GeroScience (2022) 44:39–52
https://doi.org/10.1007/s11357-021-00479-y

EDITORIAL

Metabolism in the Midwest: research from the Midwest Aging Consortium at the 49th Annual Meeting of the American Aging Association

Michaela E. Murphy · Akilavalli Narasimhan · Alexis Adrian · Ankur Kumar · Cara L. Green · Carolina Soto-Palma · Chathurika Henpita · Christina Camell · Christopher S. Morrow · Chung-Yang Yeh · Claire E. Richardson · Cristal M. Hill · Darcie L. Moore · Dudley W. Lamming · Eric R. McGregor · Heather A. Simmons · Heidi H. Pak · Hua Bai · John M. Denu · Josef Clark · Judith Simcox · Kishore Chittimalli · Korbyn Dahlquist · Kyoo-a Lee · Mariah Calubag · Mark Bouska · Matthew J. Yousefzadeh · Michelle Sonsalla · Reji Babygirija · Rong Yuan · Tadataka Tsuji · Timothy Rhoads · Vinal Menon · Yagna PR. Jarajapu · Yun Zhu

Received: 18 October 2021 / Accepted: 19 October 2021 / Published online: 29 October 2021

Introduction

As the aging population on a global and national scale reaches ever increasing highs, research is essential to reduce the burden of aging and age-related diseases. Due to the complexity of aging, geroscience research necessarily spans a variety of disciplines and requires a collaborative and interdisciplinary approach. In order to leverage the respective strengths of different programs, centers, and institutes of aging across the Midwestern region to accelerate basic and translational geroscience, and to provide a strong training environment for postdoctoral fellows and graduate students, the Midwest Aging Consortium (MAC;

M. E. Murphy · C. L. Green · C.-Y. Yeh · D. W. Lamming · E. R. McGregor · H. H. Pak · J. Clark · M. Calubag · M. Sonsalla · R. Babygirija · T. Rhoads
Department of Medicine, University of Wisconsin-Madison, Madison, WI 53705, USA

M. E. Murphy · C. L. Green · C.-Y. Yeh · D. W. Lamming · E. R. McGregor · H. H. Pak · J. Clark · M. Calubag · M. Sonsalla · R. Babygirija · T. Rhoads
William S. Middleton Memorial Veterans Hospital, Madison, WI 53705, USA

M. E. Murphy · D. W. Lamming · E. R. McGregor · H. H. Pak · J. M. Denu
Department of Nutritional Sciences, University of Wisconsin-Madison, Madison, WI 53705, USA

A. Narasimhan · C. Soto-Palma · C. Henpita · C. Camell · K. Dahlquist · K. Lee · M. J. Yousefzadeh · V. Menon
Institute On the Biology of Aging and Metabolism, University of Minnesota, Minneapolis, MN, USA

A. Adrian
Department of Urology, University of Wisconsin-Madison, Madison, WI 53705, USA

A. Adrian
Molecular and Cellular Pharmacology Program, University of Wisconsin-Madison, Madison, WI 53705, USA

A. Adrian
U54 George M. O'Brien Center for Benign Urology Research, Madison, WI 53705, USA

A. Kumar · H. Bai · M. Bouska
Department of Genetics, Development, and Cell Biology, Iowa State University, Ames, IA 50011, USA

C. S. Morrow · D. L. Moore
Department of Neuroscience, University of Wisconsin – Madison, Madison, WI 53705, USA

C. E. Richardson
Department of Genetics, University of Wisconsin – Madison, Madison, WI 53706, USA

C. M. Hill
Neurosignaling Laboratory, Pennington Biomedical Research Center, Baton Rouge, LA 70809, USA

https://midwestagingconsortium.org/) was organized in 2019, with an in-person symposium in 2019 and a virtual symposium in 2021 [1]. The MAC now spans 7 states in the Midwestern United States, and includes the Mayo Clinic; the Universities of Wisconsin–Madison, Minnesota, Iowa, Northwestern, and North Dakota; Iowa State University; Southern Illinois University; Indiana University-Bloomington; and The Ohio State University.

Many members of the MAC as well as other aging experts convened at the 2021 Annual Meeting of the American Aging Association in Madison, WI, home to the University of Wisconsin-Madison. **Dr. Rozalyn Anderson**, a MAC founder and the 2019–2021 AGE President, organized a spectacular event, with over 380 in person and virtual attendees; this article will summarize the science that members of MAC institutions presented at this meeting. The research presented can be broadly separated into a few main categories, including nutritional interventions, senescence and stem cells, metabolic homeostasis, and immunity.

Nutritional interventions to slow aging

A wide variety of dietary regimens, such as dietary protein restriction (PR), calorie restriction (CR), fasting, time-restricted feeding, and more, are under investigation for their roles in health span and lifespan extension. It is hypothesized that these variations of dietary restriction (DR) enhance longevity and metabolic health through molecular adaptations of nutrient-sensing pathways including, but not limited to, the mechanistic target of rapamycin complex 1 (mTORC1), AKT, FOXO, nicotinamide adenine dinucleotide (NAD+), and AMP-activated protein kinase (reviewed in [2]).

Calorie restriction

CR has been demonstrated to delay the impacts of aging and extend lifespan across a variety of species, from simple yeast to complex multicellular organisms such as mice and non-human primates (NHP) [2]. Despite nearly a century of research, the exact mechanism yielding improved lifespan under CR is unclear. While a CR diet is probably too abstemious for most people to follow, many MAC members are interested in achieving a better mechanistic understanding of how CR functions in order to eventually develop pharmacological or dietary interventions that will mimic the benefits of a CR diet.

As discussed by **Dr. Timothy Rhoads**, University of Wisconsin-Madison, a long-term CR study conducted at the Wisconsin National Primate Research Center at University of Wisconsin-Madison has shown that a CR diet can improve health and extend longevity [3]. In recent work, Dr. Rhoads has linked the molecular reprogramming initiated by CR to

D. W. Lamming · M. Calubag · R. Babygirija
Cellular and Molecular Biology Program, University of Wisconsin-Madison, Madison, WI 53705, USA

H. A. Simmons
Wisconsin National Primate Research Center, University of Wisconsin-Madison, Madison, WI 53175, USA

J. M. Denu
Department of Biomolecular Chemistry, University of Wisconsin-Madison, Madison, WI, USA

J. M. Denu
Wisconsin Institute for Discovery, Madison, WI, USA

J. Simcox
Department of Biochemistry, University of Wisconsin-Madison, Madison, WI 53705, USA

K. Chittimalli · Y. P. Jarajapu
Department of Pharmaceutical Sciences, College of Health Professions, North Dakota State University, Fargo, ND 58105, USA

R. Yuan · Y. Zhu (✉)
Department of Medical Microbiology, Immunology, and Cell Biology, Southern Illinois School of Medicine, Springfield, IL, USA
e-mail: yzhu59@siumed.edu

R. Yuan · Y. Zhu
Department of Internal Medicine, Southern Illinois University School of Medicine, Springfield, IL 62794, USA

T. Tsuji
Section On Integrative Physiology and Metabolism, Joslin Diabetes Center, Harvard Medical School, Boston, MA 02215, USA

changes in RNA processing in the liver of rhesus macaques [4]. At the meeting, Dr. Rhoads discussed his recent discovery that CR engages RNA processing mechanisms in subcutaneous adipose tissue, but not in visceral adipose depots. Dr. Rhoads highlighted that these changes in RNA processing take different forms, including changes in exon usage, alternative splicing, and expression changes via small RNA inference. He has also identified age and sex-dependent effects of CR on RNA processing.

As shown several years ago by researchers including **Dr. Josef Clark** from the University of Wisconsin-Madison, CR has systemic effects on lipid metabolism in mice, altering not only adipose tissue but also circulating lipids [5]. More recently, Dr. Rhoads and Dr. Clark have demonstrated that CR mediates lipid processing pathways in the skeletal muscle of aging rhesus macaques [6]. In his presentation, Dr. Clark highlighted that CR improves lipid and lipoprotein profiles in NHP.

Also from the University of Wisconsin-Madison, **Heidi Pak** has been researching how the timing of food delivery affects the physiological and molecular effects of CR. As CR mice are typically fed once per day and rapidly consume their food in about 2 h, they are subjected not only to reduced calorie intake, but also to a collaterally imposed fast. Using a series of dietary regimens to tease apart the role of reduced calories and fasting, Heidi discovered that a prolonged fast is necessary for many of the physiological, molecular, and geroprotective effects of a CR diet in mice [7]. In her presentation, Heidi described the effect of fasting length on metabolic and molecular signaling in mice.

Heidi reports that a widely conserved effect of CR in mammals — increased insulin sensitivity — is only observed at certain times relative to the last feeding. While CR-fed mice have increased insulin sensitivity 16–24 h following their last meal, they exhibited insulin resistance relative to ad libitum (AL) control mice when tested 4–12 h after their last meal. The protein kinase mTORC1 is a critical regulator of insulin sensitivity through feedback inhibition of the insulin receptor substrate [8, 9]. Heidi hypothesized that the reduced insulin sensitivity of CR-fed mice may be the result of negative feedback regulation of mTORC1 on insulin action, and has found that following feeding, mTORC1 substrates are hyperphosphorylated in CR-fed mice as compared to AL-fed mice, and reduced to the same degree during fasting for both CR- and AL-fed mice. These results reveal that the length of time that has elapsed between feeding and the time at which metabolic phenotypes are examined has profound implications for understanding and identifying the molecular processes that drive the effects of a CR diet.

Protein restriction

CR diets necessarily reduce the levels of all major dietary macronutrients. Although the beneficial effects of a CR diet are not likely due to the reduced consumption of dietary protein [10], recent studies have found that low protein diets improve the metabolic health of humans [11] and are associated with decreased rates of diabetes and other age-related diseases as well as decreased mortality in humans [12, 13]. **Dr. Cristal Hill**, a postdoc in the lab of Christopher D. Morrison at Pennington Biomedical Research Center and a collaborator of SIU investigator and MAC member Dr. Andrzej Bartke, discussed that in contrast to CR, PR induces a robust increase in metabolic hormone fibroblast growth factor 21 (FGF21). In mice, FGF21 is required for PR-induced changes in food consumption and improves energy expenditure [14, 15]; ablation of βklotho, an FGF21 co-receptor, in the brain ablates this physiological response [16]. In this same study, Dr. Hill also confirmed that i.c.v. FGF21-treatment induces behavioral adaptation for increased preference for protein in chow-fed wild-type mice. [16]. Together, these data demonstrate that FGF21 is a required endocrine signal, linking the liver and brain, to adaptively coordinate feeding behavior and improvements in metabolic health during PR.

Dr. Cara Green discussed her research evaluating how sex, genetic background, and age on onset in rodents alters the response to dietary protein limitation. Despite evidence that sex and genetic background are key factors in the response to diet [17], most protein intake studies examine only a single strain and sex of mice [18]. Using multiple strains and male and female mice, Dr. Green found that improvements in metabolic health in response to reduced dietary protein strongly depend on sex and strain. While some phenotypes were conserved across

strains and sexes, including increased glucose tolerance and energy expenditure, she observed high variability in adiposity, insulin sensitivity, and circulating hormones. Using a multi-omics approach, Dr. Green identified mega-clusters of differentially expressed hepatic genes, metabolites, and lipids associated with each phenotype, and identified sex-specific roles for the hormone FGF21. Using mice lacking *Fgf21*, she confirmed these surprising results, demonstrating that FGF21 is required for PR-induced phenotypes only in males, and not in females.

The complex intersection of lifelong dietary habits and protein consumption, metabolic health, and cognitive function remains unclear. **Reji Babygirija**, a graduate student at UW-Madison, presented a poster demonstrating that PR improves body composition, metabolic health, and cognition in the 3xTg mouse model of Alzheimer's disease (AD). PR not only improved cognitive performance, but also reduced tau phosphorylation, a marker of AD, and brain levels of p62, indicating that PR induced autophagy. Overall, Reji's results suggest that low protein diets should be investigated further for the ability to prevent or delay AD. Broadly similar results were observed in 3xTg AD mice by Michelle Sonsalla of the University of Wisconsin-Madison, who tested the ability of the geroprotector acarbose on cognition and metabolism. Acarbose decreased tau phosphorylation and reduces mTORC1 signaling in the brain of 3xTg mice. These results suggest that the use of geroprotective diets and drugs may have potential for the prevention and treatment of AD.

Branched-chain amino acid restriction

Dr. Dudley Lamming from the University of Wisconsin-Madison opened a panel discussion of nutritional interventions for aging by presenting recent data from his lab showing that the branched-chain amino acids (BCAAs) — leucine, valine, and isoleucine — play a crucial role in the metabolic response to dietary protein, and when restricted, promote fitness and longevity in a sex-specific manner [19]. Intriguingly, the individual BCAAs have distinct effects on metabolism, with reduced levels of isoleucine being the main driver of the positive effects of BCAA restriction on metabolic health [20]. Isoleucine restriction (IleR) robustly induces FGF21 and induces browning of white adipose tissue to increase energy expenditure in young male mice. The effects of IleR on food consumption and energy expenditure, but not other phenotypes, are dependent on FGF21.

Further research on IleR was presented by UW-Madison graduate student **Michaela Murphy** and postdoctoral fellow **Dr. Chung-Yang Yeh**. Michaela demonstrated that IleR reverses diet-induced obesity in mice without reduced calorie intake. IleR results in a simultaneous improvement in glucose homeostasis as revealed by both glucose and insulin tolerance tests. Late-life intervention with IleR was examined by Dr. Yeh, who showed that IleR increases energy expenditure in both young and old mice. The overall effects of IleR on fitness were unclear, and are still being investigated.

Beige and brown adipose depots get their distinctive color from a high density of mitochondria, which allow for the enhanced thermogenic capacity of these depots. Brown adipose tissue (BAT) is associated with improvements in cardiometabolic health in both mice and humans, and correlates with reduced risk of a variety of chronic diseases, even in those that are overweight and obese [21]. **Dr. Tadataka Tsuji**, a collaborator of SIU investigator Dr. Andrzej Bartke, reported on the results of recent experiments with the BAT of long-lived Ames dwarf mice [22]. Using integrated metabolomics analyses, Dr. Tsuji and colleagues showed that the BAT of Ames Dwarf mice produces lipid species that counteract the detrimental effects of a high fat diet and may contribute to extended lifespan. Male mice, but not female mice, that received transplanted BAT from Ames Dwarf donors showed sex-specific improvements in glucose tolerance and insulin sensitivity. This study demonstrates the importance of lipid-derived mediators on overall metabolic health and perhaps lifespan.

Senescence, immunity, and inflammaging

Biological aging is traditionally thought of as the progressive loss of functional characteristics on a cellular and organismal level. Both impaired stem cell function and accumulated senescent cells contribute to this process [23–26]. Cells that reach the point of irreversible cell arrest are considered to be senescent. Heterochronic parabiosis in mice has been shown to decrease the senescent cell load, implying senescence is controlled in part by circulating geronic factors

[27], Additionally, age is associated with increased inflammation and immune changes that can be chronic in nature. Both senescence and aging immunity proved to be large talking points at this year's conference.

The Institute of the Biology of Aging and Metabolism (iBAM) at the University of Minnesota seeks to study aging at the molecular level as a way to promote healthy aging. It is directed by physician scientist and MAC member **Dr. Laura Niedernhofer**, whose laboratory utilizes mouse models of accelerated aging to study senescence biology and investigate potential senolytics and senomorphics. Similarly, **Dr. Paul Robbins**, another MAC and iBAM member, investigates the development of senotherapeutics and aging of the immune system. **Dr. Christina Camell**, an Assistant Professor who recently joined iBAM, studies inflammation and the dysfunctional aged immune system. Many graduate students and postdoctoral scholars from the Niedernhofer, Robbins and Camell laboratories showcased posters of their work during the AGE Conference.

Dr. Chathurika Henpita presented evidence that a decline in mitochondrial homeostasis contributes to cellular senescence, and that mitochondrial targeted drugs may alleviate that. She tested XJB-5–131, a mitochondrial free radical scavenger [28], nicotinamide mononucleotide (NMN), an NAD precursor, metformin, a novel compound that can reduce ROS generation at mitochondrial complex 1, rapamycin, an mTORC1 inhibitor, and finally IKK7 and SR12343 inhibitors of IKK/NF-kB. She measured senescence markers SA-β-gal activity, ROS production, and mitochondrial membrane potential, bioenergetics, and mass in senescent mouse embryonic fibroblasts (MEFs) following administration of these drugs. It was found that several of the compounds significantly reduced senescence and ROS levels. A subset of the compounds also significantly improved mitochondrial bioenergetics. In total, the preliminary data suggest that targeting signaling events has more impact on mitochondrial health than trying to target mitochondrial per se. These results indicate that there are a variety of promising senotherapeutics to be tested in future studies.

Dr. Caroline Soto-Palma also examined senotherapeutics — specifically novel LSD1 histone demethylase inhibitors that are currently being investigated to treat certain cancers. LSD1 plays a pivotal role in epigenetic up- or downregulation of gene expression, due to its removal of methyl groups from various lysines on histone protein 3 [29]. LSD1 also targets non-histone proteins p53 and RelA/p65, which contribute to senescence and the secretory phenotype of senescent cells (senescence-associated secretory phenotype or SASP). Preliminary data suggest that LSD1 inhibition of senescent cells reduced their pro-inflammatory SASP via downregulation of NF-kB activation and induces senolysis by p53 activation.

Aging of the immune system, or immunosenescence, contributes to morbidity and mortality of the elderly. **Dr. Matt Yousefzadeh** attempted to define the contribution of immune aging to organismal aging. He deleted *Ercc1,* which encodes a subunit of the DNA repair endonuclease ERCC1-XPF that is critical for the stability of the holoenzyme [30], in murine hematopoietic cells using the Vav promoter/S21/45 control regions to drive Cre recombinase expression. This resulted in an increased senescence in immune cells. *Vav-iCre*$^{\pm}$*;Ercc1*$^{-/fl}$ mice are healthy into adulthood, but then display premature onset of immunosenescence characterized by senescence of specific immune cell populations and impaired immune function, similar to changes that occur with aging in wild-type mice. Remarkably, non-lymphoid organs also showed increased senescence and damage, suggesting that senescent, aged immune cells can promote systemic aging. Indeed, transplantation of splenocytes from *Vav-iCre*$^{\pm}$*;Ercc1*$^{-/fl}$ or aged WT mice into young animals induced senescence in *trans*, whereas transplantation of young immune cells attenuated senescence in progeroid mice. These data demonstrate that an aged, senescent immune system plays a causal role in driving systemic aging and therefore represents a key therapeutic target to extend healthy aging [31].

It is well known that senescent cells drive aging and age-related diseases including tauopathies that model AD [32–34], where selective clearing of senescent cells improves disease phenotypes, offering a new approach to treat AD. However, there remains controversy as to which central nervous system (CNS) cells senesce. Identifying those cell types would enable the development of senolytics that are optimally suited to age-related neurodegenerative diseases. To tackle this, **Dr. Vinal Menon,** University of Minnesota, is deleting *Ercc1* in each cell type of the CNS to drive senescence as a consequence of unrepaired

DNA damage. Despite this deletion in forebrain neurons in *CamKIIαCre*$^{+/-}$*; Ercc1*$^{-/fl}$ mice, these animals developed normally into adulthood. Mutant animals at 6 and 10 months of age presented with progressive brain atrophy relative to control mice. This could explain, in part, the cognitive decline previously reported in these mice [35]. In the hippocampus and cortex of 1-year-old *CamKIIαCre*$^{\pm}$*; Ercc1*$^{-/fl}$ mice, histology revealed neuron loss and an increase in inflammation. While there were no significant changes in expression of senescence markers in the cortex of 10-month-old mutants, there was increased inflammation In contrast, in *Ercc1*$^{-/\Delta}$ mice, in which ERCC1-XPF is systemically depleted, both senescence and inflammation were increased in several brain regions including hippocampus and midbrain. Moreover, these mutant animals exhibit increased microglia priming [36]. These findings suggest that DNA damage does not cause neurons to senesce, but can drive senescence in the CNS. This further suggests that senolytics might be utilized to selectively remove senescent cell types inside the CNS that can be replenished.

With aging, there is a gradual decline in immune surveillance along with an increase in chronic inflammation. Invariant γδ T cells are less common than αβ T cells and may be triggered by different alarm signals including PAMPs and DAMPs. The presence of γδ T cells in peripheral organs makes them well positioned to contribute to immune-mediated aging. Here, **Kyoo-a Lee**, University of Minnesota, assessed how aging affects the accumulation of inflammatory γδ T cells in various organs including peripheral and central lymphoid organs. Aging was shown to increase the percentage of IL-17A-producing cells in γδ T cell population in the spleen and liver of old mice. Notably, mature γδ T cells were found to accumulate in the aged thymus and the percentage of IL-17A-producing cells dramatically increased, similar to the increase in the periphery. Similarly, in the *Ercc1*$^{-/\Delta}$ mouse model of accelerated aging due to the accumulation of DNA damage, there was a preferential increase in IL-17A-producing cells compared to IFN-γ-producing cells in γδ T cell population in spleen and liver. In the thymus of *Ercc1*$^{-/\Delta}$ mice, there were fewer immature T cells and a larger population of γδ17 T cells, similar to that of old WT mice, indicating potential connections between senescence and altered γδ T cell composition. In both animal models, the number of IL-17A and TNF-α-co-expressing γδ T cells increased. TNF-α and IL-17A co-expression from γδ T cells may be crucial in promoting the production of other SASP factors like IL-6 in senescent fibroblasts. Overall, this research contributes to a better knowledge of the immunological alterations associated with age, as well as potential therapeutic targets for slowing aging.

Inflammaging is a critical feature of aging and contributes to the age-related risk for various diseases. It is associated with the accumulation of senescent cells that secrete SASPs. **Dr. Christina Camell**, University of Minnesota, investigated the response of senescent cells to viral and bacterial infection [37]. Human cells that are induced to senesce produce more SASP after stimulation with lipopolysaccharide (LPS), then their non-senescent counterpart. They also leveraged an experimental paradigm that exposes specific pathogen free (SPF) mice to a normal microbial environment (NME). NME-exposure generates a more diverse immune system in young mice but leads to β-coronavirus, the mouse hepatitis virus (MHV) infection in the liver, increases in immune infiltrates, and elevated SASP factors within the old mice. Ultimately, old mice show 100% mortality following NME exposure. MHV immunization protects old mice from mortality following NME-exposure. There are also increased markers of senescence in multiple tissues from the old mice exposed to NME, such as p21 and p16, and RNA-sequencing shows an enrichment of the senescence pathway, indicating that senescent cell burden is increased with infection in the old mice. To address the role of senescent cells in NME-induced mortality, they used fisetin, a senolytic which selectively kills senescent cells [38] by targeting and inhibiting PI3K pathways and NF-kB activation. Administration of fisetin in aged wild-type mice results in improved age-related pathology and extended longevity [38]. Old NME-exposed mice treated with fisetin show a reduction in senescence markers, p16 and p21, SASP factors, and inflammation. The senolytic fisetin also protects old mice from death upon NME. This is likely mediated through an improved immune response, as fisetin treatment increases MHV-specific antibodies. Other effective combinations of senolytics, dasatinib and quercetin, also increase survival in the old NME-exposed mice. Thus, reducing the senescent cell burden in diseased or aged individuals should enhance resilience and

reduce mortality following viral infection, including SARS-CoV-2.

Elderly individuals have among the highest risk for morbidity to numerous infectious diseases including SARS-COV2. **Korbyn Dahlquist**, University of Minnesota, explored how the dysfunctional immune system regulates infection-induced mortality in aged mice. Using the NME model, she defined inflammatory and immune cell changes in the lymphoid and non-lymphoid tissues from young and old mice. RNA-sequencing shows distinct clustering of the individual groups of livers from young or old, unexposed or exposed mice, with strong enrichment for inflammatory and immune activation pathways. Flow cytometry showed an increased in multiple populations of immune cells. Obesity, a condition that induces inflammation and metabolic disease, was not sufficient to induce mortality to NME in middle-aged mice, suggesting that mortality is due to unique aspects of the aged immune system. Overall, this project initiates a better understanding of the aged immune system and inflammatory pathways activated during exposure to novel pathogens.

While most research focuses on identifying and delaying the functional changes that occur within immune cells in advancing age, some researchers noticed that the rapid growth period in pre-pubertal stage may already provide a window to improve the development of immune system. **Dr. Rong Yuan**, Southern Illinois University School of Medicine, investigated the innate immunity changes in young mice treated with metformin. Female and male heterogeneous mice (UM-HET3) were treated with metformin or saline between the ages of 15 and 56 days. Flowcytometry assay revealed that TLR4, a mediator of LPS-induced reaction, is significantly reduced in the circulating leukocytes of the metformin treated mice. In an ex vivo study, in the metformin treated mice, LPS treatment significantly increased the expression of CD14 but reduced the expression of TLR4. In both LPS untreated and treated samples, females had significantly lower TLR4 levels than the males. Furthermore, with LPS stimulation, leukocytes collected from the metformin treated mice produced significantly higher levels of IL-6 and IL-1β. Interestingly, in the LPS treated samples, metformin treatment was associated with significantly higher levels of IL-6 and IL-1β in males than that in females. These results indicate that metformin may regulate the immune reaction to infections in a sexually dimorphic manner.

Metabolic adaptation to the environment

It is well known that genetics, lifestyle, diet, and environmental factors play important roles in the process of aging and metabolism. CR and PR, as well as other dietary interventions, are associated with dynamic and significant fluctuations in the availability of metabolites, including essential cofactors for many chromatin modifying enzymes [39]. **Dr. John Denu** and colleagues at the University of Wisconsin-Madison are investigating how dietary composition controls gene expression via alteration in the epigenome. Recent work from the Denu lab has investigated how methyl-metabolite depletion elicits adaptive response to support heterochromatin stability and epigenetic persistence [39]. During methyl-donor depletion, SAM (S-adenosylmethionine) utilization is directed towards de novo histone methylation to preserve heterochromatin stability and ensure epigenetic persistence upon metabolic recovery. Other factors that affect metabolism and alter the epigenome includes the gut microbiome [40]. Dr. Denu reported that short-chain fatty acids (SCFAs), acetate, propionate, and butyrate produced by gut microbial fermentation of fiber can drive epigenetic changes in distal host tissues like the liver. To investigate the mechanism using cell culture models, propionate and butyrate (but not acetate) was shown to lead to similar chromatin hyperacetylation. While decades of prior observations would have suggested that hyperacetylation induced by SCFAs are attributed to inhibition of histone deacetylases (HDACs), Dr. Denu reports that propionate and butyrate instead activate the histone acetyltransferase p300 [41]. Propionate and butyrate are rapidly converted to the corresponding acyl-CoAs which are then used by p300 to catalyze auto-acylation, activating the enzyme for histone acetylation. This data reveals a previously unknown mechanism of histone acetyltransferase activation.

People tend to become sensitive to cold temperature in old age. The impact of age dependent changes in circulating lipids on BAT thermogenesis was discussed by **Dr. Judith Simcox**, University of Wisconsin-Madison. In indirect calorimetry measurements in mice, a doubling of energy expenditure via shivering

and non-shivering thermogenesis is observed when a mouse has to maintain body temperature with cold challenge at 4 °C compared to thermoneutrality at 30 °C. The respiratory quotient (RQ) was lower at the colder exposure, indicating that the primary fuel being utilized was lipids. In aged mice, the thermogenic response is impaired, resulting in an impaired ability to maintain body temperature and therefore, a lower core body temperature. This impaired cold response coincides with increased lipid droplet deposition in the BAT and decreased plasma lipid plasticity in response to cold exposure in aging. During cold exposure, there is a shift in lipid species to metabolize acylcarnitines by the liver, and this response to cold exposure is also ablated in aged mice. Acylcarnitines localize to lipid droplets in activated brown adipocytes and it could partially rescue cold intolerance in aged mice. Ceramides, a known regulator of thermogenesis in the BAT, are increased in the plasma in acute cold exposure in 3-month age mice with the changes being ablated in 24-month age mice.

Dr. Simcox also tested these principles in humans; she measured fasting body temperature in the Midlife in the United States (MIDUS) study and found that body temperature is positively associated with increased Cer(18:1/22:0). Adipose tissue specific inhibition of ceramide synthesis increases beige adipocyte differentiation [42]. Ceramide synthesis is associated with BAT function, thermogenic gene expression and mitochondrial abundance [43]. Ceramide levels are decreased in BAT with cold exposure [42]. Acute inhibition of ceramide synthesis with myriocin causes cold intolerance. The Simcox laboratory is continuing to explore the functional role of plasma ceramides in thermogenesis and healthy aging.

In response to stress, damaged mitochondria are repaired and recycled through the mechanisms of mitochondrial dynamics, which have emerged as a novel regulator of aging in recent years [44]. During aging, alterations in mitochondrial morphology and structure have been observed. In fruit fly, genetic manipulations of genes involved in mitochondrial fission and fusion could extend lifespan [45]. However, the causes of the age-dependent alteration in mitochondrial dynamics remain unanswered. The focus of **Ankur Kumar**, Iowa State University, is to explore the involvement of the peroxisome in maintaining mitochondrial homeostasis during animal aging. Their recent studies have shown mitochondrial morphology and function alteration due to impaired peroxisomal protein import in aging oenocytes (hepatocytes) of fruit flies. Knockdown of peroxisome adaptor *Pex5* led to increased mitochondrial size in oenocytes, which is similarly seen in aged flies. Interestingly, peroxisomal plasmalogen levels decrease with age, while knocking down genes involved in peroxisomal plasmalogen synthesis resulted in enlarged mitochondria. The future goal of this study is to understand how peroxisome-derived plasmalogen contributes to age-related alterations of mitochondrial dynamics and functions.

It is well known that, in addition to genetic factors [46], the risk of adult diseases importantly depends on parental, nutritional, and other environmental effects during pre- and post-natal development [47–52]. Importantly, across mouse inbred strains, delayed sexual maturation is associated with extended lifespan, while in humans early pubertal onset is associated with increased risk of adult obesity and cardiovascular disease [53, 54]. **Yun Zhu**, Southern Illinois School of Medicine, investigated the effect of metformin treatment on aging-related developmental and metabolic phenotypes in juvenile mice. The results show that early life treatment with metformin has profound effects on developmental and metabolic traits. Body weight and food consumption are increased in both sexes. Age of sexual maturation is significantly delayed in females, but not affected in males. Interestingly, tail length and circulating insulin-like growth factor 1 (IGF1) levels, which controls numerous metabolic processes throughout the body, are significantly increased in both sexes. Circulating adiponectin and insulin levels are altered by metformin treatment in a sex-specific manner. Analysis of quantitative insulin sensitivity check index (QUICKI) suggests that metformin treatment significantly increases insulin sensitivity in female pups, but, unexpectedly, had opposite effect in male pups. This study reveals that early life metformin treatment alters development and metabolism of mice in both sex-specific and non-specific manners. Based on the age-depended role of hormones in age-related diseases, cognition, and lifespan [55], these effects of metformin may have long-term impacts on aging-related traits.

Aging in different organs and organelles

As a whole picture of assembled individual cells, organs also start to lose functions during aging. One example is increased weakness caused by aging of skeletal muscle. MAC members presented excellent work of innovative discoveries on organ-specific aging during the meeting.

The loss of skeletal muscle mass and function with age, known as sarcopenia, is accompanied by reduced muscle strength and physical performance leading to increased risk for impaired mobility, falls, fracture, and mortality, as well as metabolic consequences including sarcopenic obesity, diabetes, and cardiovascular disorders [56, 57]. Currently, there is no effective pharmacological intervention for sarcopenia. Adiponectin, an adipose tissue derived hormone, has been shown to stimulate mitochondrial metabolism in target tissues and has been linked to delayed aging with caloric restriction [5, 58, 59]. AdipoRon, an adiponectin agonist, has been shown to stimulate expression of genes involved in metabolism skeletal muscle in young mice [60]; however, its effects on skeletal muscle in older mice and its functional consequence are still largely unknown. **Katie Osterbauer**, University of Wisconsin-Madison, investigated if AdipoRon could be used as a novel agent to treat or reverse the effects of sarcopenia by preserving muscle metabolism, mass, and function. Male and female mice presenting with early or late stage sarcopenia were treated with AdipoRon for 4 months. At advanced age, AdipoRon improved fasting glucose in both males and females and improved indices of whole-body metabolism. In addition, AdipoRon treatment prevented age-related changes in body composition in aged females and males. AdipoRon treatment attenuated age-related declines in functional performance in aged males, but not females. These data show sex dimorphism in skeletal muscle aging, and that AdipoRon as a therapeutic has potential to correct metabolic and functional declines linked to sarcopenia.

Neurons live as long as the animal in which they reside. Just as animals show characteristic signs of aging, neuronal aging is likewise accompanied by characteristic cellular features, including morphological alterations, synapse loss, and metabolic dysfunction. The mechanisms in which these features develop remain unclear, though loss of neuronal cellular homeostasis likely contributes to the increased risk of neurodegenerative disorders observed with age. **Dr. Claire Richardson**, University of Wisconsin-Madison, investigated how aging affects neuron cell biology by exploiting the ability of the nematode *Caenorhabditis* elegans to de-couple the symptoms of aging from chronological age [61]. Under standard laboratory growth conditions, *C. elegans* live in a proliferative state, in which they develop, age, and die in two weeks. Animals, on the other hand, can live for months in an alternative organismal stage known as dauer. Importantly, their neurons continue to function. This raises the question of how neuron aging is slowed in the dauer stage. They discovered that morphological aging is cell-intrinsically controlled by focusing on features of neuron morphological aging. A neuron-specific genetic modification (dauerization) was used to generate a dauer-like suspension of neuron morphological aging within aging (non-dauer) animals. The constitutive endocytic process is strongly suppressed by dauerization. This physiological inhibition of the constitutive endocytic pathway may block numerous pro-aging activities supported by the endosomal system at the same time. The well-established link between aberrant endocytic pathway function and neurodegenerative disorders, on the other hand, suggests that there is a conserved interaction between the endocytic system and aging.

Recent evidence suggests that senescent cells may contribute to retinopathy, in particular senescence of the retinal pigment epithelium (RPE) [62]. RPE is a single layer of pigmented cells that act as a selective barrier and nourishes the retinal cells. **Dr. Akilavalli Narasimhan**, University of Minnesota, extended this to a novel murine model of adult onset diabetes. Knock-out of *Ercc1* in β-cells resulted in many phenotypes associated with diabetes. Eyes from these hyperglycemic mice were examined to identify the morphologic and molecular alterations in the RPE and retinal layers. Preliminary data suggest that both apoptosis and cell senescence contribute to retinal changes in response to chronic hyperglycemia, including loss of photoreceptors and vascular degeneration. Further studies are needed to characterize retina in these and other tissue-specific *Ercc1* deleted mice.

As the engine of the cell, mitochondrial dysfunction has been proposed as a hallmark of the aging process [63]. Specifically, as a function of aging, mitochondria appear to have decreased enzyme activity and respiratory capacity and increase reactive oxygen

species production. The ubiquitously expressed transcriptional coactivator peroxisome proliferator-activated receptor gamma-coactivator 1 (PGC-1a) has been described as the master regulator of mitochondrial function [64, 65]. Despite the emerging connections between PGC-1a and disease vulnerability, the regulation of PGC-1a in the CNS is not well defined. This is particularly true in the brain, where PGC-1a is enriched in neurons, and alterations in expression levels have been linked to neurodegenerative disorders. **Eric McGregor**, from the University of Wisconsin-Madison, reported that astrocytes and neurons differ substantially in mitochondrial status and the transcript variants of PGC-1a expressed, including using a neuron-specific promoter. Taking advantage of the ability of the tau-kinase GSK-3b to influence PGC-1a expression [66, 67], differential regulation of PGC-1a transcription in primary neurons and astrocytes has been investigated. Neuronal PGC-1a responds robustly to GSK3b inhibition by lithium, switching the dominant promoter, leading to changes in isoform distribution and abundance, while astrocytes are refractory to lithium treatment. This highlight the key mechanisms for neuron-specific metabolic regulation that are likely to be relevant to neurodegenerative diseases that have a link to mitochondrial dysfunction.

Molecular aging clocks have become accurate predictors of organismal and tissue age. Dissecting the effects of individual genes found in these clocks could help us understand the mechanisms behind age-related disease. Comparing methylation and blood protein aging clocks [68, 69] with early middle aged brain transcriptome trajectory turning points [70], identified 5 candidate genes shared among these studies. To begin to examine the possible effects of identified genes on lifespan and health-span, **Mark Bouska**, from Iowa State University, knocked down the human orthologs in fruit fly, *Drosophila melanogaster*. Constitutive knockdown of fz3, Glo1, and Loxl2 improved fly lifespan. Because Loxl2 plays a role in cardiac aging in humans [71], cardiac arrhythmia and fibrotic measures were examined under Loxl2 RNAi. Age-related changes in collagen filament width and arrhythmia were improved in Loxl2 knockdown flies. Furthermore, Loxl2 regulates transcription of many genes in humans [72] and RT-qPCR confirmed *Drosophila* CadN2 levels corresponded with Loxl2 reduction. Finally, heart-specific Loxl2 knockdown improved the lifespan of flies. These findings point to conserved pathways and potential mechanisms by which Loxl2 inhibition might benefit heart health and aging.

Benign prostatic hyperplasia (BPH) is characterized by proliferation, smooth muscle changes, and fibrosis of the prostate. The single greatest risk factor for BPH is age, with 90% of men in their eighties impacted. Many men with BPH will develop lower urinary tract symptoms, which reduce their quality of life as disease severity progresses. Given the multifactorial nature of the disease, treatments have thus far been limited. While BPH has been clearly linked to aging, the molecular mechanisms have yet to be fully elucidated. **Alexis Adrian**, University of Wisconsin-Madison, specifically examined how mitochondrial dysfunction caused by aging may contribute to fibrosis in BPH. To evaluate how mitochondrial dysfunction may contribute to fibrosis, both in vivo and in vitro models have been used. Results showed decreased levels of NDUSF3 in prostate, suggesting reduced mitochondrial function, specifically associated with complex I of the electron transport chain (ETC). Furthermore, PINK1 was also decreased, indicating a reduction in Parkin-dependent mitophagy. qPCR experiments on rotenone treated BHPrS1 cells revealed increased gene expression for both Col1a1 and Col3a1, suggesting complex I dysfunction can contribute to increased collagen production, and therefore fibrosis. Finally, idebenone ameliorated overexpression, supporting the role of complex I in dysfunction. Combined, these data suggests that mitochondrial dysfunction, potentially originating from complex I of the ETC, is contributing to the production of collagen, potentially leading to a progression of fibrosis in BPH in aging men.

Compromised barrier functions of intestinal epithelium (leaky gut) with aging leads to systemic inflammation [73]. Recent studies point to the beneficial effects of the protective axis of Renin Angiotensin System (RAS), which constitutes Angiotensin converting enzyme-2 (ACE2)/Angiotensin-(1–7) (Ang-(1–7)/Mas receptor (MasR) [74]. This is known to be counter-regulatory to the classical axis of RAS consisting of ACE/Ang II/AT1 receptor (AT1R). **Kishore Chittimalli**, North Dakota State University, tested the hypothesis that leaky gut with aging is associated with ACE2/ACE imbalance and that activation of MasR with Ang-(1–7) would restore gut barrier integrity. ACE2 protein and

activity were decreased in old group while that of ACE were unaltered. AT1R and MasR expressions in the gut wall were higher in the old compared to the Young. Gut permeability was higher in old mice that was abolished by Ang-(1–7) treatment. Hematoxylin & eosin staining indicated that aging was linked with structural disintegrity in the gut wall, which Ang-(1–7) restored. Importantly, plasma levels of Zonulin-1, IL-6, and TNF-α were higher in the old group compared to the Young, which were reversed by Ang-(1–7). Ang-(1–7) activation of MasR appears to be a potential strategy for correcting leaky gut and reducing systemic inflammation with age.

While researchers (Dr. Ladiges) provided excellent introduction to geropathology and the use of organ composite scores for rodent studies, **Dr. Heather Simmons**, University of Wisconsin-Madison, discussed the ongoing development of a geropathology grading system in NHP. Because the strong similarities to humans, a non-human primate species such as the Rhesus macaque is a favored model for aging. The average lifespan of macaque is about 25 years and they have a maximum lifespan about 40.6 years. Similar to humans, they undergo menopause at about 20 years old age, a large portion of life that is non-reproductive, in females. The mouse and the marmoset have ovarian cycles rather than menstrual cycles and undergo reproductive senescence rather than menopause. Alterations in hormone levels affect ovarian function causing decreasing ovarian cycle frequency. Both macaques and marmoset have similar age-related changes to humans including changes in fragility, metabolism, and cognition (macaques at ~ 20 years and marmosets at ~ 7 to 8 years). Aged macaques lose dermal elasticity, develop arthritis, and have progressive sarcopenia (loss of muscle mass). NHP studies are usually longitudinal and use of a grading system will improve comparisons between animals and studies. Composite index scoring of kidney and heart in NHP increases with age [75]. Dr. Simmons and Dr. Olstad are performing expanded grading studies on cardiac, renal, lung, and pancreatic tissues. The long-term goals of the Geropathology Research Network are to develop a NHP geropathology grading system that includes multiple NHP species comparable to the mouse grading system that includes ~ 25 organs. Eventually, a NHP Geropathology Atlas could be a national digital resource.

The formation of newborn neurons by neural stem cells (NSCs) in the adult brain, referred to as adult neurogenesis, dramatically declines during early mammalian aging, heavily limiting the brain's intrinsic capacity to rejuvenate [76]. Recent evidence suggests that NSCs are yet present in the adult brain, but that the decline in adult neurogenesis is caused largely by a decreased capacity of NSCs to exit quiescence in the aging brain [77, 78]. **Dr. Christopher Morrow**, University of Wisconsin-Madison, presented work aimed at identifying the molecular mechanisms mediating NSC quiescence exit. NSCs were recently observed to remodel protein homeostasis during quiescence exit, clearing aggregated proteins present in quiescent NSCs [79]. Dr. Morrow and colleagues found that NSCs exiting quiescence use a protein degradation mechanism called the aggresome to efficiently exit quiescence, a pathway which involves proteins destined for degradation being trafficked to the centrosome [80]. Vimentin, an intermediate filament protein which cages the aggresome, was critical for localizing proteasomes to the aggresome for efficient protein degradation [81]. Vimentin KO NSCs in vitro and in vivo exhibited delayed NSC quiescence exit, and vimentin KO mice experienced a faster age-dependent decline in adult neurogenesis. These experiments contribute to our understanding of the rate-limiting factors in adult neurogenesis which may lead to a greater understanding of how to increase the production of newborn neurons to improve brain aging.

Conclusion

Recent advancements in scientific technology and innovation have resulted in exciting and diverse discoveries in geroscience, which have the potential to lead to long-term improvements in preventing and treating chronic age-related diseases and easing the financial burden and human cost of the graying population. Despite navigating the challenges brought on by the ongoing COVID-19 pandemic, this year's 49th Annual Meeting of the American Aging Association was a great success — in large part due to the contributions of members of the MAC. The contributions, which span a wide variety of aging disciplines, bode well for the progression of geroscience and highlights the strength and diversity of aging research in

the Midwestern United States. We hope to see these early career researchers and many others over the next year at the next in-person meeting of the MAC, as well as at the 50th Anniversary meeting of the American Aging Association, which will be held in May of 2022 in San Antonio, Texas. Information on Registration for the 50th Anniversary meeting of AGE will be available early next year at https://www.americanagingassociation.org/annual-meeting.

Acknowledgements We would like to thank Drs. Rozalyn Anderson, Paul Robbins, and Laura Nidernhofer for their role in the establishment of the MAC and their contributions to this meeting as well as this report. In addition, we would like to thank the American Aging Association for organizing the meeting at which these talks were presented, and a special thanks to all sponsors of the 49th Annual AGE Metabolism of Aging Conference, including Gentech; School of Medicine and Public Health at University of Wisconsin-Madison; Geriatrics and Gerontology at University of Wisconsin-Madison; American Aging Association; American Federation for Aging Research; The Glenn Foundation for Medical Research; Morgridge Institute for Research; Orentreich Foundation for the Advancement of Science, Inc.; National Institutes of Health: National Institute on Aging; Healthy Aging & Longevity Research Institute; and Calico.

Funding C.L.G. is supported by a Glenn/AFAR Foundation for Medical Research Postdoctoral Fellowship in Aging Research. M.J.Y. is supported by the Irene Diamond Fund/American Federation for Aging Research Postdoctoral Transition Award and in part by funds from the NIH/NIA (AG063543, AG059676, and AG056278 to Drs. Laura Niedernhofer and Paul Robbins). H.H.P. is supported by a NIA F31 predoctoral fellowship (NIA F31 AG066311). M.E.M. is supported by a Research Supplement to Promote Diversity in Health-Related Research (R01AG062328-03S1). C-Y.Y. is supported by the T32 Biology of Aging Program (T32 AG000213). D.W.L. and the Lamming laboratory are supported in part by research grants and funds from the National Institutes of Health (AG056771, AG062328 and DK125859) the University of Wisconsin-Madison School of Medicine and Public Health and Department of Medicine, and the U.S. Department of Veterans Affairs (I01-BX004031). T.W.R. is supported in part by funds from the NIH/NIA (AG070686 to TWR and AG057408 to Dr. Rozalyn Anderson) and the U.S. Department of Veterans Affiars (I01-BX003846 to Dr. Rozalyn Anderson). J.P.C. has received support from the T32 Biology of Aging Program (T32 AG000213). H.B., A.K., M.B., and the Bai laboratory is supported by NIH/NIA R01AG058741. J.M.D. is supported by GM059785, GM65386 and DK125859. C.S.M. has received support for this work by a NIH T32 T32GM008688, D.L.M. has received support for this work by an AFAR Young Investigator Award, a Sloan Foundation fellowship, and a DP2 NIH New Innovator Award. Y.P.R.J has received funding from NIGMS/NIA (AG056881). JS has received support from the NIH BIRCWH K12HD101368, start up funds from the Department of Biochemistry, and the NIH DRC P30DK020579. K.L. has received support from the T32 AG029796. V.M. has received support from NIH/NIA R01 AG063543, U19 AG056278, P01 AG062413, and Aligning Science across Parkinson's ASAP-000592.Y.Z. and R.Y. are supported by William E. McElroy Charitable Foundation, NIA R21AG062985, ADA 1–19-IBS-126 and Research seed grant of Southern Illinois University School of Medicine. The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIH. This work does not represent the views of the Department of Veterans Affairs or the United States Government.

Declarations

Conflict of interest DWL has received funding from, and is a scientific advisory board member of, Aeovian Pharmaceuticals, which seeks to develop novel, selective mTOR inhibitors for the treatment of various diseases. The University of Wisconsin-Madison has applied for a patent based in part on the findings reported here, for which DWL is an inventor. JMD consults for Evrys Bio and is co-founder of Galilei Biosciences.

References

1. Green CL, et al. The 2 nd Annual Symposium of the Midwest Aging Consortium: The Future of Aging Research in the Midwestern United States. J Gerontol A Biol Sci Med Sci. 2021. https://doi.org/10.1093/gerona/glab210.
2. Green CL, Lamming DW, Fontana L. Molecular mechanisms of dietary restriction promoting health and longevity. Nat Rev Mol Cell Biol. 2021. https://doi.org/10.1038/s41580-021-00411-4.
3. Colman RJ, et al. Caloric restriction reduces age-related and all-cause mortality in rhesus monkeys. Nat Commun. 2014;5:3557.
4. Rhoads TW, et al. Caloric restriction engages hepatic RNA processing mechanisms in rhesus monkeys. Cell Metab. 2018;27(3):677-688 e5.
5. Miller KN, et al. Aging and caloric restriction impact adipose tissue, adiponectin, and circulating lipids. Aging Cell. 2017;16(3):497–507.
6. Rhoads TW, et al. Molecular and functional networks linked to sarcopenia prevention by caloric restriction in rhesus monkeys. Cell Syst. 2020;10(2):156-168 e5.
7. Pak HH, et al. Distinct roles of fasting and calories in the metabolic, molecular, and geroprotective effects of a calorie restricted diet. Nat Met. 2021, in press.
8. Hsu PP, et al. The mTOR-regulated phosphoproteome reveals a mechanism of mTORC1-mediated inhibition of growth factor signaling. Science. 2011;332(6035):1317–22.
9. Lamming DW. Diminished mTOR signaling: a common mode of action for endocrine longevity factors. Springerplus. 2014;3:735.
10. Speakman JR, Mitchell SE, Mazidi M. Calories or protein? The effect of dietary restriction on lifespan in rodents is explained by calories alone. Exp Gerontol. 2016;86:28–38.

11. Fontana L, et al. Decreased consumption of branched-chain amino acids improves metabolic health. Cell Rep. 2016;16(2):520–30.
12. Levine ME, et al. Low protein intake is associated with a major reduction in IGF-1, cancer, and overall mortality in the 65 and younger but not older population. Cell Metab. 2014;19(3):407–17.
13. Sluijs I, et al. Dietary intake of total, animal, and vegetable protein and risk of type 2 diabetes in the European Prospective Investigation into Cancer and Nutrition (EPIC)-NL study. Diabetes Care. 2010;33(1):43–8.
14. L'Vov MV, Dzhanpoladian EG. Effect of different pharmacological substances on arrhythmia caused by poisoning with cardiac glycosides. Zh Eksp Klin Med. 1974;14(4):3–8.
15. Laeger T, et al. FGF21 is an endocrine signal of protein restriction. J Clin Invest. 2014;124(9):3913–22.
16. Hill CM, et al. FGF21 signals protein status to the brain and adaptively regulates food choice and metabolism. Cell Rep. 2019;27(10):2934-2947 e3.
17. Mitchell SJ, et al. Effects of sex, strain, and energy intake on hallmarks of aging in mice. Cell Metab. 2016;23(6):1093–112.
18. Solon-Biet SM, et al. The ratio of macronutrients, not caloric intake, dictates cardiometabolic health, aging, and longevity in ad libitum-fed mice. Cell Metab. 2014;19(3):418–30.
19. Richardson NE, et al. Lifelong restriction of dietary branched-chain amino acids has sex-specific benefits for frailty and life span in mice. Nat Aging. 2021;1(1):73–86.
20 Yu D, et al. The adverse metabolic effects of branched-chain amino acids are mediated by isoleucine and valine. Cell Metab. 2021;33(5):905-922 e6.
21. Becher T, et al. Brown adipose tissue is associated with cardiometabolic health. Nat Med. 2021;27(1):58–65.
22. Bartke A, Brown-Borg H. Life extension in the dwarf mouse. Curr Top Dev Biol. 2004;63:189–225.
23. Hu Y, et al. Deletion of Nrip1 delays skin aging by reducing adipose-derived mesenchymal stem cells (ADMSCs) senescence, and maintaining ADMSCs quiescence. Geroscience. 2021;43(4):1815–33.
24. van Deursen JM. The role of senescent cells in ageing. Nature. 2014;509(7501):439–46.
25. Behrens A, et al. Impact of genomic damage and ageing on stem cell function. Nat Cell Biol. 2014;16(3):201–7.
26. Joshi S, et al. ACE2/ACE imbalance and impaired vasoreparative functions of stem/progenitor cells in aging. Geroscience. 2021;43(3):1423–36.
27. Yousefzadeh MJ, et al. Heterochronic parabiosis regulates the extent of cellular senescence in multiple tissues. Geroscience. 2020;42(3):951–61.
28. Robinson AR, et al. Spontaneous DNA damage to the nuclear genome promotes senescence, redox imbalance and aging. Redox Biol. 2018;17:259–73.
29. Perillo B, et al. LSD1: more than demethylation of histone lysine residues. Exp Mol Med. 2020;52(12):1936–47.
30 Yousefzadeh M, et al. DNA damage-how and why we age? Elife. 2021;10:e62852.
31. Yousefzadeh MJ, et al. An aged immune system drives senescence and ageing of solid organs. Nature. 2021;594(7861):100–5.
32. Zhang P, et al. Senolytic therapy alleviates Abeta-associated oligodendrocyte progenitor cell senescence and cognitive deficits in an Alzheimer's disease model. Nat Neurosci. 2019;22(5):719–28.
33. Musi N, et al. Tau protein aggregation is associated with cellular senescence in the brain. Aging Cell. 2018;17(6):e12840.
34. Bussian TJ, et al. Clearance of senescent glial cells prevents tau-dependent pathology and cognitive decline. Nature. 2018;562(7728):578–82.
35. Borgesius NZ, de Waard MC, van der Pluijm I, Omrani A, Zondag GC, van der Horst GT, Melton DW, Hoeijmakers JH, Jaarsma D, Elgersma Y. Accelerated age-related cognitive decline and neurodegeneration, caused by deficient DNA repair. J Neurosci. 2011;31(35):12543–53.
36. Raj DD, Jaarsma D, Holtman IR, Olah M, Ferreira FM, Schaafsma W, Brouwer N, Meijer MM, de Waard MC, van der Pluijm I, Brandt R, Kreft KL, Laman JD, de Haan G, Biber KP, Hoeijmakers JH, Eggen BJ, Boddeke HW. Priming of microglia in a DNA-repair deficient model of accelerated aging. Neurobiol Aging. 2014;35(9):2147–60.
37 Camell CD, et al. Senolytics reduce coronavirus-related mortality in old mice. Science. 2021;373(6552):eabe4832.
38. Yousefzadeh MJ, et al. Fisetin is a senotherapeutic that extends health and lifespan. EBioMedicine. 2018;36:18–28.
39. Haws SA. Leech CM, Denu JM. Metabolism and the epigenome: a dynamic relationship. Trends Biochem Sci. 2020. https://doi.org/10.1016/j.tibs.2020.04.002.
40. Krautkramer KA, et al. Diet-microbiota interactions mediate global epigenetic programming in multiple host tissues. Mol Cell. 2016;64(5):982–92.
41. Thomas SP, Denu JM. Short-chain fatty acids activate acetyltransferase p300. bioRxiv. 2021: 2021.07.21.453192.
42. Chaurasia B, Summers SA. Ceramides in metabolism: key lipotoxic players. Annu Rev Physiol. 2021;83:303–30.
43. Chaurasia B, et al. Targeting a ceramide double bond improves insulin resistance and hepatic steatosis. Science. 2019;365(6451):386–92.
44 Sharma A, et al. Causal roles of mitochondrial dynamics in longevity and healthy aging. EMBO Rep. 2019;20(12):e48395.
45. Rana A, et al. Promoting Drp1-mediated mitochondrial fission in midlife prolongs healthy lifespan of Drosophila melanogaster. Nat Commun. 2017;8(1):448.
46. Mendenhall AR, et al. Cell-to-cell variation in gene expression and the aging process. Geroscience. 2021;43(1):181–96.
47. Zheng T, et al. Effects of environmental exposures on fetal and childhood growth trajectories. Ann Glob Health. 2016;82(1):41–99.
48. Langley-Evans SC. Nutrition in early life and the programming of adult disease: a review. J Hum Nutr Diet. 2015;28(Suppl 1):1–14.
49. McMullen S, Mostyn A. Animal models for the study of the developmental origins of health and disease. Proc Nutr Soc. 2009;68(3):306–20.
50. Dekker MC, et al. Developmental trajectories of depressive symptoms from early childhood to late adolescence:

gender differences and adult outcome. J Child Psychol Psychiatry. 2007;48(7):657–66.
51. Monaghan P. Early growth conditions, phenotypic development and environmental change. Philos Trans R Soc Lond B Biol Sci. 2008;363(1497):1635–45.
52. Power C, Lake JK, Cole TJ. Measurement and long-term health risks of child and adolescent fatness. Int J Obes Relat Metab Disord. 1997;21(7):507–26.
53. Widen E, et al. Pubertal timing and growth influences cardiometabolic risk factors in adult males and females. Diabetes Care. 2012;35(4):850–6.
54. Yuan R, et al. Genetic coregulation of age of female sexual maturation and lifespan through circulating IGF1 among inbred mouse strains. Proc Natl Acad Sci U S A. 2012;109(21):8224–9.
55. Colon G, et al. The enigmatic role of growth hormone in age-related diseases, cognition, and longevity. Geroscience. 2019;41(6):759–74.
56. Cruz-Jentoft AJ, Sayer AA. Sarcopenia. Lancet. 2019;393(10191):2636–46.
57. Cleasby ME, Jamieson PM, Atherton PJ. Insulin resistance and sarcopenia: mechanistic links between common co-morbidities. J Endocrinol. 2016;229(2):R67-81.
58. Shetty S, et al. Enhanced fatty acid flux triggered by adiponectin overexpression. Endocrinology. 2012;153(1):113–22.
59. Stern JH, Rutkowski JM, Scherer PE. Adiponectin, leptin, and fatty acids in the maintenance of metabolic homeostasis through adipose tissue crosstalk. Cell Metab. 2016;23(5):770–84.
60. Okada-Iwabu M, et al. A small-molecule AdipoR agonist for type 2 diabetes and short life in obesity. Nature. 2013;503(7477):493–9.
61. Richardson CE, Yee C, Shen K. A hormone receptor pathway cell-autonomously delays neuron morphological aging by suppressing endocytosis. PLoS Biol. 2019;17(10):e3000452.
62. Chae JB, et al. Targeting senescent retinal pigment epithelial cells facilitates retinal regeneration in mouse models of age-related macular degeneration. Geroscience. 2021. https://doi.org/10.1007/s11357-021-00457-4.
63. López-Otín C, et al. The hallmarks of aging. Cell. 2013;153(6):1194–217.
64. Miller KN, Clark JP, Anderson RM. Mitochondrial regulator PGC-1a-modulating the modulator. Curr Opin Endocr Metab Res. 2019;5:37–44.
65 Miller KN, et al. PGC-1a integrates a metabolism and growth network linked to caloric restriction. Aging Cell. 2019;18(5):e12999.
66. Martin SA, et al. GSK3β regulates brain energy metabolism. Cell Rep. 2018;23(7):1922-1931.e4.
67. Souder DC, Anderson RM. An expanding GSK3 network: implications for aging research. Geroscience. 2019;41(4):369–82.
68. Horvath S. DNA methylation age of human tissues and cell types. Genome Biol. 2013;14(10):R115.
69. Lehallier B, et al. Undulating changes in human plasma proteome profiles across the lifespan. Nat Med. 2019;25(12):1843–50.
70 Skene PJ, Henikoff S. An efficient targeted nuclease strategy for high-resolution mapping of DNA binding sites. Elife. 2017;6:e21856.
71 Erasmus M, et al. Linking LOXL2 to cardiac interstitial fibrosis. Int J Mol Sci. 2020;21(16):5913.
72. Wen B, Xu LY, Li EM. LOXL2 in cancer: regulation, downstream effectors and novel roles. Biochim Biophys Acta Rev Cancer. 2020;1874(2):188435.
73. Britton E, McLaughlin JT. Ageing and the gut. Proc Nutr Soc. 2013;72(1):173–7.
74. Garg M, et al. Imbalance of the renin-angiotensin system may contribute to inflammation and fibrosis in IBD: a novel therapeutic target? Gut. 2020;69(5):841–51.
75. Olstad KJ, et al. Development of a geropathology grading platform for nonhuman primates. Aging Pathobiol Ther. 2020;2(1):16–9.
76. Kuhn HG, Dickinson-Anson H, Gage FH. Neurogenesis in the dentate gyrus of the adult rat: age-related decrease of neuronal progenitor proliferation. J Neurosci. 1996;16(6):2027–33.
77 Kalamakis G, et al. Quiescence modulates stem cell maintenance and regenerative capacity in the aging brain. Cell. 2019;176(6):1407-1419 e14.
78. Ibrayeva A, et al. Early stem cell aging in the mature brain. Cell Stem Cell. 2021. https://doi.org/10.1016/j.stem.2021.03.018.
79. Leeman DS, et al. Lysosome activation clears aggregates and enhances quiescent neural stem cell activation during aging. Science. 2018;359(6381):1277–83.
80. Johnston JA, Ward CL, Kopito RR. Aggresomes: a cellular response to misfolded proteins. J Cell Biol. 1998;143(7):1883–98.
81. Morrow CS, et al. Vimentin coordinates protein turnover at the aggresome during neural stem cell quiescence exit. Cell Stem Cell. 2020. https://doi.org/10.1016/j.stem.2020.01.018.