IGF-I and insulin, respectively. This proposes that ursolic acid either facilitates hormone-mediated receptor autophosphorylation or suppress receptor dephosphorylation. The latter possibility is supported by previous in vitro data showing that ursolic acid directly suppresses protein tyrosine phosphatase 1B, a tyrosine phosphatase that dephosphorylates and thereby inactivates the IGF-I and insulin receptors. More research is required to clarify the impact of supplementation with ursolic acid in skeletal muscle in the attenuation of muscle atrophy.

3 Caloric Restriction (CR)

CR typically involves consuming 20–40% fewer calories than normal intake as a means of maintaining mitochondrial health and attenuating sarcopenia. CR is recognized as the most important intervention that delays primary (natural age-associated deterioration) and secondary (related to disease and negative lifestyle behaviors) aging, thereby increasing lifespan in many species. Studies in rodents have consistently indicated that CR extends maximum lifespan by up to 50% and decreases the occurrence of many age-related diseases. These protective impacts are attributable to the ability of CR to decrease the incidence of mitochondrial abnormalities and also decrease oxidative stress. In rodents, CR seems to alter mitochondrial efficiency, content and function via reduced proton leakage which, in turn, is enabled by a shift to a less oxidative milieu. With regards of mitochondrial content and function, CR does not influence gene expression, protein level, or activity of citrate synthase [108]. Lanza et al. [109] indicated that CR maintains mitochondrial function by protecting the integrity and function of existing cellular components rather than by elevating mitochondrial biogenesis. Moreover, CR appears to combat the age-associated increases in pro-apoptotic signaling in skeletal muscle [110]. Importantly, CR has been demonstrated to modulate the majority of the apoptotic

pathways involved in age-related skeletal muscle loss, such as mitochondrial-, cytokine/receptor-, and Ca^{2+}/ER-stress-mediated signaling [110]. Therefore, CR notably inhibits increases in several mediators of the TNF-mediated pathway of apoptosis (TNF-α, TNF-receptor 1, cleaved caspase-3 and -8), possibly by elevating production of a muscle-derived anabolic cytokine, IL-15, which competes with TNF-mediated signaling. Furthermore, the combination of CR with exercise training has been suggested to combat the apoptosis related to sarcopenia more effectively.

It is interesting that Baker et al. indicated a significant increase in PGC-1α in gastrocnemius muscle of rats after a 40% CR diet beginning at 16 weeks of age [111]. It has become apparent that PGC-1α binds to and co-activates many transcription factors in addition to PPARγ, including most nuclear factors. Therefore, PGC-1α has various roles, such as in fatty acid oxidation, myokine secretion, activation of autophagy, and neuromuscular junction (NMJ) gene induction, as well as up-regulation of mitochondrial biogenesis [112]. Valdez et al. [113] indicated that lifelong CR significantly reduced the incidence of pre- and postsynaptic abnormalities in 24- month-old mice as well as the age-associated loss of motor neurons, likely due to PGC-1α induction. Since the level of basal autophagy in the skeletal muscle has been shown to be decreased with age [5, 114], normal function of autophagy by CR may weaken the atrophy of muscle fibers during aging. However, CR in mice did not modulate the level of several autophagy-linked molecules (Beclin-1, Atg9, LC3) at the protein level, except for Atg7 in sarcopenic muscles of rats [5]. However, one study [115] demonstrated that CR has no useful effect on health and survival in rhesus monkeys in contrast to many other reports from studies using the same species [116, 117]. More studies are required to evaluate whether CR is effective in counteracting the age-associated loss of muscle in human subjects and to what extent dietary intervention can be applied in human populations. Because excessive CR (over 50%) may have side effects, milder CR conditions should be applied in the elderly population.

4 Dietary Patterns

The eating habits of elderly individuals are affected by several factors, including food preferences that have been formed throughout life, physiological alterations related to aging, socioeconomic conditions, being institutionalized or not, physical disability, and living with a spouse or alone. Food insecurity and hunger are issues of concern for many elderly individuals, especially for those having low socioeconomic status or from minority ethnic groups [118, 119]. Energy requirements decrease with advancing age, and elevated physical activity or exercise may be important to combat this trend. In addition, with higher energy intake by those with increased energy requirements, it is easier to provide the amount of food necessary to meet the nutritional recommendations, especially for micronutrients [120]. The modern Western-type diet is rich in animal products and limited in fruits and vegetables [121], which leads to a net acid production, in contrast with diets abundant in potassium that possess an alkalinizing effect [121]. Moreover, a sufficient

potassium intake and an alkaline diet may favor lean tissue mass in elderly people [122], while acidosis [121] can intensify the reduction in muscle mass. This is also particularly important considering that the normal reduction in kidney function related to age may also favor acidosis [121]. In addition to being important for potassium intake, consumption of fruit and vegetables is negatively related to inflammation in the elderly population [75], and ensuring sufficient intake of these foods is also important to achieve sufficient ingestion of anti-oxidants, including carotenoids [123], polyphenols, tocopherols, ascorbate and selenium [124].

Many of the components previously indicated as having beneficial effects of inflammation and redox status, especially n-3 LCPUFAs and dietary anti-oxidants, are natural constituents of the Mediterranean diet, considering its high content of vegetables, legumes, fruit, nuts, seeds, whole grain cereals, olive oil, fish and herbal infusions [125]. Hence, nutritional strategies are required to limit muscle atrophy and to combat decreases in muscle mass and function. When evaluating relationships between grip strength and empirically healthy dietary patterns such as the prudent dietary pattern, grip strength was positively associated with prudent diet score in community-dwelling elderly population [126]. This diet is generally characterized by high consumption of vegetables, fruit, fatty fish and whole grains, and a low consumption of white bread, chips, sugar and full-fat dairy products. Looking for nutrients and foods using a whole dietary pattern approach may present several advantages over a "single nutrient approach", considering the high number of interactions and synergies that may exist between food components, and future studies are required to investigate this in detail.

5 Exercise Training

Resistance exercise can promote muscle protein synthesis within 1 h of training, which can last for up to 72 h after exercise. Resistance training has been shown to be the most promising among interventions aimed at reducing the impacts of sarcopenia, since it elevates strength, power and mobility function, and induces different degrees of skeletal muscle hypertrophy [127]. For example, 12 weeks of whole-body resistance training led to an increase in type II muscle fiber area in men aged 64–86 years and 65–72 years. A 2-year longitudinal trial of resistance training reported increases in leg press (32%) and military press (90%), single-repetition maximum weight lifted and knee extensor muscle cross-sectional area (9%) in elderly people aged 60–80 years [128]. The functional advantages of resistance training have been investigated in a large trial of 72–98-year-olds and frail nursing home residents, with resistance training elevating muscle strength (113%), stair-climbing power (28%), gait velocity (12%), and spontaneous physical activity [129]. In the elderly, resistance training induces the muscle levels of IGF-I, myogenic regulatory agents, and IL-6, which lead to muscular hypertrophy by regulating the activation, proliferation, and differentiation of satellite cells. However, several studies using humans and rodents demonstrated a lower degree of activation

in mitogen-activated protein kinase (MAPK) and Akt-mTOR pathways after muscle contraction or mechanical overload than occurs in young adults [114]. However, Mayhew et al. [130] reported that resistance exercise induced a similar extent of activation in translational signaling (Akt, p70S6K, ribosomal protein S6, and 4E-BP1) between young and old participants. It might appear surprising that physical activity can influence muscle inflammation. The evidence indicates that chronic resistance physical training leads to the control of locally-derived inflammation via adaptations to repeated and acute elevations in pro-inflammatory mRNA within muscle. Several studies [131] have indicated that the addition of intensive strength training for the elderly reduces the effective gain of muscle strength and mass especially in women. Hence, careful attention should be paid when estimating the amount and intensity of resistance training in this advanced age group.

6 Malnutrition-Sarcopenia Syndrome

Malnutrition has been explained as a status of an imbalance of energy, protein and other nutrients that result in negative impacts on body composition, physical function and clinical conditions [132]. One vital clinical aspect often not evaluated in nutrition screening or assessment is the loss of lean body or muscle mass. Lean body mass is explained as that portion of the body mass except for the fat and includes water, mineral, muscle and other protein-rich structures (including viscera, enzymes, red cells, and connective tissues) [133]. Skeletal muscle mass constitutes the majority of lean body mass and provides strength, mobility and balance [134]. Muscle mass also plays a vital role in whole-body protein metabolism and affects quality of life in patients with chronic diseases [135]. The balance between muscle protein anabolism and catabolism is critically important for maintaining skeletal muscle mass, especially in elderly people who lose muscle mass as a consequence of aging and/or illness [135, 136]. Sarcopenia has been explained as an age-related loss of muscle mass, combined with loss of strength, functionality or both [137].

Research has demonstrated that reductions in handgrip strength are common in individuals who have sarcopenia as well as in individuals who are malnourished [137, 138]. Many elderly individuals are malnourished or at high risk for malnutrition due to many factors. Reduced appetite and food intake, poor dentition, an increased frequency and severity of acute and chronic medical states, multiple medications, social and economic challenges, and cognitive decrease can all play a role in the etiology of malnutrition among older adults. Advanced age is an independent risk factor for malnutrition and is related to a lower body weight, body mass index, and serum albumin levels [139–141].

In many patients, malnutrition and sarcopenia occur in parallel and manifest clinically through a combination of reduced nutrient intake and reduced body weight, along with a decrease in muscle mass, strength, and/or physical function. This has led to coining of the proposed clinical condition as the Malnutrition-Sarcopenia Syndrome. This is the clinical presentation of both malnutrition and

accelerated age-related loss of lean body mass, strength, and/or physical performance. Malnutrition and sarcopenia are each independently related to negative health outcomes that affect older adults across healthcare settings. Patients with malnutrition and/or sarcopenia are at risk of elevated morbidity and mortality, reduced functioning and quality of life, and increased re-hospitalization, length of hospital stay and higher healthcare costs [142–146].

A prospective observational study of a cohort of older adults indicated that higher lean mass predicted lower mortality with an 85% reduction in the risk, proposing that alterations in lean mass, rather than body mass index, are better predictors of mortality in elderly people [147]. This highlights the role of lean muscle mass loss in defining malnutrition.

Hence, examining both of the patient's nutritional and functional status through screening and evaluation for both malnutrition and sarcopenia will enable healthcare practitioners to better determine the presence of the Malnutrition-Sarcopenia Syndrome in their patients and prescribe interventions tailored to fit individual requirements. In addition, as the world is aging and older adults will utilize healthcare services at an increased rate, this could finally lead to better patient care and outcomes in this unique and expanding patient population.

7 Conclusions

To develop strategies to combat or retard sarcopenia, a better understanding of the lifestyle factors that affect the rate of muscle mass and functional losses in older age is required. Current data demonstrates the importance of sufficient quality and quantity of the diet in this process. The high prevalence of low nutrient intake among elderly population has made this a major concern. In addition, much has demonstrated that regular exercise can minimize the physiological impacts of an otherwise sedentary lifestyle by limiting the development and progression of chronic disease and disabling conditions, but only a limited proportion of older adults are physically active. Hence, older adults should optimize both nutrition and exercise as both are important modifiable factors that elevate muscle strength and mass, and contribute to the maintenance of muscle mass and function and the prevention and treatment of sarcopenia. Because the elderly portion of the population has undergone a steady rise over the last century, future work in this area should be designed in younger as well as in older populations.

References

1. von Haehling S, Morley JE, Anker SD (2010) An overview of sarcopenia: facts and numbers on prevalence and clinical impact. J Cachexia Sarcopenia Muscle 1(2):129–133
2. Sakuma K, Yamaguchi A (2010) Molecular mechanisms in aging and current strategies to counteract sarcopenia. Curr Aging Sci 3(2):90–101

3. Sakuma K, Yamaguchi A (2012) Sarcopenia: molecular mechanisms and current therapeutic strategy. In: Perloft JW, Wong AH, (eds) Cell aging. Nova Science Pub Inc., (March 30, 2012), pp 93–152, ISBN-10: 1613243693
4. McKay BR, Ogborn DI, Bellamy LM, Tarnopolsky MA, Parise G (2012) Myostatin is associated with age-related human muscle stem cell dysfunction. FASEB J 25(6):2509–2521
5. Wohlgemuth SE, Seo AY, Marzetti E, Lees HA, Leeuwenburgh C (2010) Skeletal muscle autophagy and apoptosis during aging: effects of calorie restriction and life-long exercise. Exp Gerontol 45:138–148
6. Zhou J, Freeman TA, Ahmad F, Shang X, Mangano E, Gao E et al (2013) GSK-3α is a central regulator of age-related pathologies in mice. J Clin Invest 123(4):1821–1832
7. Sakuma K, Aoi W, Yamaguchi A (2015) Current understanding of sarcopenia: possible candidates modulating muscle mass. Pflügers Arch 467(2):213–229
8. Abiri B, Vafa M (2019) Nutrition and sarcopenia: a review of the evidence of nutritional influences. Crit Rev Food Sci Nutr 59(9):1456–1466
9. Timmerman KL, Volpi E (2008) Amino acid metabolism and regulatory effects in aging. Curr Opin Clin Nutr Metab Care 11(1):45–49
10. Walker DK, Dickinson JM, Timmerman KL, Drummond MJ, Reidy PT, Fry CS et al (2011) Exercise, amino acids, and aging in the control of human muscle protein synthesis. Med Sci Sports Exerc 43(12):2249–2258
11. Esmarck B, Andersen JL, Olsen S, Richter EA, Mizuno M, Kjaer M (2005) Timing of postexercise protein intake is important for muscle hypertrophy with resistance training in elderly humans. J Physiol 567(Pt 1):301–311
12. Drummond MJ, Dreyer HC, Pennings B, Fry CS, Dhanani S, Dillon EL et al (2008) Skeletal muscle protein anabolic response to resistance exercise and essential amino acids is delayed with aging. J Appl Physiol 104(5):1452–1461
13. Walrand S, Short KR, Bigelow ML, Sweatt AJ, Hutson SM, Nair KS (2008) Functional impact of high protein intake on healthy elderly people. Am J Physiol Endocrinol Metab 295(4):E921–E928
14. Godard MP, Williamson DL, Trappe SW (2002) Oral amino-acid provision does not affect muscle strength or size gains in older men. Med Sci Sports Exerc 34(7):1126–1131
15. Nicastro H, Artioli GG, Dos Santos Costa A, Sollis MY, Da Luz CR, Blachier F et al (2011) An overview of the therapeutic effects of leucine supplementation on skeletal muscle under atrophic conditions. Amino Acids 40(2):287–300
16. Waterlow JC (1984) Protein turnover with special reference to man. Q J Exp Physiol 69(3):409–438
17. Young VR, Steffee WP, Pencharz PB, Winterer JC, Scrimshaw NS (1975) Total human body protein synthesis in relation to protein requirements at various ages. Nature 253(5488):192–194
18. Tang JE, Phillips SM (2009) Maximizing muscle protein anabolism: the role of protein quality. Curr Opin Clin Nutr Metab Care 12(1):66–71
19. Breen L, Phillips SM (2011) Skeletal muscle protein metabolism in the elderly: interventions to counteract the "anabolic resistance" of ageing. Nutr Metab (Lond) 8:68. https://doi.org/10.1186/1743-7075-8-68
20. Churchward-Venne TA, Burd NA, Phillips SM (2012) Nutritional regulation of muscle protein synthesis with resistance exercise: strategies to enhance anabolism. Nutr Metab (Lond) 9(1):40. https://doi.org/10.1186/1743-7075-9-40
21. Vafa MR, Abiri B, Haghifar S, Malkami A, Esmaeili F, Arefazar A et al (2017) The association of food intake and physical activity with body composition, muscle strength and muscle function in postmenopausal women. Nutr Food Sci Res 4(1):11–16
22. Millward DJ, Fereday A, Gibson N, Pacy PJ (1997) Aging, protein requirements, and protein turnover. Am J Clin Nutr 66(4):774–786
23. Gersovitz M, Motil K, Munro HN, Scrimshaw NS, Young VR (1982) Human protein requirements: assessment of the adequacy of the current Recommended Dietary Allowance for dietary protein in elderly men and women. Am J Clin Nutr 35(1):6–14

24. Campbell WW, Trappe TA, Wolfe RR, Evans WJ (2001) The recommended dietary allowance for protein may not be adequate for older people to maintain skeletal muscle. J Gerontol A Biol Sci Med Sci 56(6):M373–M380
25. Houston DK, Nicklas BJ, Ding J, Harris TB, Tylavsky FA, Newman AB et al (2008) Dietary protein intake is associated with lean mass change in older, community-dwelling adults: the Health, Aging, and Body Composition (Health ABC) Study. Am J Clin Nutr 87(6):150–155
26. Volkert D (2011) The role of nutrition in the prevention of sarcopenia. Wien Med Wochenschr 161(17–18):409–415
27. Evans WJ (2004) Protein nutrition, exercise and aging. J Am Coll Nutr 23(6 Suppl):601S–609S
28. Paddon-Jones D, Rasmussen BB (2009) Dietary protein recommendations and the prevention of sarcopenia. Curr Opin Clin Nutr Metab Care 12(1):86–90
29. Tieland M, Borgonjen-Van den Berg KJ, van Loon LJ, de Groot LC (2012) Dietary protein intake in community-dwelling, frail, and institutionalized elderly people: scope for improvement. Eur J Nutr 51(2):173–179
30. Nissen SL, Abumrad NN (1997) Nutritional role of the leucine metabolite β-hydroxy β-methylbutyrate (HMB). J Nutr Biochem 8(6):300–311
31. Zanchi NE, Gerlinger-Romero F, Guimarães-Ferreira L, de Siqueira Filho MA, Felitti V, Lira FS et al (2011) HMB supplementation: clinical and athletic performance-related effects and mechanisms of action. Amino Acids 40(4):1015–1025
32. Flakoll P, Sharp R, Baier S, Levenhagen D, Carr C, Nissen S (2004) Effect of β-hydroxy-β-methylbutyrate, arginine, and lysine supplementation on strength, functionality, body composition, and protein metabolism in elderly women. Nutrition 20(5):445–451
33. Candow DG (2011) Sarcopenia: current theories and the potential beneficial effect of creatine application strategies. Biogerontology 12(4):273–281
34. Smith SA, Montain SJ, Matott RP, Zientara GP, Jolesz FA, Fielding RA (1998) Creatine supplementation and age influence muscle metabolism during exercise. J Appl Physiol (1985) 85(4):1349–1356
35. Campbell WW, Joseph LJ, Davey SL, Cyr-Campbell D, Anderson RA, Evans WJ (1999) Effects of resistance training and chromium picolinate on body composition and skeletal muscle in older men. J Appl Physiol 86(1):29–39
36. Conley KE, Jubrias SA, Esselman PC (2000) Oxidative capacity and ageing in human muscle. J Appl Physiol 526(1):203–210
37. Rawson ES, Clarkson PM, Price TB, Miles MP (2002) Differential response of muscle phosphocreatine to creatine supplementation in young and old subjects. Acta Physiol Scand 174(1):57–65
38. Tesch PA, Thorsson A, Fujitsuka N (1989) Creatine phosphate in fiber types of skeletal muscle before and after exhaustive exercise. J Appl Physiol (1985) 66(4):1756–1759
39. Lexell J, Taylor CC (1991) Variability in muscle fibre areas in whole human quadriceps muscle: effects of increasing age. J Anat 174:239–249
40. Larsson PO, Mosbach K (1979) Affinity precipitation of enzymes. FEBS Lett 98(2):333–338
41. Lanza IR, Befroy DE, Kent-Braun JA (2005) Age-related changes in ATP-producing pathways in human skeletal muscle in vivo. J Appl Physiol (1985) 99(5):1736–1744
42. Brose A, Parise G, Tarnopolsky MA (2003) Creatine supplementation enhances isometric strength and body composition improvements following strength exercise training in older adults. J Gerontol A Biol Sci Med Sci 58(1):11–19
43. Vandenberghe K, Goris M, Van Hecke P, Van Leemputte M, Vangerven L, Hespel P (1987) Long-term creatine intake is beneficial to muscle performance during resistance training. J Appl Physiol (1985) 83(6):2055–2063
44. Volek JS, Duncan ND, Mazzetti SA, Staron RS, Putukian M, Gómez AL et al (1999) Performance and muscle fiber adaptations to creatine supplementation and heavy resistance training. Med Sci Sports Exerc 31(8):1147–1156

45. Eijnde BO, Van Leemputte M, Goris M, Labarque V, Taes Y, Verbessem P et al (2003) Effects of creatine supplementation and exercise training on fitness in men 55–75 yr old. J Appl Physiol (1985) 95(2):818–828
46. Wyss M, Felber S, Skladal D, Koller A, Kremser C, Sperl W (1998) The therapeutic potential of oral creatine supplementation in muscle disease. Med Hypotheses 51(4):333–336
47. 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
48. Rawson ES, Clarkson PM (2000) Acute creatine supplementation in older men. Int J Sports Med 21(1):71–75
49. Wiroth JB, Bermon S, Andreï S, Dalloz E, Hébuterne X, Dolisi C (2001) Effects of oral creatine supplementation on maximal pedalling performance in older adults. Eur J Appl Physiol 84(6):533–539
50. Gotshalk LA, Volek JS, Staron RS, Denegar CR, Hagerman FC, Kraemer WJ (2002) Creatine supplementation improves muscular performance in older men. Med Sci Sports Exerc 33(12):2111–2117
51. Stout JR, Sue Graves B, Cramer JT, Goldstein ER, Costa PB, Smith AE 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
52. Gotshalk LA, Kraemer WJ, Mendonca MA, Vingren JL, Kenny AM, Spiering BA et al (2008) Creatine supplementation improves muscular performance in older women. Eur J Appl Physiol 102(2):223–231
53. Cañete S, San Juan AF, Pérez M, Gómez-Gallego F, López-Mojares LM, Earnest CP et al (2006) Does creatine supplementation improve functional capacity in elderly women? J Strength Cond Res 20(1):22–28
54. Candow DG, Chilibeck PD (2008) Timing of creatine or protein supplementation and resistance training in the elderly. Appl Physiol Nutr Metab 33(1):184–190
55. Tarnopolsky M, Zimmer A, Paikin J, Safdar A, Aboud A, Pearce E et al (2007) Creatine monohydrate and conjugated linoleic acid improve strength and body composition following resistance exercise in older adults. PLoS One 2(10):e991. https://doi.org/10.1371/journal.pone.0000991
56. Dalbo VJ, Roberts MD, Lockwood CM, Tucker PS, Kreider RB, Kerksick CM (2009) The effects of age on skeletal muscle and the phosphocreatine energy system: can creatine supplementation help older adults. Dyn Med 8:6. https://doi.org/10.1186/1476-5918-8-6
57. Jensen GL (2008) Inflammation: roles in aging and sarcopenia. JPEN J Parenter Enteral Nutr 32(6):656–659
58. Calder PC (2006) N-3 polyunsaturated fatty acids, inflammation, and inflammatory diseases. Am Am J Clin Nutr 83(6 Suppl):1505S–1519S
59. Podbregar M, Lainscak M, Prelovsek O, Mars T (2013) Cytokine response of cultured skeletal muscle cells stimulated with proinflammatory factors depends on differentiation stage. ScientificWorldJournal 2013:617170. https://doi.org/10.1155/2013/617170
60. Toth MJ, Matthews DE, Tracy RP, Previs MJ (2005) Age-related differences in skeletal muscle protein synthesis: relation to markers of immune activation. Am J Physiol Endocrinol Metab 288(5):E883–E891
61. Lang CH, Frost RA, Nairn AC, MacLean DA, Vary TC (2002) TNF-alpha impairs heart and skeletal muscle protein synthesis by altering translation initiation. Am J Physiol Endocrinol Metab 282:E336–E347
62. Villani AM, Crotty M, Cleland LG, James MJ, Fraser RJ, Cobiac L et al (2013) Fish oil administration in older adults: is there potential for adverse events? A systematic review of the literature. BMC Geriatr 13:41. https://doi.org/10.1186/1471-2318-13-41
63. Calder PC (2013) Omega-3 polyunsaturated fatty acids and inflammatory processes: nutrition or pharmacology? Br J Clin Pharmacol 75(3):645–662
64. Robinson SM, Jameson KA, Batelaan SF, Martin HJ, Syddall HE, Dennison EM et al (2008) Diet and its relationship with grip strength in community-dwelling older men and women: the Hertfordshire cohort study. J Am Geriatr Soc 56(1):84–90

65. Smith GI, Atherton P, Reeds DN, Mohammed BS, Rankin D, Rennie MJ et al (2011) Dietary omega-3 fatty acid supplementation increases the rate of muscle protein synthesis in older adults: a randomized controlled trial. Am J Clin Nutr 93(2):402–412
66. Robinson SM, Jameson KA, Batelaan SF, Martin HJ, Syddall HE, Dennison EM et al (2008) Diet and its relationship with grip strength in community–dwelling older men and women: the Hertfordshire cohort study. J Am Geriatr Soc 56(1):84–90
67. Galli C, Calder PC (2009) Effects of fat and fatty acid intake on inflammatory and immune responses: a critical review. Ann Nutr Metab 55(1–3):123–139
68. Anderson BM, Ma DW (2009) Are all n-3 polyunsaturated fatty acids created equal? Lipids Health Dis 8:33. https://doi.org/10.1186/1476-511X-8-33
69. Harris WS, Mozaffarian D, Lefevre M, Toner CD, Colombo J, Cunnane SC et al (2009) Towards establishing dietary reference intakes for eicosapentaenoic and docosahexaenoic acids. J Nutr 139(4):804S–819S
70. Eslick GD, Howe PR, Smith C, Priest R, Bensoussan A (2009) Benefits of fish oil supplementation in hyperlipidemia: a systematic review and meta-analysis. Int J Cardiol 136(1):4–16
71. Smith GI, Atherton P, Reeds DN, Mohammed BS, Rankin D, Rennie MJ et al (2011) Dietary omega-3 fatty acid supplementation increases the rate of muscle protein synthesis in older adults: a randomized controlled trial. Am J Clin Nutr 93(2):402–412
72. Hiona A, Leeuwenburgh C (2008) The role of mitochondrial DNA mutations in aging and sarcopenia: implications for the mitochondrial vicious cycle theory of aging. Exp Gerontol 43(1):24–33
73. Marzetti E, Calvani R, Bernabei R, Leeuwenburgh C (2012) Apoptosis in skeletal myocytes: a potential target for interventions against sarcopenia and physical frailty – a mini-review. Gerontology 58(2):99–106
74. Arthur ST, Cooley ID (2012) The effect of physiological stimuli on sarcopenia; impact of notch and Wnt signaling on impaired aged skeletal muscle repair. Int J Biol Sci 8(5):731–760
75. Semba RD, Varadhan R, Bartali B, Ferrucci L, Ricks MO, Blaum C et al (2007) Low serum carotenoids and development of severe walking disability among older women living in the community: the women's health and aging study I. Age Ageing 36(1):62–67
76. Bonorden WR, Pariza MW (1994) Antioxidant nutrients and protection from free radicals. In: Kotsonis F, Mackey M, Hjelle J (eds) Nutritional toxicology. Raven Press, New York, pp 19–48. ISBN-10: 0415248655
77. Volkert D (2011) The role of nutrition in the prevention of sarcopenia. Wien Med Wochenschr 161(17–18):409–415
78. Chaput JP, Lord C, Cloutier M, Aubertin Leheudre M, Goulet ED et al (2007) Relationship between antioxidant intakes and class I sarcopenia in elderly men and women. J Nutr Health Aging 11(4):363–369
79. Ryan MJ, Jackson JR, Hao Y, Williamson CL, Dabkowski ER, Hollander JM et al (2010) Suppression of oxidative stress by resveratrol after isometric contractions in gastrocnemius muscles of aged mice. J Gerontol A Biol Sci Med Sci 65(8):815–831
80. Ryan MJ, Dudash HJ, Docherty M, Geronilla KB, Baker BA, Haff GG et al (2010) Vitamin E and C supplementation reduces oxidative stress, improves antioxidant enzymes and positive muscle work in chronically loaded muscles of aged rats. Exp Gerontol 45(11):882–895
81. Semba RD, Blaum C, Guralnik JM, Moncrief DT, Ricks MO, Fried LP (2003) Carotenoid and vitamin E status are associated with indicators of sarcopenia among older women living in the community. Aging Clin Exp Res 15(6):482–487
82. Marzani B, Balage M, Vénien A, Astruc T, Papet I, Dardevet D et al (2008) Antioxidant supplementation restores defective leucine stimulation of protein synthesis in skeletal muscle from old rats. J Nutr 138(11):2205–2211
83. Bonetto A, Penna F, Muscaritoli M, Minero VG, Rossi Fanelli F, Baccino FM et al (2009) Are antioxidants useful for treating skeletal muscle atrophy? Free Radic Biol Med 47(7):906–916
84. Martin C, Dubouchaud H, Mosoni L, Chardigny JM, Oudot A, Fontaine E et al (2007) Abnormalities of mitochondrial functioning can partly explain the metabolic disorders encountered in sarcopenic gastrocnemius. Aging Cell 6(2):165–177

85. Baraibar MA, Gueugneau M, Duguez S, Butler-Browne G, Bechet D, Friguet B (2013) Expression and modification proteomics during skeletal muscle ageing. Biogerontology 14(3):339–352
86. Bonetto A, Penna F, Muscaritoli M, Minero VG, Fanelli FR, Baccino FM et al (2009) Are antioxidants useful for treating skeletal muscle atrophy? Free Radic Biol Med 47(7):906–916
87. Morley JE, Abbatecola AM, Argiles JM, Baracos V, Bauer J, Bhasin S et al (2011) Sarcopenia with limited mobility: an international consensus. J Am Med Dir Assoc 12(6):403–409
88. Snijder MB, Van Schoor NM, Pluijm SM, Van Dam RM, Visser M, Lips P (2006) Vitamin D status in relation to one-year risk of recurrent falling in older men and women. J Clin Endocrinol Metab 91(8):2980–2985
89. Annweiler C, Schott AM, Berrut G, Fantino B, Beauchet O (2009) Vitamin D-related changes in physical performance: a systematic review. J Nutr Health Aging 13(10):893–898
90. Sanders KM, Stuart AL, Williamson EJ, Simpson JA, Kotowicz MA, Young D et al (2010) Annual high-dose oral vitamin D and falls and fractures in older women: a randomized controlled trial. JAMA 291(18):1815–1822
91. Cesari M, Incalzi RA, Zamboni V, Pahor M (2011) Vitamin D hormone: a multitude of actions potentially influencing the physical function decline in older persons. Geriatr Gerontol Int 11:133–142
92. Abiri B, Vafa MR (2017) Vitamin D and sarcopenia. Adv Obes Weight Manag Control 6(3):00155. https://doi.org/10.15406/aowmc.2017.06.00155
93. Schubert L, DeLuca HF (2010) Hypophosphatemia is responsible for skeletal muscle weakness of vitamin D deficiency. Arch Biochem Biophys 500(2):157–161
94. Rejnmark L (2011) Effects of vitamin d on muscle function and performance: a review of evidence from randomized controlled trials. Ther Adv Chronic Dis 2(1):25–37
95. Bischoff HA, Borchers M, Gudat F, Duermueller U, Theiler R, Stähelin HB et al (2001) In situ detection of 1,25-dihydroxyvitamin D3 receptor in human skeletal muscle tissue. Histochem J 33(1):19–24
96. Stockton KA, Mengersen K, Paratz JD, Kandiah D, Bennell KL (2011) Effect of vitamin D supplementation on muscle strength: a systematic review and meta-analysis. Osteoporos Int 22(3):859–871
97. Muir SW, Montero-Odasso M (2011) Effect of vitamin D supplementation on muscle strength, gait and balance in older adults: a systematic review and meta-analysis. J Am Geriatr Soc 59(12):2291–2300
98. Abiri B, Vafa MR, Dehghani M, Moslehi N, Sarrafzadeh J (2016) Effect of vitamin D supplement consumption on muscle strength, muscle function and body composition in vitamin D-deficient middle- aged women: a randomized clinical trial. Nutr Food Sci Res 3(3):17–24
99. Faulkner KA, Cauley JA, Zmuda JM, Landsittel DP, Newman AB, Studenski SA et al (2006) Higher 1,25-dihydroxyvitamin D3 concentrations associated with lower fall rates in older community-dwelling women. Osteoporos Int 17(9):1318–1328
100. Snijder MB, van Schoor NM, Pluijm SM, van Dam RM, Visser M, Lips P (2006) Vitamin D status in relation to one-year risk of recurrent falling in older men and women. J Clin Endocrinol Metab 91(8):2980–2985
101. Bischoff-Ferrari HA, Dawson-Hughes B, Willett WC, Staehelin HB, Bazemore MG, Zee RY et al (2004) Effect of vitamin D on falls: a meta-analysis. JAMA 291(16):1999–2006
102. Ceglia L, Harris SS (2013) Vitamin D and its role in skeletal muscle. Calcif Tissue Int 92(2):151–162
103. Janssen HC, Samson MM, Verhaar HJ (2002) Vitamin D deficiency, muscle function, and falls in elderly people. Am J Clin Nutr 75(4):611–615
104. van der Wielen RP, Löwik MR, van den Berg H, de Groot LC, Haller J, Moreiras O et al (1995) Serum vitamin D concentrations among elderly people in Europe. Lancet 346(8969):207–210
105. Lips P (2007) Vitamin D status and nutrition in Europe and Asia. J Steroid Biochem Mol Biol 103(3–5):620–625

106. Sakuma K, Yamaguchi A (2012) Sarcopenia and age-related endocrine function. Int J Endocrinol 2012:127362. https://doi.org/10.1155/2012/127362
107. Kunkel SD, Suneja M, Ebert SM, Bongers KS, Fox DK, Malmberg SE et al (2011) mRNA expression signatures of human skeletal muscle atrophy identify a natural compound that increases muscle mass. Cell Metab 13(6):627–638
108. Hancock CR, Han DH, Higashida K, Kim SH, Holloszy JO (2011) Does calorie restriction induce mitochondrial biogenesis? A reevaluation. FASEB J 25(2):785–791
109. Lanza IR, Zabielski P, Klaus KA, Morse DM, Heppelmann CJ, Bergen HR 3rd et al (2012) Chronic caloric restriction preserves mitochondrial function in senescence without increasing mitochondrial biogenesis. Cell Metab 16(6):777–788
110. Dirks AJ, Leeuwenburgh C (2004) Aging and lifelong calorie restriction result in adaptations of skeletal muscle apoptosis repressor, apoptosis-inducing factor, X-linked inhibitor of apoptosis, caspase-3, and caspase-12. Free Radic Biol Med 36(1):27–39
111. Baker DJ, Betik AC, Krause DJ, Hepple RT (2006) No decline in skeletal muscle oxidative capacity with aging in long-term calorically restricted rats: effects are independent of mitochondrial DNA integrity. J Gerontol A Biol Sci Med Sci 61:675–684
112. Chan MC, Arany Z (2014) The many roles of PGC-1α in muscle–recent developments. Metabolism 63(4):441–451
113. Valdez G, Tapia JC, Kang H, Clemenson GD Jr, Gage FH, Lichtman JW et al (2010) Attenuation of age-related changes in mouse neuromuscular synapses by caloric restriction and exercise. Proc Natl Acad Sci U S A 107(33):14863–14868
114. Sakuma K, Aoi W, Yamaguchi A (2014) The intriguing regulators of muscle mass in sarcopenia and muscular dystrophy. Front Aging Neurosci 6:230. https://doi.org/10.3389/fnagi.2014.00230
115. Mattison JA, Roth GS, Beasley TM, Tilmont EM, Handy AM, Herbert RL et al (2012) Impact of caloric restriction on health and survival in rhesus monkeys from the NIA study. Nature 489(7415):318–322
116. McKiernan SH, Colman RJ, Lopez M, Beasley TM, Aiken JM, Anderson RM et al (2011) Caloric restriction delays aging-induced cellular phenotypes in rhesus monkey skeletal muscle. Exp Gerontol 46(1):23–29
117. McKiernan SH, Colman RJ, Aiken E, Evans TD, Beasley TM, Aiken JM et al (2012) Cellular adaptation contributes to calorie restriction-induced preservation of skeletal muscle in aged rhesus monkeys. Exp Gerontol 47(3):229–236
118. Kuczmarski MF, Weddle DO, American Dietetic Association (2005) Position paper of the American Dietetic Association: nutrition across the spectrum of aging. J Am Diet Assoc 105(4):616–633
119. Bernstein M, Munoz N, Academy of Nutrition and Dietetics (2012) Position of the Academy of Nutrition and Dietetics: food and nutrition for older adults: promoting health and wellness. J Acad Nutr Diet 112(8):1255–1277
120. Schlenker ED (1992) Nutrition for aging and the aged. In: Williams SR, Worthington-Roberts BS (eds) Nutrition through the life cycle. Mosby-Year Book, St Louis, pp 344–383. ISBN-10: 0801664772
121. Adeva MM, Souto G (2011) Diet-induced metabolic acidosis. Clin Nutr 30(4):416–421
122. Dawson-Hughes B, Harris SS, Ceglia L (2008) Alkaline diets favor lean tissue mass in older adults. Am J Clin Nutr 87(3):662–665
123. Doria E, Buonocore D, Focarelli A, Marzatico F (2012) Relationship between human aging muscle and oxidative system pathway. Oxidative Med Cell Longev 2012:830257
124. Buonocore DS, Rucci S, Vandoni M, Negro M, Marzatico F (2011) Oxidative system in aged skeletal muscle. Muscles Ligaments Tendons J 1(3):85–90
125. Bach-Faig A, Berry EM, Lairon D, Reguant J, Trichopoulou A, Dernini S et al (2011) Mediterranean diet pyramid today. Science and cultural updates. Public Health Nutr 14(12A):2274–2284

126. Robinson SM, Jameson KA, Batelaan SF, Martin HJ, Syddall HE, Dennison EM et al (2008) Diet and its relationship with grip strength in community–dwelling older men and women: the Hertfordshire cohort study. J Am Geriatr Soc 56(1):84–90
127. Fiatarone MA, O'Neil EF, Ryan ND, Clements KM, Solares GR, Nelson ME et al (1994) Exercise training and nutritional supplementation for physical frailty in very elderly people. N Engl J Med 330(25):1769–1775
128. McCartney N, Hicks AL, Martin J, Webber CE (1996) A longituidinal trial of weight training in the elderly: continued improvements in year 2. J Gerontol A Biol Sci Med Sci 51(6):B425–B433
129. Fiatarone MA, O'Neil EF, Ryan ND, Clements KM, Solares GR, Nelson ME et al (1994) Exercise training and nutritional supplementation for physical frailty in very elderly people. N Engl J Med 330(25):1769–1775
130. Mayhew DL, Kim JS, Cross JM, Bamman MM (2009) Translational signaling responses preceding resistance training-mediated myofiber hypertrophy in young and old humans. J Appl Physiol (1985) 107(5):1655–1662
131. Kosek DJ, Kim JS, Petrella JK, Cross JM, Bamman MM (2006) Efficacy of 3 days/wk resistance training on myofiber hypertrophy and myogenic mechanisms in young vs. older adults. J Appl Physiol (1985) 101(2):531–544
132. Elia M (2000) Guidelines for detection and management of malnutrition. BAPEN; Maidenhead. ISBN-10: 1899467459
133. Demling RH (2009) Nutrition, anabolism, and the wound healing process: an overview. Eplasty 9:e9. Epub 2009 Feb 3
134. Wolfe RR (2006) The underappreciated role of muscle in health and disease. Am J Clin Nutr 84(3):475–482
135. McCarthy JJ, Esser KA (2010) Anabolic and catabolic pathways regulating skeletal muscle mass. Curr Opin Clin Nutr Metab Care 13(3):230–235
136. Nair KS (1995) Muscle protein turnover: methodological issues and the effect of aging J Gerontol A Biol Sci Med Sci 50 Spec No:107–112
137. Cruz-Jentoft AJ, Baeyens JP, Bauer JM, Boirie Y, Cederholm T, European Working Group on Sarcopenia in Older People et al (2010) Sarcopenia: European consensus on definition and diagnosis. Age Ageing 39(4):412–423
138. Norman K, Stobäus N, Gonzalez MC, Schulzke JD, Pirlich M (2011) Hand grip strength: outcome predictor and marker of nutritional status. Clin Nutr 30(2):135–142
139. Pirlich M, Schütz T, Norman K, Gastell S, Lübke HJ, Bischoff SC et al (2006) The German hospital malnutrition study. Clin Nutr 25(4):563–572
140. Pirlich M, Schütz T, Kemps M, Luhman N, Minko N, Lübke HJ et al (2005) Social risk factors for hospital malnutrition. Nutrition 21(3):295–300
141. Forster S, Gariballa S (2005) Age as a determinant of nutritional status: a cross sectional study. Nutr J 4:28. https://doi.org/10.1186/1475-2891-4-28
142. Cederholm T, Jagren C, Hellstrom K (1995) Outcome of protein-energy malnutrition in elderly medical patients. Am J Med 98(1):67–74
143. Newman AB, Yanez D, Harris T, Duxbury A, Enright PL, Cardiovascular Study Research Group et al (2001) Weight change in old age and its association with mortality. J Am Geriatr Soc 49(10):1309–1318
144. Wallace JI, Schwartz RS, LaCroix AZ, Uhlmann RF, Pearlman RA (1995) Involuntary weight loss in older outpatients: incidence and clinical significance. J Am Geriatr Soc 43(4):329–337
145. Williamson DF, Pamuk ER (1993) The association between weight loss and increased longevity: a review of the evidence. Ann Intern Med 119(7 Pt 2):731–736
146. French SA, Folsom AR, Jeffery RW, Williamson DF (1999) Prospective study of intentionality of weight loss and mortality in older women: the Iowa Women's Health Study. Am J Epidemiol 149(6):504–514
147. Han SS, Kim KW, Kim KI, Na KY, Chae DW, Kim S et al (2010) Lean mass index: a better predictor of mortality than body mass index in elderly Asians. J Am Geriatr Soc 58(2):312–317

Chapter 13
The Use of Metformin to Increase the Human Healthspan

Veronika Piskovatska, Kenneth B. Storey, Alexander M. Vaiserman, and Oleh Lushchak

1 Introduction

Metformin or dimethylbiguanide, a medication that occurs in significant amounts in *Galega officinalis*, is the most useful medication in type 2 diabetes (T2D) management. It was initially approved for treatments in the United Kingdom (UK; 1958) and in the United States by the Food and Drug Administration (FDA; 1995) [1] and became the most prescribed anti-diabetic drug worldwide after the UK Prospective Diabetes Study [2] that showed significant benefits of metformin for treating cardiovascular diseases [3]. Currently, metformin is used as the primary choice for the pharmacological treatment of T2D [4, 5]. Being inexpensive and safe for management of glucose it is primary prescribed for elder patients because of the reduced risk of hypoglycemia and non-fatal cardiovascular pathologies compared to other antidiabetic drugs [6].

Along with the antidiabetic properties, metformin may be used for the prevention and treatment of pathologies related to aging. It may be effective to manage glucose tolerance [7] and obesity [8] or any complications related to cardiovascular system

V. Piskovatska
Clinic for Heart Surgery, University Clinic of the Martin Luther University, Halle, Germany

K. B. Storey
Institute of Biochemistry, Carleton University4, Ottawa, ON, Canada

A. M. Vaiserman
D.F. Chebotarev Institute of Gerontology, NAMS, Kyiv, Ukraine

O. Lushchak (✉)
Department of Biochemistry and Biotechnology, Vasyl Stefanyk Precarpathian National University, Ivano-Frankivsk, Ukraine
e-mail: olehl@pu.if.ua

P. C. Guest (ed.), *Reviews on New Drug Targets in Age-Related Disorders*,
Advances in Experimental Medicine and Biology 1260,
https://doi.org/10.1007/978-3-030-42667-5_13

such as hypertension [9], atherosclerosis [10] or endothelial dysfunction [11]. Long-term metformin treatment has reduced the risk of cognitive decline and dementia among older diabetic patients [12]. Moreover, metformin may be affective in decreasing the chronic inflammation that is among the main factors triggering age-related health complications [13]. Anticancer properties of metformin were shown in experimental models and epidemiological studies [14, 15]. Interestingly, diabetic patients treated with metformin were shown to have longer lifespan even if compared with healthy individuals [16]. This fact underlines the importance to understand the main targets of metformin action in aging and related complications.

In the current review, we have summarized the metabolic effects of metformin in humans and the possibilities of using metformin as a therapeutic intervention for cardiovascular diseases, inflammation, frailty and cancer. Moreover, modulation of the microbiome, safety considerations and the lifespan promoting effects of metformin are discussed.

2 Metabolic Effects of Metformin

The antidiabetic effects of metformin are mediated through reduction of hyperglycemia and enhancement of insulin sensitivity via mutiple molecular mechanisms. Metformin primarily targets mitochondrial metabolism in hepatocytes. Metformin inhibits mitochondrial glycerophosphate dehydrogenase of hepatocytes, thus reducing gluconeogenesis and hyperglycemia [17, 18]. Metformin inhibits complex I of the electron transport chain, preventing mitochondrial ATP production and leading to energy deficiency and subsequent activation of 5' AMP-activated protein kinase (AMPK). Activated AMPK phosphorylates the acetyl-CoA carboxylases 1 and 2 (ACC1, ACC2), inhibiting fat synthesis and enhancing fat oxidation. These metabolic changes result in reduction of hepatic steatosis and improve hepatic insulin sensitivity [19]. Recent studies have also explored the gastrointestinal effects of metformin and the potential antidiabetic effects mediated by gut microbiota. These mechanisms are highlighted in detail in Sect. 7.

In patients at high risk of developing T2DM, metformin reduced or delayed the risk of diabetes compared to placebo or diet and exercise. However, metformin treatment was not more beneficial in preventing T2DM, when compared with intensive diet and exercise [20]. Metformin has recently re-gained attention as a perspective treatment for patients with type 1 diabetes. As an insulin-sensitizing agent it improves glycemic control, reduces body weight and improves lipid parameters of the patients [21–23]. However, trials addressing the long-term outcomes of such interventions remain scarce.

Multiple years of clinical use have revealed pleiotropic metabolic effects of metformin, other than the hypoglycemic effect. Meta-analysis of studies conducted among patients with pre-diabetes showed that metformin allocation resulted in favorable reduction of triglycerides and low density lipoprotein-cholesterol

(LDL-C) and increased high density lipoprotein cholesterol (HDL-C) [24]. The use of metformin was associated with reduction in total cholesterol and atherogenic LDL-C levels in elderly individuals [25]. Favorable effects of metformin on lipid profiles have included ACC1 and ACC2-mediated inhibition of de-novo fatty acid synthesis [26] and AMPK- and glucogon-like peptide (GLP)-1-mediated reduction of the biosynthesis of lipoproteins, triglycerides and chylomicrons [27].

Weight loss effects of metformin were demonstrated in multiple studies of T2DM patients and non-diabetic individuals. The results of several meta-analyses have shown that metformin use is associated with modest, yet significant weight loss in T2DM patients and non-diabetic obese individuals [25, 28, 29]. In women with polycystic ovary syndrome (PCOS), metformin leads to reduction in body mass index (BMI), subcutaneous adipose tissue and testosterone levels, and an increase in the number of menstrual cycles [30]. A systemic review and meta-analysis of studies in adults and children administered with atypical antipsychotic medications showed that metformin caused a significant reduction of the gained body weight [31]. Metformin increases insulin sensitivity, reduces leptin levels and provides lipolytic and anorectic effects by increased GLP-1 production [32]. *In vitro* and clinical data have shown that metformin may induce secretion of growth differentiating factor 15 (GDF15), a factor possessing central anorexigenic effects, promoting appetite reduction in association with weight loss [33].

3 Cardiovascular Disease

Metformin reduces risk of cardiovascular disease in T2DM patients and non-diabetics. Sub-analysis of obese patients from one of the largest trials, the United Kingdom Prospective Diabetes Study [2], showed that metformin treatment led to a 33% reduction of myocardial infarction risk, compared to patients who received conventional treatment [34]. Over 10 years of follow-up showed a sustainable reduction in microvascular risk, and a reduction of risk of myocardial infarction and death from any cause was observed among the overweight patients. This effect was thought to be exerted due to pleiotropic effects of metformin, not just to glycemic control alone [35]. A recent meta-analysis showed substantial reduction of cardiovascular mortality, all-cause mortality and cardiovascular events in patients with coronary artery disease allocated to metformin treatment [36]. Similar results were demonstrated in the later meta-analysis, pooling data from studies in T2DM patients allocated to metformin [37].

Metformin contributes to cardiovascular risk reduction by improving glycaemia with a favorable impact on the lipid profile. In specific populations of patients, metfomin was shown to reduce cardiac fibrosis [11, 38], improve endothelial function [39] and reduce myocardial hypertrophy [40]. In a recent randomized controlled trial (RCT), metformin treatment was demonstrated to significantly reduce levels of biomarkers of endothelial dysfunction [von Willebrand factor (vWF), soluble

vascular cell adhesion molecule-1 (sVCAM-1), tissue plasminogen activator (t-PA), plasminogen activator inhibitor-1 (PAI-1), C-reactive protein (CRP) and soluble intercellular adhesion molecule-1 (sICAM-1)] in T2DM patients. During 4.3 years of follow-up, metformin-allocated patients also demonstrated a 34% reduction in cardiovascular morbidity and mortality [41]. In patients with heart failure and reduced ejection fraction, metformin was considered safe and effective to control glycemia, even in patients with kidney function decline [42].

4 Inflammation

Multiple age-related diseases and conditions are instigated by chronic low-grade inflammation. In patients with T2DM several studies have provided evidence of anti-inflammatory effects of metformin. In patients with T2DM, metformin monotherapy is associated with significantly lower levels of inflammatory molecules [tumor necrosis factor alpha (TNFα), soluble TNF receptor 1 (sTNFRI), and sTNFRII] in comparison with other antidiabetic monotherapies [43]. A study by Chen and colleagues showed a significant reduction of proinflammatory cytokines [interleukin-6 (IL-6) and TNF-α] in serum and urinary MCP-1 compared to other antidiabetic drugs (gliclazide, acarbose, or repaglinide) [44]. This reduction in pro-inflammatory serum and urinary markers was time- and dose-dependent. In another study, involving 3575 treatment naïve T2DM patients, metformin in comparison to sulfonylurea significantly reduced the mean neutrophil to lymphocyte ratio, a marker of systemic inflammation and predictor of all-cause mortality and cardiac events [45].

5 Cancer

Metformin has been widely discussed as a prospective agent to be repurposed as an adjuvant treatment of malignancies such as cancer and/or cancer recurrence chemoprevention [46, 47]. In vitro and in vivo studies have shown that metformin might sensitize tumor cells to chemotherapeutic agents that with time have lost their efficacy due to multi-drug resistance effects [48]. Numerous studies have confirmed an association between T2DM, hyperglycemia, insulin resistance, hyperinsulinemia, concomitant obesity and metabolic syndrome with significantly higher risks of liver, pancreatic, endometrial, colorectal, breast, and bladder cancer [49, 50].

The results of a recent meta-analysis combining data from cohort studies, suggested lower risk of cancer incidence in T2DM patients taking metformin compared to sulfonylureas [51]. A systematic review by Franciosi and co-authors pooled the data on cancer mortality and cancer risk from 41 observational studies including 1,029,389 patients. Metformin was associated with a 35% reduction in cancer

mortality risk, and a 31% reduction in risk of any cancer [52]. Zhang and colleagues demonstrated that metformin is preventive against liver cancer in T2DM patients [53].

Observational studies have shown a reduced incidence of endometrial cancer (EC) and an improved overall survival in metformin-treated women with T2DM [54]. In another systematic review and meta-analysis of studies, Meireles and colleagues showed reversion of atypical endometrial hyperplasia under metformin treatment. Biomarkers of cell proliferation were also significantly decreased in these patients. Patients with EC treated with metformin had higher overall survival compared to non-metformin-treated and non-diabetic patients. The authors hypothesized that patients with EC can potentially benefit from adjuvant administration of metformin via reduction of cell proliferation, reversal of atypical hyperplasia, and improved overall survival [55]. Short-term presurgical administration of metformin in women with EC contributed to significant reduction in the tumor expression of Ki-67 [56]. Anti-proliferating effects of metformin, targeting the PI3K/AKT/mTOR signaling pathway were shown in EC patients allocated to metformin with a daily dose of 1500 mg 3–4 weeks prior to hysterectomy [57].

A meta-analysis including 7 studies and 7178 patients with T2DM evaluated the impact of metformin treatment on the occurrence of colorectal adenoma (CRA) [58]. Treatment with metformin correlated with a significant decrease in the risk of CRA in diabetic subjects, showing a 27% reduction in comparison with non-metformin antidiabetic agents. A meta-analysis by Menget et al. showed better overall survival in metformin-treated colorectal cancer patients with no effect on cancer-specific survival [59].

A recent meta-analysis of RCTs, combining data from 1520 breast cancer (BC) patients showed that metformin treatment did not significantly influence overall survival or progression-free survival compared to control study arms. However, metformin treatment substantially modified biomarkers of insulin resistance, inflammation, dyslipidemia and tumor proliferation (Ki-67, p-Akt) [60]. A protective effect of metformin against BC in postmenopausal diabetic women was also indicated by a meta-analysis of 11 studies, which included 5464 BC patients with diabetes (2760 patients who had received metformin and 2704 patients who had not) [61]. This analysis showed that metformin treatment was associated with a 47% decreased risk of death from all causes in BC patients with diabetes, as well as reduced cancer-related mortality. After adjusting for the expression of hormonal receptors in different patients, metformin showed an even greater 65% improvement in overall survival [62]. Recently, metformin treatment also showed potential as an additional cancer-treatment option in non-diabetic BC patients, revealing indirect insulin-dependent effects of intervention. Women with newly diagnosed, treatment-naïve, early-stage BC were allocated to 1500 mg of metformin daily for 2 weeks after tumor biopsy and before the surgery. Biopsies and immunohistochemical analysis of tumor material were performed before metformin administration and after the surgery. After metformin administration, the tumor tissue showed markedly reduced insulin receptor expression along with significantly reduced

phosphorylation of PKB/Akt, ERK1/2 and reduced PI3K and Ras-MAPK signaling. Dowling and colleagues proposed that tumor insulin receptor expression and fasting plasma insulin levels could be biomarkers used to allocate patients to metformin treatment [63].

According to recent meta-analyses, metformin treatment does not influence the incidence of prostate cancer (PC) [64, 65]. However metformin therapy significantly improved overall survival, prostate-cancer-specific survival and recurrence-free survival in patients with PC, compared to non-metformin treatment [64].

Data about cancer incidence and mortality among metformin -treated patients should be taken into consideration with caution. Studies included in meta-analyses are often heterogeneous, have short follow-up, and may contain causal interpretations of findings [66, 61]. Even though epidemiological data confirms possible anticancer effects of metformin, the exact molecular mechanism of tumor growth suppression remains unknown. Suggested effects include AMPK-activation and m-TOR inhibition, as well as possible inhibition of HER2 and NF-κB signaling [61, 67].

6 Frailty

Frailty is a complex geriatric syndrome, including progredient reduction of muscle mass, muscle quality and strength [68, 69]. Frailty substantially aggravates other age-related diseases, reducing mobility, increasing risk of falls, and inevitably worsening the prognosis of the patient [70]. Accumulating evidence indicates that frailty could potentially be prevented and/or affected by metformin [71]. One study showed that use of metformin at a 1500 mg/day dose for 6 weeks resulted in significantly improved gait speed but did not significantly affect quality of life or indices of muscle strength in non-diabetic elderly patients [72]. Elderly T2DM patients treated with metformin had a reduced risk of frailty and comorbidity, compared to metformin-naïve patients. Metformin-treated patients in this study also demonstrated better muscle strength and body balance characteristics [73]. Musi and colleagues suggested that enhanced phosphorylation of AMPK and glucose uptake are the underlying mechanisms for better muscular function and reduction in frailty indices [74]. A study by Gore et al. showed enhanced muscle protein anabolism in response to metformin in patients with severe burns in intensive care units [75].

Metformin was shown to prevent osteoporosis in experimental models [76–78]. Administration of metformin in T2DM appears to have positive effects on bone mineral density and prevent diabetes-related bone tissue loss [79]. A recent meta-analysis showed an inverse correlation between metformin use and risk of fractures [80]. High-quality RCTs with clear criteria, defining the frailty syndrome standards and compounds use are warranted to determine whether or not metformin can indeed have a protective influence in populations of elderly patients.

7 Modulation of the Microbiome

There is a mounting evidence suggesting that metformin has potential to tackle bacteria-bacteria and host-microbiome interactions. A double-blind placebo-controlled study by Wu and colleagues showed a shift in the gut microbiota composition after 2 and 4 months of metformin treatment in previously treatment-naïve T2DM patients [81]. The microbial landscape was significantly altered with an increased abundance of *Escherichia* and *Intestinibacter* [82, 81]. An increase in the population of *Bifidobacterium adolescentis* also correlated with glycated hemoglobin (HbA1c) levels, suggesting potential contribution of this bacterial strain to anti-diabetic effects of metformin. Changes in microbial subpopulations were also reflected by increased production of short-chain fatty acids (SCFA) and changes in bile acid metabolism. In this study, plasma bile acid concentrations also correlated with HbA1c reduction, suggesting a role in glucose homeostasis regulation [82].

8 Safety Considerations

About one-third of patients, allocated to metformin suffer from gastrointestinal adverse effects including nausea, vomiting, bloating, and diarrhea. Mechanisms behind these adverse events are poorly understood, although the symptoms rarely lead to treatment discontinuation. Metformin in its extended release (XR) form seems to be an alternative that is more tolerable and equivalently effective for glycemic control. XR forms of metformin include specific polymers, expanding in the fluid and ensuring the slow absorption of the drug into the blood stream [83]. XR-metformin can potentially ensure better compliance and reduce the incidence of gastrointestinal side effects. A small trial, conducted in T2DM patients showed that XR- metformin provided better glycemic control, lipid profiles, and levels of certain adipokines in comparison with immediate-release metformin [84]. Another randomized head-to-head trial in pharmacologically naïve T2DM patients demonstrated equal therapeutic effects and adverse event rates in both groups [85]. High-quality RCTs with longer follow-up is necessary to draw conclusions about safety and efficacy of the XR forms of metformin in terms of clinical outcomes.

Individual response to metformin varies, and about 35% patients with T2DM fail to reach glycemic goal with standard doses using monotherapy [86, 87]. Some genetic polymorphisms in transport proteins, like the organic cation transporter 1 (OCT1), might affect individual metformin responses and mediate occurrence of gastrointestinal-side-effects. Polypharmacy and concomitant treatment with other drugs, blocking OCT1 may also result in decreased tolerability of metformin treatment [88, 89].

Lactic acidosis (LA) used to be a major considerable adverse event associated to metformin treatment. However, recent studies suggest that LA occurs extremely rarely, with a similar incidence in T2DM patients taking metformin or in those prescribed with other glucose-lowering agents [90, 42].

Metformin is known to deplete vitamin B12 and cause deficiency with anemia and polyneuropathy [91, 92]. These disturbances occur at higher doses (1500–2000 mg/day) and with long-term use of metformin [93]. Patients allocated to high doses of metformin, exposed to other risk factors (systematic alcohol use, unbalanced diet) could benefit from regular vitamin-B12 deficiency screening and appropriate supplementation.

It is highly likely that metformin can impair muscle regeneration and muscular energetics, especially in older adults. A recent trial demonstrated that metformin inhibits the mitochondrial adaptation to aerobic exercise in older adults [94]. In the MASTERS trial, allocation to metformin prevented muscle hypertrophy induced by resistance exercise in healthy older adults [95]. The combination of metformin with exercise should be further investigated before recommending such a program to slow down age-related decline.

Optimal daily dosing for off-label metformin treatment and the optimal age for treatment initiation remain unknown. Evidence from high-quality RCTs, conducted in non-diabetic patients is still scarce or missing.

9 Metformin, Survival and Longevity

There is mounting evidence suggesting that metformin increases lifespan and healthspan in model organisms such as worms [96–99] and mice [100, 101]. Evidence showing that metformin can reduce all-cause mortality in humans has been obtained in several human observational studies. The United Kingdom Prospective Diabetes Study [2] revealed a 42% lower risk of diabetes-related death compared to the effects seen with conventional diabetes treatments. Remarkably, the risk for all-cause mortality was also reduced by 36% in the metformin -treated group. Reduction in all-cause mortality was observed in diabetic patients taking metformin in comparison to subjects with T2DM allocated to sulfonylurea treatment. The same study also suggested that metformin use invokes health benefits beyond glycemic control since T2DM patients on metformin monotherapy had better survival than age- and sex-matched non-diabetic controls [16]. A systematic review and meta-analysis by Campbell and coauthors summarizing the data from 53 studies, showed that T2DM patients taking metformin had significantly lower all-cause mortality than non-diabetics (hazard ratio = 0.93, 95% CI 0.88–0.99) [102].

10 Conclusions and Future Perspectives

The majority of the clinical data about health benefits of metformin have been obtained from the study of patients with diabetes, insulin resistance, obesity, often having comorbidities and taking multiple medications to control concomitant diseases. Long-term treatment with metformin seems to be safe, allows effective

glycemic control and offers additional health benefits, not attributed to glycemia management alone. Metformin exerts multiple metabolic effects, affecting neuroendocrine regulation, mediating weight reduction, improving insulin sensitivity and favorably modifying the lipid profile. Nevertheless, side effects of metformin can occur in about 30% of patients and this necessitates careful monitoring with dose and formulation (extended release forms) adjustment. Data about safety and efficacy of metformin in healthy and young individuals remains scarce. Well-designed RCTs are warranted to clarify whether or not prescription of metformin at a younger age is safe and really contributes to optimal health maintenance and longevity.

Acknowledgments This work was partially supported by the Ministry of Education and Science of Ukraine (#0117 U006426) to OL and Discovery grant from the Natural Sciences and Engineering Research Council of Canada (#6793) to KBS.

References

1. Miles JM, Rule AD, Borlaug BA (2014) Use of metformin in diseases of aging. Curr Diab Rep 14(6):490. https://doi.org/10.1007/s11892-014-0490-4
2. UK Prospective Diabetes Study (UKPDS) Group (1998) Effect of intensive blood-glucose control with metformin on complications in overweight patients with type 2 diabetes (UKPDS 34). Lancet 352:854–865
3. Bailey CJ (2017) Metformin: historical overview. Diabetologia 60(9):1566–1576
4. Larsen JR, Dima L, Correll CU, Manu P (2018) The pharmacological management of metabolic syndrome. Expert Rev Clin Pharmacol 11:397–410
5. Samocha-Bonet D, Debs S, Greenfield JR (2018) Prevention and treatment of type 2 diabetes: a pathophysiological-based approach. Trends Endocrinol Metab 29:370–379
6. Schlender L, Martinez YV, Adeniji C, Reeves D, Faller B, Sommerauer C et al (2017) Efficacy and safety of metformin in the management of type 2 diabetes mellitus in older adults: a systematic review for the development of recommendations to reduce potentially inappropriate prescribing. BMC Geriatr 17(Suppl 1):227. https://doi.org/10.1186/s12877-017-0574-5
7. Hostalek U, Gwilt M, Hildemann S (2015) Therapeutic use of metformin in prediabetes and diabetes prevention. Drugs 75:1071–1094
8. Berstein LM (2012) Metformin in obesity, cancer and aging: addressing controversies. Aging (Albany NY) 4:320–329
9. Zhou L, Liu H, Wen X, Peng Y, Tian Y, Zhao L (2017) Effects of metformin on blood pressure in nondiabetic patients: a meta-analysis of randomized controlled trials. J Hypertens 35:18–26
10. Jenkins AJ, Welsh P, Petrie JR (2018) Metformin, lipids and atherosclerosis prevention. Curr Opin Lipidol 29:346–353
11. Nesti L, Natali A (2017) Metformin effects on the heart and the cardiovascular system: a review of experimental and clinical data. Nutr Metab Cardiovasc Dis 27(8):657–669
12. Ng TP, Feng L, Yap KB, Lee TS, Tan CH, Winblad B (2014) Long-term metformin usage and cognitive function among older adults with diabetes. J Alzheimers Dis 41(1):61–68
13. Saisho Y (2015) Metformin and inflammation: its potential beyond glucose-lowering effect. Endocr Metab Immune Disord Drug Targets 15:196–205
14. Heckman-Stoddard BM, DeCensi A, Sahasrabuddhe VV, Ford LG (2017) Repurposing metformin for the prevention of cancer and cancer recurrence. Diabetologia 60(9):1639–1647

15. Safe S, Nair V, Karki K (2018) Metformin-induced anticancer activities: recent insights. Biol Chem 399:321–335
16. Bannister CA, Holden SE, Jenkins-Jones S, Morgan CL, Halcox JP, Schernthaner G (2014) Can people with type 2 diabetes live longer than those without? A comparison of mortality in people initiated with metformin or sulphonylurea monotherapy and matched, non-diabetic controls. Diabetes Obes Metab 16:1165–1173
17. Madiraju AK, Erion DM, Rahimi Y, Zhang XM, Braddock DT, Albright RA et al (2014) Metformin suppresses gluconeogenesis by inhibiting mitochondrial glycerophosphate dehydrogenase. Nature 510:542–546
18. Foretz M, Guigas B, Bertrand L, Pollak M, Viollet B et al (2014) Metformin: from mechanisms of action to therapies. Cell Metab 20(6):953–966
19. Rena G, Hardie DG, Pearson ER (2017) The mechanisms of action of metformin. Diabetologia 60:1577–1585
20. Madsen KS, Chi Y, Metzendorf MI, Richter B, Hemmingsen B (2019) Metformin for prevention or delay of type 2 diabetes mellitus and its associated complications in persons at increased risk for the development of type 2 diabetes mellitus. Cochrane Database Syst Rev (12). https://doi.org/10.1002/14651858.CD008558.pub2
21. Snaith JR, Holmes-Walker DJ, Greenfield JR (2020) Reducing type 1 diabetes mortality: role for adjunctive therapies? Trends Endocrinol Metab 31:150. https://doi.org/10.1016/j.tem.2019.11.007. [Epub ahead of print]
22. Beysel S, Unsal IO, Kizilgul M, Caliskan M, Ucan B, Cakal E (2018) The effects of metformin in type 1 diabetes mellitus. BMC Endocr Disord 18(1):1. https://doi.org/10.1186/s12902-017-0228-9
23. Livingstone R, Boyle JG, Petrie JR, REMOVAL Study Team (2017) A new perspective on metformin therapy in type 1 diabetes. Diabetologia 60(9):1594–1600
24. Salpeter SR, Buckley NS, Kahn JA, Salpeter EE et al (2008) Meta-analysis: metformin treatment in persons at risk for diabetes mellitus. Am J Med 121(2):149–157
25. Solymar M, Ivic I, Poto L, Hegyi P, Garami A, Hartmann P et al (2018) Metformin induces significant reduction of body weight, total cholesterol and LDL levels in the elderly–a meta-analysis. PLoS One 13(11):e0207947. https://doi.org/10.1371/journal.pone.0207947
26. Zhou J, Massey S, Story D, Li L (2018) Metformin: an old drug with new applications. Int J Mol Sci 19(10):2863. https://doi.org/10.3390/ijms19102863
27. van Stee MF, de Graaf AA, Groen AK (2018) Actions of metformin and statins on lipid and glucose metabolism and possible benefit of combination therapy. Cardiovasc Diabetol 17(1):94. https://doi.org/10.1186/s12933-018-0738-4
28. Ning HH, Le J, Wang Q, Young CA, Deng B, Gao PX et al (2018) The effects of metformin on simple obesity: a meta-analysis. Endocrine 62:528–534
29. Hui F, Zhang Y, Ren T, Li X, Zhao M, Zhao Q (2019) Role of metformin in overweight and obese people without diabetes: a systematic review and network meta-analysis. Eur J Clin Pharmacol 75(4):437–450
30. Naderpoor N, Shorakae S, de Courten B, Misso ML, Moran LJ, Teede HJ (2016) Metformin and lifestyle modification in polycystic ovary syndrome: systematic review and meta-analysis. Hum Reprod Update 21(5):560–574
31. Björkhem-Bergman L, Asplund AB, Lindh JD (2011) Metformin for weight reduction in non-diabetic patients on antipsychotic drugs: a systematic review and meta-analysis. J Psychopharmacol 25:299–305
32. Malin SK, Kashyap SR (2014) Effects of metformin on weight loss: potential mechanisms. Curr Opin Endocrinol Diabetes Obes 21(5):323–329
33. Day EA, Ford RJ, Smith BK, Mohammadi-Shemirani P, Morrow MR, Gutgesell RM et al (2019) Metformin-induced increases in GDF15 are important for suppressing appetite and promoting weight loss. Nature Metabol 1(12):1202–1208
34. American Diabetes Association (2002) Implications of the United Kingdom prospective diabetes study. Diabetes Care 25(Suppl 1):28–32

35. Holman RR, Sanjoy KP, Bethel MA, Matthews DR, Neil AW (2008) 10-year follow-up of intensive glucose control in type 2 diabetes. N Engl J Med 359:1577–1589
36. Han Y, Xie H, Liu Y, Gao P, Yang X, Shen Z (2019) Effect of metformin on all-cause and cardiovascular mortality in patients with coronary artery diseases: a systematic review and an updated meta-analysis. Cardiovasc Diabetol 18:96. https://doi.org/10.1186/s12933-019-0900-7
37. Zhang K, Yang W, Dai H, Deng Z (2020) Cardiovascular risk following metformin treatment in patients with type 2 diabetes mellitus: results from meta-analysis. Diabetes Res Clin Pract 160:108001. https://doi.org/10.1016/j.diabres.2020.108001
38. Dziubak A, Wójcicka G, Wojtak A, Bełtowski J (2018) Metabolic effects of metformin in the failing heart. Int J Mol Sci 19(10):2869. https://doi.org/10.3390/ijms19102869
39. Sardu C, Paolisso P, Sacra C, Mauro C, Minicucci F, Portoghese M et al (2019) Effects of metformin therapy on coronary endothelial dysfunction in patients with prediabetes with stable angina and nonobstructive coronary artery stenosis: the CODYCE multicenter prospective study. Diabetes Care 42(10):1946–1955
40. Mohan M, Al-Talabany S, McKinnie A, Mordi IR, Singh JS, Gandy SJ et al (2019) A randomized controlled trial of metformin on left ventricular hypertrophy in patients with coronary artery disease without diabetes: the MET-REMODEL trial. Eur Heart J 40(41):3409–3417
41. de Jager J, Kooy A, Schalkwijk C, van der Kolk J, Lehert P, Bets D et al (2014) Long-term effects of metformin on endothelial function in type 2 diabetes: a randomized controlled trial. J Intern Med 275:59–70
42. Eurich DT, Weir DL, Majumdar SR, Tsuyuki RT, Johnson JA, Tjosvold L (2013) Comparative safety and effectiveness of metformin in patients with diabetes mellitus and heart failure. Systematic review of observational studies involving 34,000 patients. Circ Heart Fail 6:395–402
43. Tizazu AM, Zin NMS, Olivier C, Suku K, Mok E, Xian CH et al (2019) Metformin monotherapy downregulates diabetes-associated inflammatory status and impacts on mortality. Front Physiol 10:572. https://doi.org/10.3389/fphys.2019.00572
44. Chen W, Liu X, Ye S (2016) Effects of metformin on blood and urine pro-inflammatory mediators in patients with type 2 diabetes. J Inflamm Res 13(1):34. https://doi.org/10.1186/s12950-016-0142-3
45. Cameron AR, Morrison VL, Levin D, Mohan M, Forteath C, Beall C et al (2016) Anti-inflammatory effects of metformin irrespective of diabetes status. Circ Res 119:652–665
46. Vancura A, Bu P, Bhagwat M, Zeng J, Vancurova I (2018) Metformin as an anticancer agent. Trends Pharmacol Sci 39(10):867–878
47. Kobayashi Y, Banno K, Kunitomi H, Tominaga E, Aoki D (2019) Current state and outlook for drug repositioning anticipated in the field of ovarian cancer. J Gynecol Oncol 30(1):e10. https://doi.org/10.3802/jgo.2019.30.e10
48. Roshan MH, Shing YK, Pace NP (2019) Metformin as an adjuvant in breast cancer treatment. SAGE Open Med 7:2050312119865114. https://doi.org/10.1177/2050312119865114
49. Giovannucci E, Harlan DM, Archer MC, Bergenstal RM, Gapstur SM, Habel L et al (2010) Diabetes and cancer: a consensus report. Diabetes Care 33(7):1674–1685
50. Tsilidis KK, Kasimis JC, Lopez DS, Ntzani EE, Ioannidis JP (2015) Type 2 diabetes and cancer: umbrella review of meta-analyses of observational studies. BMJ 350:g7607. https://doi.org/10.1136/bmj.g7607
51. Mekuria AN, Ayele Y, Tola A, Mishore KM (2019) Monotherapy with metformin versus sulfonylureas and risk of cancer in type 2 diabetic patients: a systematic review and meta-analysis. J Diabetes Res 2019:7676909. https://doi.org/10.1155/2019/7676909
52. Franciosi M, Lucisano G, Lapice E, Strippoli GFM, Pellegrini F, Nicolucci A (2013) Metformin therapy and risk of cancer in patients with type 2 diabetes: systematic review. PLoS One 8:e71583. https://doi.org/10.1371/journal.pone.0071583
53. Zhang ZJ, Zheng ZJ, Shi R, Su Q, Jiang Q, Kip KE (2012) Metformin for liver cancer prevention in patients with type 2 diabetes: a systematic review and meta-analysis. J Clin Endocrinol Metab 97:2347–2353

54. Tang YL, Zhu LY, Li Y, Wang J, Zeng XX, Hu KX et al (2017) Metformin use is associated with reduced incidence and improved survival of endometrial cancer: a meta-analysis. Biomed Res Int 2017:5905384. https://doi.org/10.1155/2017/5905384
55. Meireles CG, Pereira SA, Valadares LP, Rêgo DF, Simeoni LA, Guerra ENS et al (2017) Effects of metformin on endometrial cancer: systematic review and meta-analysis. GynecolOncol 147:167–180
56. Sivalingam VN, Kitson S, McVey R et al (2016) Measuring the biological effect of presurgical metformin treatment in endometrial cancer. Br J Cancer 114(3):281–289
57. Zhao Y, Sun H, Feng M, Zhao J, Zhao X, Wan Q et al (2018) Metformin is associated with reduced cell proliferation in human endometrial cancer by inhibiting PI3K/AKT/mTOR signaling. Gynecol Endocrinol 34(5):428–432
58. Hou YC, Hu Q, Huang J, Fang JY, Xiong H (2017) Metformin therapy and the risk of colorectal adenoma in patients with type 2 diabetes: a meta-analysis. Oncotarget 8:8843–8853
59. Meng F, Song L, Wang W (2017) Metformin improves overall survival of colorectal cancer patients with diabetes: a meta-analysis. J Diabetes Res 2017:5063239. https://doi.org/10.1155/2017/5063239
60. Zhang ZJ, Yuan J, Bi Y, Wang C, Liu Y (2019) The effect of metformin on biomarkers and survivals for breast cancer- a systematic review and meta-analysis of randomized clinical trials. Pharmacol Res 141:551–555
61. Col NF, Ochs L, Springmann V, Aragaki AK, Chlebowski RT (2012) Metformin and breast cancer risk: a meta-analysis and critical literature review. Breast Cancer Res Treat 135:639–646
62. Xu H, Chen K, Jia X, Tian Y, Dai Y, Li D et al (2015) Metformin use is associated with better survival of breast cancer patients with diabetes: a meta-analysis. Oncologist 20:1236–1244
63. Dowling RJ, Niraula S, Chang MC, Done SJ, Ennis M, McCready DR et al (2015) Changes in insulin receptor signaling underlie neoadjuvant metformin administration in breast cancer: a prospective window of opportunity neoadjuvant study. Breast Cancer Res 17(1):32. https://doi.org/10.1186/s13058-015-0540-0
64. He K, Hu H, Ye S, Wang H, Cui R, Yi L et al (2019) The effect of metformin therapy on incidence and prognosis in prostate cancer: a systematic review and meta-analysis. Sci Rep 9:2218. https://doi.org/10.1038/s41598-018-38285-w
65. Feng Z, Zhou X, Liu N, Wang J, Chen X, Xu X (2019) Metformin use and prostate cancer risk: a meta-analysis of cohort studies. Medicine 98(12):e14955. https://doi.org/10.1097/MD.0000000000014955
66. Stevens RJ, Ali R, Bankhead CR, Bethel MA, Cairns BJ, Camisasca RP et al (2012) Cancer outcomes and all-cause mortality in adults allocated to metformin: systematic review and collaborative meta-analysis of randomized clinical trials. Diabetologia 55:2593–2603
67. Lei Y, Yi Y, Liu Y, Liu X, Keller ET, Qian CN et al (2017) Metformin targets multiple signaling pathways in cancer. Chin J Cancer 36:17. https://doi.org/10.1186/s40880-017-0184-9
68. Wilson D, Jackson T, Sapey E, Lord JM (2017) Frailty and sarcopenia: the potential role of an aged immune system. Ageing Res Rev 36:1–10
69. Cruz-Jentoft AJ, Kiesswetter E, Drey M, Sieber CC (2017) Nutrition, frailty, and sarcopenia. Aging Clin Exp Res 29:43. https://doi.org/10.1007/s40520-016-0709-0
70. Li G, Thabane L, Papaioannou A, Ioannidis G, Levine MA, Adachi JD (2017) An overview of osteoporosis and frailty in the elderly. BMC Musculoskelet Disord 18(1):46. https://doi.org/10.1186/s12891-017-1403-x
71. Espinoza SE, Jiwani R, Wang J, Wang CP (2019) Review of interventions for the frailty syndrome and the role of metformin as a potential pharmacologic agent for frailty prevention. Clin Ther 41(3):376–386
72. Laksmi PW, Setiati S, Tamin TZ (2017) Effect of metformin on handgrip strength, gait speed, myostatin serum level, and health-related quality of life: a double blind randomized controlled trial among non-diabetic pre-frail elderly patients. Acta Med Indones Apr 49(2):118–127

73. Sumantri S, Setiati S, Purnamasari D, Dewiasty E (2014) Relationship between metformin and frailty syndrome in elderly people with type 2 diabetes. Acta Med Indones 46:183–188
74. Musi N, Hirshman MF, Nygren J, Svanfeldt M, Bavenholm P, Rooyackers O (2002) Metformin increases AMP-activated protein kinase activity in skeletal muscle of subjects with type 2 diabetes. Diabetes 51:2074–2081
75. Gore DC, Wolf SE, Sanford A, Herndon DN, Wolfe RR (2005) Influence of metformin on glucose intolerance and muscle catabolism following severe burn injury. Ann Surg 241:334–342
76. Gao Y, Li Y, Xue J, Jia Y, Hu J (2010) Effect of the anti-diabetic drug metformin on bone mass in ovariectomized rats. Eur J Pharmacol 635:231–236
77. Mai QG, Zhang ZM, Xu S, Lu M, Zhou RP, Zhao L et al (2011) Metformin stimulates osteoprotegerin and reduces RANKL expression in osteoblasts and ovariectomized rats. J Cell Biochem 112:2902–2909
78. Tolosa MJ, Chuguransky SR, Sedlinsky C, Schurman L, McCarthy AD, Molinuevo MS et al (2013) Insulin-deficient diabetes-induced bone microarchitecture alterations are associated with a decrease in the osteogenic potential of bone marrow progenitor cells: preventive effects of metformin. Diabetes Res Clin Pract 101:177–186
79. Ferrari S, Abrahamsen B, Napoli N, Akesson K, Chandran M, Eastell R et al (2018) Diagnosis and management of bone fragility in diabetes: an emerging challenge. Osteoporos Int 29:2585–2596
80. Salari-Moghaddam A, Sadeghi O, Keshteli AH, Larijani B, Esmaillzadeh A (2019) Metformin use and risk of fracture: a systematic review and meta-analysis of observational studies. Osteoporos Int 30:1167–1173
81. Wu H, Esteve E, Tremaroli V, Khan MT, Caesar R, Mannerås-Holm L et al (2017) Metformin alters the gut microbiome of individuals with treatment-naive type 2 diabetes, contributing to the therapeutic effects of the drug. Nat Med 23:850–858
82. Forslund K, Hildebrand F, Nielsen T, Falony G, Le Chatelier E, Sunagawa S et al (2015) Disentangling type 2 diabetes and metformin treatment signatures in the human gut microbiota. Nature 528:262–266
83. Jabbour S, Ziring B (2011) Advantages of extended-release metformin in patients with type 2 diabetes mellitus. Postgrad Med 123(1):15–23
84. Derosa G, D'Angelo A, Romano D, Maffioli P (2017) Effects of metformin extended release compared to immediate release formula on glycemic control and glycemic variability in patients with type 2 diabetes. Drug Des Devel Ther 11:1481–1488
85. Aggarwal N, Singla A, Mathieu C, Montanya E, Pfeiffer AFH, Johnsson E et al (2018) Metformin extended-release versus immediate-release: an international, randomized, double-blind, head-to-head trial in pharmacotherapy-naïve patients with type 2 diabetes. Diabetes Obes Metab 20(2):463–467
86. Florez JC (2011) Does metformin work for everyone? A genome-wide association study for metformin response. Curr Diab Rep 11:467. https://doi.org/10.1007/s11892-011-0220-0
87. van Leeuwen N, Swen JJ, Guchelaar HJ, t Hart LM (2013) The role of pharmacogenetics in drug disposition and response of oral glucose-lowering drugs. Clin Pharmacokinet 52(10):833–854
88. Dujic T, Zhou K, Donnelly LA, Tavendale R, Palmer CN, Pearson ER (2015) Association of organic cation transporter 1 with intolerance to metformin in type 2 diabetes: a GoDARTS study. Diabetes 64(5):1786–1793
89. Dujic T, Causevic A, Bego T, Malenica M, Velija-Asimi Z, Pearson E et al (2016) Organic cation transporter 1 variants and gastrointestinal side effects of metformin in patients with type 2 diabetes. Diabet Med 33(4):511–514
90. Salpeter SR, Greyber E, Pasternack GA, Salpeter EE (2010) Risk of fatal and nonfatal lactic acidosis with metformin use in type 2 diabetes mellitus. Cochrane Database Syst Rev 4:CD002967. https://doi.org/10.1002/14651858.CD002967.pub3
91. Aroda VR, Edelstein SL, Goldberg RB, Knowler WC, Marcovina SM, Orchard TJ et al (2016) Diabetes prevention program research group. Long-term metformin use and vitamin B12 deficiency in the diabetes prevention program outcomes study. J Clin Endocrinol Metabol 101:1754–1761

92. Alharbi TJ, Tourkmani AM, Abdelhay O, Alkhashan HI, Al-Asmari AK, Bin Rsheed AM et al (2018) The association of metformin use with vitamin B12 deficiency and peripheral neuropathy in Saudi individuals with type 2 diabetes mellitus. PLoS One 13(10):e0204420. https://doi.org/10.1371/journal.pone.0204420
93. De Jager J, Kooy A, Lehert P, Wulffelé MG, Van der Kolk J, Bets D et al (2010) Long term treatment with metformin in patients with type 2 diabetes and risk of vitamin B-12 deficiency: randomised placebo controlled trial. BMJ 340:c2181. https://doi.org/10.1136/bmj.c2181
94. Konopka AR, Laurin JL, Schoenberg HM, Reid JJ, Castor WM, Wolff CA et al (2019) Metformin inhibits mitochondrial adaptations to aerobic exercise training in older adults. Aging Cell 18(1):e12880. https://doi.org/10.1111/acel.12880
95. Walton RG, Dungan CM, Long DE, Tuggle SC, Kosmac K, Peck BD et al (2019) Metformin blunts muscle hypertrophy in response to progressive resistance exercise training in older adults: a randomized, double-blind, placebo-controlled, multicenter trial: the MASTERS trial. Aging Cell 18(6):e13039. https://doi.org/10.1111/acel.13039
96. Onken B, Driscoll M (2010) Metformin induces a dietary restriction-like state and the oxidative stress response to extend C. elegans Healthspan via AMPK, LKB1, and SKN-1. PLoS One 5:e8758. https://doi.org/10.1371/journal.pone.0008758
97. Cabreiro F, Au C, Leung KY, Vergara-Irigaray N, Cochemé HM, Noori T et al (2013) Metformin retards aging in C. elegan Metformin retards aging in C. elegans by s by altering microbial folate and methionine metabolism. Cell 153:228–239
98. De Haes W, Frooninckx L, Van Assche R, Smolders A, Depuydt G, Billen J et al (2014) Metformin promotes lifespan through mitohormesis via the peroxiredoxin PRDX-2. Proc Natl Acad Sci U S A 111(24):E2501–E2509
99. Wu L, Zhou B, Oshiro-Rapley N, Li M, Paulo JA, Webster CM (2016) An ancient, unified mechanism for metformin growth inhibition in C elegans and cancer. Cell 167:1705–1718
100. Anisimov VN, Berstein LM, Egormin PA, Piskunova TS, Popovich IG, Zabezhinski MA (2008) Metformin slows down aging and extends life span of female SHR mice. Cell Cycle 7:2769–2773
101. Martin-Montalvo A, Mercken EM, Mitchell SJ, Palacios HH, Mote PL, Scheibye-Knudsen M (2013) Metformin improves healthspan and lifespan in mice. Nat Commun 4:2192. https://doi.org/10.1038/ncomms3192
102. Campbell JM, Bellman SM, Stephenson MD, Lisy K (2017) Metformin reduces all-cause mortality and diseases of ageing independent of its effect on diabetes control: a systematic review and meta-analysis. Ageing Res Rev 40:31–44

Index

P. C. Guest (ed.), *Reviews on New Drug Targets in Age-Related Disorders*,
Advances in Experimental Medicine and Biology 1260,
https://doi.org/10.1007/978-3-030-42667-5