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REVIEW

Metabolism and metabolomics in senescence, aging, and age-related diseases: a multiscale perspective

Ziyi Wang[1], Hongying Zhu (✉)[1,2,3,4], Wei Xiong (✉)[1,2,3,4]

[1]Center for Advanced Interdisciplinary Science and Biomedicine of IHM, Division of Life Sciences and Medicine, University of Science and Technology of China, Hefei 230027, China; [2]Anhui Province Key Laboratory of Biomedical Imaging and Intelligent Processing, Institute of Artificial Intelligence, Hefei Comprehensive National Science Center, Hefei 230088, China; [3]CAS Key Laboratory of Brain Function and Disease, Hefei 230026, China; [4]Anhui Province Key Laboratory of Biomedical Aging Research, Hefei 230026, China

Abstract **The pursuit of healthy aging has long rendered aging and senescence captivating. Age-related ailments, such as cardiovascular diseases, diabetes, and neurodegenerative disorders, pose significant threats to individuals. Recent studies have shed light on the intricate mechanisms encompassing genetics, epigenetics, transcriptomics, and metabolomics in the processes of senescence and aging, as well as the establishment of age-related pathologies. Amidst these underlying mechanisms governing aging and related pathology metabolism assumes a pivotal role that holds promise for intervention and therapeutics. The advancements in metabolomics techniques and analysis methods have significantly propelled the study of senescence and aging, particularly with the aid of multiscale metabolomics which has facilitated the discovery of metabolic markers and therapeutic potentials. This review provides an overview of senescence and aging, emphasizing the crucial role metabolism plays in the aging process as well as age-related diseases.**

Keywords metabolism; aging; senescence; age-related diseases; metabolomics

Introduction

In the 1960s, Hayflick pioneered the understanding of cellular senescence, igniting a vast body of research that has since delved into its intricate complexities [1]. Cellular senescence encompasses diverse alterations in the genome, epigenome, transcriptome, proteome, and metabolome. Prior investigations have extensively mapped the complexity and heterogeneity underlying the establishment and characteristics of senescence. While distinct inducers can trigger various cellular senescence types, they often share common features such as elevated SA-β-Gal activity, growth arrest, and the secretion of senescent-associated secretory phenotypes (SASPs). However, each specific senescence type exhibits unique attributes in the genome, epigenome, transcriptome, and beyond. Given metabolism's pivotal role in cellular function and biological activities, it serves as a direct reflection of cellular functional states. As such, the regulation of metabolism in senescence and aging has emerged as a novel frontier in aging science. Senescent cells and aged animals exhibit specific metabolic signatures, highlighting the evolution of the metabolic landscape during senescence and aging. Conversely, metabolism also contributes to the onset and progression of senescence, aging, and related diseases.

The burgeoning field of metabolomics, a relatively new area of research, offers comprehensive analysis of the entire spectrum of cellular, tissue, and even organism-wide metabolites [2]. Recent technological advancements in metabolomics have enabled us to profile metabolism at the individual organelle, cellular, and tissue levels. The elucidation of intricate metabolic mechanisms across various senescence and aging models has provided invaluable insights into age-related diseases. Through metabolic profiling, a more holistic understanding of the regulatory role of metabolism in the aging process is emerging. Diverse strategies involving metabolic intervention are being explored in various aging and age-related disease models to uncover their therapeutic potential.

In this review, we delve into the commonalities and distinguishing features of diverse senescence types, alongside the intricate interplay between cellular

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Correspondence: Hongying Zhu, zhuhy62@ustc.edu.cn;
Wei Xiong, wxiong@ustc.edu.cn

senescence and aging. We succinctly outline the crucial role of metabolism in senescence, aging, and age-related diseases. Additionally, we conduct an in-depth analysis of studies focused on metabolic intervention strategies aimed at combating aging and age-related diseases. Finally, we introduce the latest advancements and practical applications of multiscale metabolomic techniques.

An overview of senescence and aging

The process of aging is marked by the accumulation of senescent cells and the progressive decline in physical functions. Cellular senescence occupies a pivotal role in organismal aging, significantly contributing to functional deterioration. Gaining a comprehensive understanding of the underlying mechanisms of cellular senescence is crucial in the field of aging research. Senescence represents a distinct cellular state characterized by growth arrest, initially identified by Hayflick [1]. Fibroblasts cultured *in vitro* exhibit a finite proliferative capacity, after which they cease dividing and exit the cell cycle. This growth arrest constitutes a crucial hallmark of cellular senescence. This seminal discovery not only piqued researchers' interest but also dispelled the misconception of unlimited passaging in cultured cells. A diverse array of factors has been implicated in inducing cellular senescence, including prominent types such as replicative senescence [1], DNA damage-induced senescence [3], oncogene-induced senescence (OIS) [4], oxidative stress-induced senescence [5], and mitochondrial dysfunction-induced senescence [6]. Additionally, chemotherapeutic drugs [7], epigenetic modifications [7], and the paracrine signaling of the senescence-associated secretory phenotype (SASP) also contribute to this intricate cellular aging process.

The complexity of cellular senescence manifests in the diverse characteristics exhibited by senescent cells. Typically, these cells display distinct features encompassing alterations in morphology, genetics, epigenetics, cell signaling, stress response, and secretory phenotypes. Among these, the enlarged and irregular shape, coupled with growth arrest, are the most prevalent attributes. Other notable features include the activation of DNA damage response (DDR), the induction of endoplasmic reticulum (ER) stress, the initiation of antiapoptotic signaling, chromatin remodeling events, and the secretion of inflammatory factors, primarily the SASP. In the following, we will elaborate on the underlying mechanisms governing several common aspects of senescence in detail.

Growth arrest

Growth arrest, a crucial aspect of senescence, is intricately linked to cyclins and related kinases [8]. The inhibition of these kinases halts the cell cycle's progression, thereby inducing growth arrest in senescent cells. Among the key inhibitors of the cell cycle during senescence are CDKN1A (or p16) and CDKN2A (or p21). These two proteins employ distinct mechanisms to inhibit the cell cycle. Specifically, p16 directly interacts with CDK4/6, suppressing its activity and ultimately leading to growth arrest. Consequently, p16 is recognized as a specific marker of senescence and is widely used in the identification of senescent cells [9]. The expression of p16 is governed by a diverse array of factors. Several transcription factors, such as Sp1, Ets, AP1, and PPARγ, bind to the p16 promoter and upregulate its expression [10–13]. Conversely, elements like INK4A transcription silence element (ITSE), YB1, ID1, and AP-1 inhibit p16 transcription [11,14–16], creating a stable and homeostatic regulatory system. Additionally, certain RNA binding proteins regulate p16 expression. For instance, hnRNP A1 and hnRNP A2 bind to and stabilize the p16 transcript [17], while AUF1 binds to p16 mRNA, promoting its degradation [18].

The protein p21, a pivotal inhibitor of the cell cycle, is prominently expressed in numerous senescence models [5] and is subject to regulation by the tumor suppressor p53. It serves as a crucial component of the DDR orchestrated by the p53-p21 signaling pathway. Nonetheless, p21 can also be activated independently of p53, mediated by mechanisms such as transcriptional regulation by Sp1 and TNF-β signaling [19,20]. Furthermore, miRNAs contribute to the regulation of p21, both transcriptionally and post-transcriptionally [21]. Remarkably, AUF1 specifically targets mRNA encoding p21, enhancing its degradation. This suggests that by indirectly inhibiting AUF1 activity, p16 may modulate the expression of p21 [22], indicating a cooperative regulatory mechanism between these two cell cycle inhibitory proteins during cellular senescence.

SASP

The secretion of SASP, encompassing a diverse array of cytokines, chemokines, and proteinases, stands as a defining characteristic of senescent cells, exhibiting striking heterogeneity [5]. In the process of senescence, DDR signaling orchestrates the activation of NF-κB, ultimately triggering the transcriptional upregulation of SASP. Notably, while NF-κB predominantly fuels the expression of pro-inflammatory cytokines within SASP, specific anti-inflammatory components, including IL-10, IL-13, M-CSF, GM-CSF, and CXCL1, are intricately regulated via the Jak2/Stat3 pathway [23].

Epigenetic modification emerges as a pivotal regulatory mechanism governing the SASP in aging cells. Specifically, the H3K9me2 modification orchestrates the

regulation of IL-6 and IL-8 promoters, both of which are crucial components of SASP. Research indicates that during cellular senescence, the methylation status of these promoters is downregulated due to DDR signaling [24]. Furthermore, acetylation plays a pivotal role in modulating SASP production. During senescence, the deacetylase enzyme sirtuin-1 exhibits reduced activity, leading to a diminished deacetylation process on IL-6 and IL-8 promoters, thus promoting their transcriptional activation [23]. mTOR exercises post-transcriptional regulation over SASP, enhancing the expression and translation of IL-A1, which in turn activates NF-κB and facilitates the induction of SASP [25]. Additionally, mTOR directly inhibits ZFP36L1, a DNA binding protein, to safeguard mRNAs associated with SASP from degradation [26]. The induction of oxidative stress triggers the activation of the p38/MAPK pathway, engaging RNA binding proteins, leading to the stabilization of SASP-related mRNAs and ultimately enhancing the expression of SASP [27].

The SASP, due to its heterogeneity, comprises diverse components secreted by varying senescent cell types. Profiling its components can offer profound insights into the specific senescence subtype. For example, PDGF-A, VEGF, and matrix metalloproteinases (MMPs) are primarily involved in senescence linked to tissue repair [28,29], whereas inflammatory cytokines contribute significantly to age-related senescence [30].

The SASP exerts a broad range of biological effects in cellular senescence and other vital biological processes. It can autocrinely modulate senescence by secreting IL-1 and IL-6, thereby strengthening its own senescent state [31]. Additionally, it exerts a paracrine influence on neighboring cells and actively contributes to the establishment of microenvironments, playing an indispensable role in embryonic reconstruction, tissue regeneration, and immune surveillance [32,33]. Furthermore, the SASP possesses the capability to regulate distant cells or organs through vesicles secreted by senescent cells. These vesicles enable the SASP to traverse long distances from its original location to remote sites. A noteworthy example is the stimulatory effect of SASP on distant tumor growth [28].

Morphological changes

During the process of senescence, cells undergo profound transformations in their size and shape, manifesting as a remarkable morphological feature. Culturing senescent cells *in vitro* results in irregular forms, enlarged dimensions, and a flattened appearance. Notably, the mTORC1 signaling pathway plays a pivotal role in orchestrating these morphological alterations in senescent cells. Moreover, senescence-inducing agents further modulate cellular morphology by activating the mTORC1 pathway [34,35]. Additionally, the rearrangement of scaffold proteins contributes significantly to the morphological changes observed in senescent cells. Specifically, ATF6α participates in this process by modulating COX-2/PGE2 signaling and activating NF-κB, thereby influencing the ER size and transcriptional regulation of scaffold proteins [36,37].

Lysosomes

The lysosome is a vital cellular component, essential for the degradation and recycling of outdated cellular materials, dysfunctional organelles, and participating in diverse signal transduction pathways. However, during senescence, the lysosome's functionality undergoes profound changes. These alterations include a remarkable accumulation of lysosomal proteins and an intensified retention of undegraded waste. Particularly noteworthy is the significant increase in the activity of senescence-associated beta-galactosidase (SA-β-Gal), a classical biomarker for senescence, which is assayed at a pH of 6.0 [38,39]. Alongside these functional alterations, there is also an augmentation in the number of dysfunctional lysosomes due to the accumulation of lipofuscins [40]. This intricate interplay between senescence and lysosomal function underscores the importance of lysosomes in cellular homeostasis and senescence-related processes.

Mitochondrial dysfunction

Mitochondria, being the most crucial organelle within a cell, bestows energy upon biological processes and actively participates in signal transduction. During senescence, there is an increase in the number of mitochondria; however, their functionality declines due to impaired mitophagy [41,42]. The membrane potential of mitochondria significantly diminishes during senescence, consequently leading to the liberation of endonuclease G alongside other mitochondrial components. Additionally, malfunctioning mitochondria generate reactive oxygen species (ROS), thereby initiating oxidative stress as yet another hallmark feature of cellular senescence [43,44].

Cellular senescence as a driver of aging

Cellular senescence serves as a pivotal catalyst for the process of aging. Various mouse models involving either premature senescence or senescent manipulation, including the BubR1 hypomorphic mouse model, the INK-ATTAC model and the p16-3MR model, have substantiated the impact of senescent cells on organismal aging and age-related ailments [45]. The BubR1 hypomorphic mouse model exhibits a progeroid phenotype accompanied by an upregulation of senescent

features such as p16 expression in multiple tissues encompassing adipose tissue, muscle tissue, and ocular tissue. The INK-ATTAC model facilitates the elimination of senescent cells in mice and enhances physical functionality during old age while simultaneously extending both lifespan and healthspan in mice. Similar to the INK-ATTAC model, the p16-3MR model enables clearance of senescent cells in aged mice. The p16-3MR model has validated the pivotal role played by senescent cells in age-related diseases including atherosclerosis, osteoarthritis, Alzheimer's disease (AD), Parkinson's disease (PD).

Collectively, cellular senescence is a highly heterogenous process with complicated features and mechanisms, and a main driver of aging and age-related diseases.

The critical role of metabolism in senescence and aging

The metabolism serves as a direct manifestation of cellular function and biological activities. The metabolic landscape undergoes significant transformations during the process of senescence and aging. Senescent cells manifest alterations in a multitude of metabolic features, including alterations in lipid, amino acid, nucleotide, redox, and transition metal metabolism along with organelle-specific metabolic alterations (Table 1).

Lipid metabolism

Lipid metabolism has been implicated in regulating cellular senescence. Specifically, the sphingomyelin-ceramide pathway exhibits heightened activity in senescent cells. Notably, the supplementation of ceramide in endothelial and fibroblast cultures decelerates cell proliferation and triggers senescent phenotypes, including the inhibition of DNA synthesis and replication, Rb dephosphorylation and activation, and elevated SA-β-Gal expression [46,47]. The mechanism underlying ceramide's ability to induce senescence involves the activation of specific protein phosphatases, leading to the dephosphorylation of cyclin-dependent kinase 2 (CDK2) and a concurrent increase in p21 expression [48]. Furthermore, β-oxidation of fatty acids contributes to the generation of SASP, a crucial marker of senescence [49]. During senescence, altered lipid metabolism is characterized by an increased incorporation of polyunsaturated fatty acids (PUFAs) [50]. In some cells, senescence leads to the accumulation of lipid droplets due to the assimilation of exogenous lipids.

Furthermore, p38-dependent phospholipase A2 effectively cleaves PUFAs in the membrane and subsequently incorporates them into triglycerides [51]. This process exhibits significant activity in mitochondrial-related senescence. Eicosapentanoic acid (EPA), arachidonic acid (AA), and dihomo-γ-linolenic acid (DGLA) are all

Table 1 Metabolic features in cellular senescence

Metabolic features	Changes/Effects on senescence
Metabolic process	
Lipid metabolism	Elevated sphingomyelin-ceramide pathway leads to senescence; β-oxidation of fatty acids contributes to the production of SASP; increased PUFAs incorporation; increased LDL and VLDL; decreased HDL
Amino acid metabolism	Increased tyrosine; decreased cysteine, threonine, serine, tryptophan, and methionine in senescence. There is conflict of evidence in the overall change of amino acids in senescence
Nucleotide metabolism	Deficiency of dNTPs leads to cell cycle arrest and senescence
Redox metabolism	Oxidative stress; decline of acylcarnitines, glutathione/oxidized glutathione ratio, glutathione, and ophthalmic acid leads to redox imbalance
Transition metal metabolism	Accumulation of iron copper, and zinc
Metabolism in organelles	
Mitochondrial metabolism	Defects in oxidative phosphorylation, fatty acid oxidation and lipid storage; depletion of SOD, SIRT3, and HSPA9; inhibition of electron transport chain; depletion of ATP, NAD^+/NADH; activation of AMPK
Lysosome metabolism	Downregulation of amino acids and accumulation of lipids; accumulation of SA-β-Gal
Endoplasmic reticulum metabolism	Lipid metabolism and reprogramming in ER stress and senescence
Key metabolites and metabolic enzyme	
NAD^+	Depleted in senescence; regulates poly ADP-ribose polymerase (PAPR); promotes lifespan extension
α-ketoglutarate	Extending both lifespan and healthspan; fight against osteoporosis
β-hydroxybutyrate	Reducing senescent markers in blood vessel smooth muscle cells and endothelial cells
Methionine	Restriction of methionine uptake leads to reduction in SASP production
HMG-CoA	Alleviate SASP production, cell cycle arrest, and senescence

Table 1 summarizes the metabolic features in senescent cells along with key metabolites in cellular senescence.

upregulated in mitochondrial related senescence, giving rise to a specific metabolic phenotype [52]. Lipoproteins are proteins that bind with lipids and assist in lipid transportation, while also playing a role in signal transduction. The association between lipoproteins and aging is characterized by an increase in very-low-density lipoprotein (VLDL) and low-density lipoprotein (LDL), as well as a decrease in high-density lipoprotein (HDL). In aged individuals, there is a significant elevation of total lipoproteins and lipids [53], whereas people with longer lifespans tend to have lower levels of VLDL [54]. Mass spectrometry studies have revealed elevated cholesterol [55] and PUFAs [56] during the aging process, along with alterations in the lipid profile such as downregulation of vitamin D2-related compounds and age-correlated lipids [57].

Amino acid metabolism

The metabolism of amino acids plays a pivotal regulatory role in senescence, undergoing various directional changes as one ages. A particular study indicates that tyrosine levels increase with age, whereas cysteine, threonine, serine, tryptophan, and methionine levels decrease [58]. Another study singles out methionine as a strong predictor of aging, given its elevated concentration in individuals who experience accelerated aging [59]. Nevertheless, there exists conflicting evidence about the overall changes in amino acid abundance during aging, as various studies present contradictory trends. Given the rapid protein turnover and the complexity of amino acid metabolism and catabolism, a deeper and more systematic exploration utilizing cutting-edge techniques like isotope tracing or metabolic flux analysis is imperative to clearly understand the specific amino acid alterations during the aging process.

Nucleotide metabolism

Nucleotide metabolism undergoes significant changes during cellular senescence, playing a key role in cell cycle arrest. Deoxynucleotide triphosphates (dNTPs) are primarily synthesized from adenosine triphosphate (ATP), guanosine triphosphate (GTP), and cytosine triphosphate (CTP) through the catalytic action of ribonucleotide reductase. However, in senescent cells, ribonucleotide reductase expression is downregulated [60], resulting in a deficiency of dNTPs and subsequent impairment of DNA repair and replication processes. In OIS, the downregulation of ribonucleotide reductase paradoxically stimulates cell replication. Nevertheless, due to the lack of dNTPs, the replication fork and DNA chain often collapse, leading to DNA damage and, consequently, cell cycle arrest. Interestingly, supplementation with dNTPs can restore cell replication [60]. Additionally, another study found that when p53 or p16 is knocked down, cells acquire substances necessary for nucleotide synthesis, thereby alleviating cell cycle arrest in oncogene *ras*-induced cellular senescence [61,62]. Collectively, these studies underscore the central role of nucleotide metabolism in senescence and implicate dNTP deficiency as a key factor leading to cell cycle arrest.

Redox metabolism

Oxidative stress is a hallmark of senescence that is closely intertwined with metabolism. Studies examining metabolic shifts associated with oxidative stress during aging have identified several key metabolites. The glutathione/oxidized glutathione ratio, glutathione disulfide, glutathione, and ophthalmic acid all decline with age, indicating a disruption in redox balance systems as we age [63,64]. Acylcarnitines, which play a role in the mitochondrial carnitine-acylcarnitine shuttle and help alleviate oxidative stress, are downregulated during aging [65]. Additionally, the abundance of sphingomyelins increases under oxidative stress during aging, involving the conversion of sphingomyelins to ceramides [66]. Other metabolites related to redox balance, such as carnosine and vitamin E, also undergo changes during aging-related oxidative stress [67].

Transition metal metabolism

Transition metal metabolism also plays a part in the aging process and is linked to certain senescent characteristics. Previous research suggests an accumulation of transition metals, including iron, zinc, magnesium, and copper, in senescent cells compared to non-senescent or immortalized cell lines. These metals are essential building blocks for the organism. Iron, for instance, binds to transferrin for cellular transport or uptake. It plays a pivotal role in cellular respiration and oxygen transportation, forming the basis of life. However, senescent cells upregulate the transferrin receptor and internalize iron bound to transferrin through endocytosis. Due to impaired autophagy and lysosomal function, iron and transferrin accumulate in senescent cells instead of being degraded [68]. Copper is a cofactor for a wide range of metabolic enzymes and is crucial for maintaining enzyme bioactivity. Excess copper, however, can be harmful to cells. It has been reported to induce cellular senescence in fibroblasts and glioblastomas [69]. Similar to iron accumulation, copper accumulation is related to lysosomal dysfunction and impaired autophagy. Copper often combines with other molecules and relies on the lysosome for degradation. Thus, lysosomal malfunction and impaired autophagy lead to copper accumulation. One theory for copper accumulation in senescent cells is

that it aids in combating oxidative stress during aging and senescence [70]. Zinc is vital for life, but a previous report indicates an inverse correlation between zinc abundance and the lifespan of *Caenorhabditis elegans* [71]. Excessive zinc has been shown to induce cellular senescence in endothelial cells, fibroblasts, and blood vessel smooth muscle cells [72–74]. Transition metals have been reported to be involved in cell cycle arrest, a key feature of senescence. Nevertheless, the precise role of transition metals in cellular senescence remains to be elucidated.

Organelle specific metabolism

Mitochondrial metabolism undergoes significant alterations during senescence and serves as a key driver of cellular senescence. The early stages of cellular senescence are marked by defects in oxidative phosphorylation (OXPHOS) [75]. Additionally, mitochondrial metabolism's homeostasis becomes compromised in senescence, manifesting as a deficiency in fatty acid oxidation and lipid storage [76]. Senescence involves the depletion of mitochondrial protein deacetylase SIRT3, mitochondrial chaperone protein HSPA9, mtDNA mutations, and electron transport chain inhibition —all factors contributing to mitochondrial dysfunction. Consequently, NADH accumulates in the cytosol, reducing the NAD^+/NADH ratio, depleting ATP, and activating AMPK, ultimately leading to cell cycle arrest [6]. This phenomenon is also known as mitochondrial dysfunction-associated senescence (MiDAS). Mitochondria are a primary source of ROS, accelerators of senescence and aging. The depletion of mitochondrial superoxide dismutase (SOD) induces cellular senescence in mouse models [77]. The accumulation of mitochondrial oxidative damage gives rise to the "free radical theory of aging" [78], which postulates that oxidative damage drives mitochondrial and organ malfunction during aging.

Lysosomes, crucial organelles involved in autophagy and signal transduction, exhibit profound metabolic alterations in senescent cells. Proteins and enzymes such as SA-β-Gal, a classical marker of senescence, accumulate within lysosomes in senescent cells [38,39]. These findings suggest that lysosomes undergo malfunction and metabolic impairment during cellular senescence, providing valuable insights into the metabolic changes associated with this biological process. A recently devised single-lysosome mass spectrometry (SLMS) technique has unlocked the door to exploring the metabolomic shifts that occur within aging lysosomes [79]. Leveraging bioinformatic algorithms, this technique has not only revealed the metabolic heterogeneity present in different types of lysosomes but has also pinpointed five distinct subpopulations. Furthermore, it has uncovered subpopulation-specific alterations in metabolites during cellular senescence, with significant changes observed in lipids, amino acids, and organic acids. For instance, metabolites such as CDP-DG (43:2), CL (72:10), and PIP2 (38:3) exhibit a remarkable upregulation of approximately twofold in the endolysosomes of senescent cells. Conversely, amino acids like serine, leucine, threonine, arginine, glutamine, and hypotaurine are downregulated by about twofold in the autolysosomes of these senescent cells, highlighting the intricate metabolic reprogramming that accompanies cellular aging.

ER is a crucial organelle in lipid metabolism, playing a pivotal role in a wide array of biological processes, such as calcium homeostasis, lipid biogenesis, lipid homeostasis, and protein folding. It also provides compartments, enzymes, and microenvironments essential for various biochemical reactions. Notably, the ER is a key regulator in senescence and aging. This organelle is responsible for composing a diverse range of glycerophospholipids within a cell and is abundant in phosphatidylinositol (PI), phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylserine (PS), and phosphatidic acid (PA) [80]. ER stress holds a vital role in senescence and aging, with a tight correlation to cellular metabolism. Studies have shown that ER reductive stress can lead to cellular senescence [81]. Furthermore, ER stress is implicated in β cell senescence, which is associated with diabetes, an age-related disease [82]. Lipid metabolism and distribution are regulated by the ER, and ER stress evokes the unfolded protein response (UPR), promoting reprogramming of lipid metabolism that can lead to age-related metabolic diseases like diabetes [83]. The ER also actively interacts with other organelles within a cell. For instance, the ER and mitochondria can form close contact sites known as mitochondria-associated membranes (MAM), which play a critical role in senescence and metabolism. The MAM harbors several enzymes for lipid biosynthesis along with lipid transfer proteins that govern the metabolism of lipids, including cholesterol [84,85], PE [84], and ceramides [86]. These metabolites may further regulate senescence and aging, highlighting the intricate interplay between the ER and cellular metabolism in the context of aging and related diseases.

Key metabolites regulating senescence and aging

Through detailed investigations into senescence and aging, several crucial metabolites that regulate the aging process have been uncovered. For instance, NAD^+ stands out as a significant modulator of both senescence and aging. NAD^+ acts as the rate-limiting metabolite for poly ADP-ribose polymerase (PARP), which plays a pivotal role in DDR signaling and DNA repair mechanisms.

Inhibiting PARP activity elevates NAD^+ levels, thereby enhancing mitochondrial function and extending the lifespan of *C. elegans* [87]. Cells isolated from patients with progeria demonstrate persistent PARP activation and depleted NAD^+ levels, resulting in a range of progeria symptoms. Supplementing with NAD^+ has been shown to mitigate these symptoms, indicating that NAD^+ regulates premature senescence and prevents its progression, as noted in [88]. Another noteworthy example is the supplementation of α-ketoglutarate, an intermediate in the tricarboxylic acid cycle (TCA cycle), which has been found to prolong both the lifespan and healthspan of mice [89]. Furthermore, α-ketoglutarate (α-KG) improves osteoporosis in aged mice by modulating epigenetics [90]. α-KG inhibits H3K9me3 and H3K27me3 accumulation, leading to better proliferation, migration, and osteogenic potential in bone marrow mesenchymal stromal/stem cells in aged mice, resulting in an acceleration of bone regeneration. This study bridges the gap between metabolism and gene expression regulation, uncovering the mechanisms behind metabolism's regulatory effects on aging. Additionally, ketone bodies have been identified as modulators of cellular senescence and aging. Specifically, the injection of β-hydroxybutyrate reduces senescent markers in blood vessel smooth muscle cells and endothelial cells in mice [91]. Modulating certain amino acids, such as restricting methionine uptake, can also modulate cellular senescence by reducing the production of some SASP components [92]. These findings further underscore the regulatory influence of metabolism on senescence and aging. Beyond metabolites, regulating specific metabolic enzymes has emerged as a novel approach for senescence and aging intervention. For instance, statins, which inhibit HMG-CoA reductase and consequently cholesterol biosynthesis, alleviate SASP production, cell cycle arrest, and senescence of endothelial progenitor cells (EPCs) [93,94]. The inhibition of HMG-CoA reductase prevents the activation of rho-GTPase enzymes like RAS and RAC, which are essential for SASP production. Moreover, intervening in glucose metabolism, such as through the inhibition of the sodium-glucose cotransporter, reduces glucose transport into cells, thereby preventing cellular senescence [95].

In summary, senescence and aging encompass sophisticated characteristics and mechanisms, with metabolism playing an essential role in their development and serving as a fundamental aspect of aging (Fig. 1).

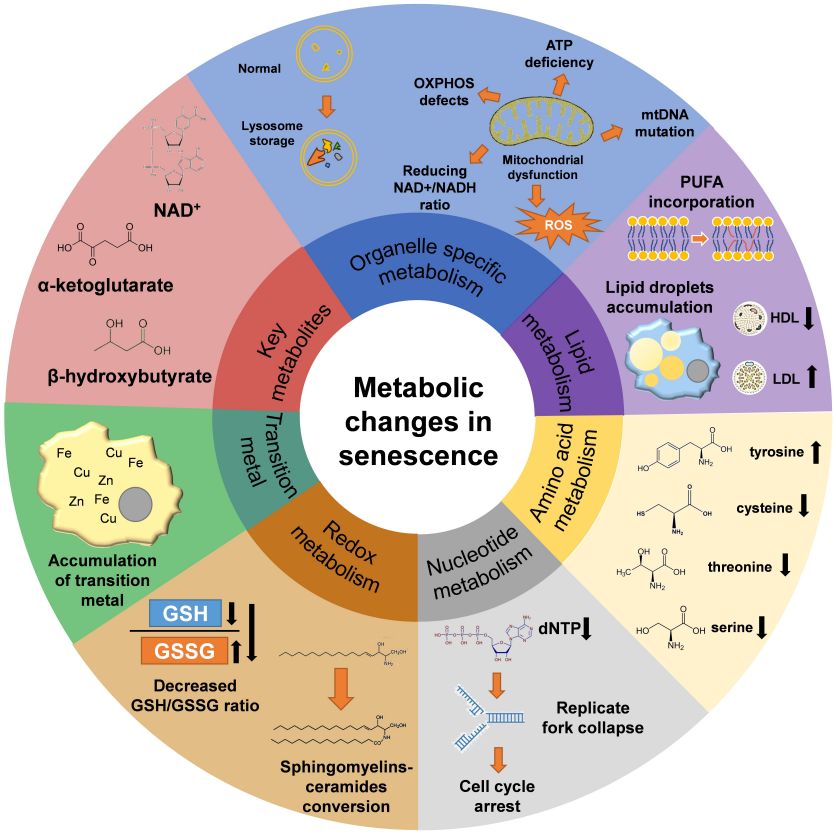

Fig. 1 An overview of senescence related metabolic changes. Senescent cells undergo modifications in their organelles, primarily affecting lysosomes and mitochondria, resulting in distinct alterations in metabolites. Additional shifts in the metabolome encompass changes in lipid, nucleotide, and amino acid metabolism. Other modifications include imbalances in redox metabolism as well as accumulation of transition metals. Several crucial metabolites have been identified to be involved in regulating senescence.

Metabolism, age-related diseases, and metabolic interventions

Metabolic dysregulation is a salient characteristic of age-related diseases, with metabolism playing a pivotal role in their pathological processes. Research focusing on metabolism in such diseases has revealed its regulatory function and potential clinical applications. In this section, we delve into common age-related diseases and the underlying metabolic mechanisms (Fig. 2).

Metabolism in common age-related diseases

Cardiovascular disease

Aging is a common risk factor for vascular diseases, where oxidative stress is a key feature of vascular aging. Atherosclerosis is a common cardiovascular disease and a risk factor of ischemic heart disease. Dysfunctional endothelial cells exhibit elevated ROS levels, initiating the pathological changes that lead to atherosclerosis [96,97]. Studies suggest that impaired mitochondrial function contributes to the elevated ROS levels, resulting in an inflammatory microenvironment that promotes disease progression [98]. Animal models further illustrate the metabolic role in vascular diseases, highlighting the importance of mitochondrial enzymes like manganese-dependent superoxide dismutase (MnSOD) in redox metabolism [99]. Additionally, mutations in mitochondrial DNA (mtDNA) have been linked to the development of cardiovascular diseases, including hypertension and atherosclerosis [100,101]. Besides mitochondrial dysfunction and oxidative stress, other metabolites are significantly altered in atherosclerosis and serve as potential biomarkers for diagnostics. Studies suggest plasm trimethylamine-N-oxide to be a good predictor of atherosclerosis [102]. Trimethylamine-N-oxide impairs the reverse cholesterol transport and induces the formation of plaques, along with promoting platelet activation and aggregation [102]. Lipid metabolism has a vital role in the pathogenesis of atherosclerosis. Plasm lipids are reported to be associated with risk of atherosclerosis. 18:2 monoglyceride suggests higher risk for cardiovascular events whereas 28:1 sphingomyelin and 18:2 lysophosphatidylcholine suggest lower risks [103].

Heart failure is another common age-related disease and is tightly related to metabolism. Energy metabolism is critical in normal heart function and plays a key role in heart failure. Insufficient energy supply is involved in the

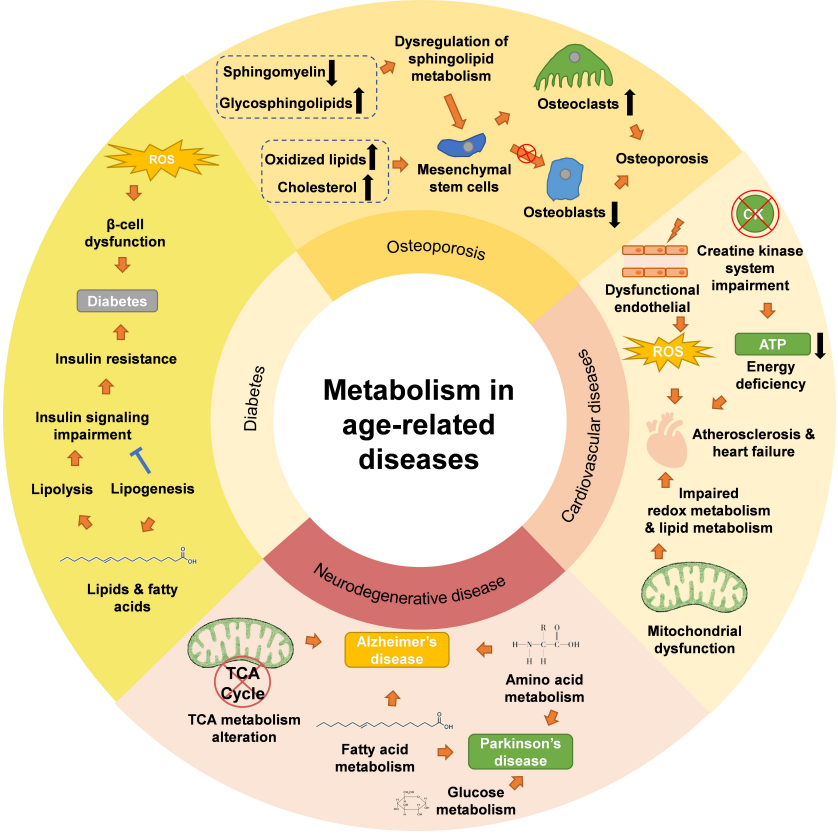

Fig. 2 Diagram of metabolic changes and mechanisms in age-related diseases summarizing metabolic regulation and pathology in cardiovascular diseases, osteoporosis, diabetes, and neurodegenerative diseases.

pathogenesis of heart failure. The creatine kinase system sustains the ATP/Phosphocreatine conversion, which buffers and storages energy for the heart. The process can generate a large amount of ATP in a rather short time, supplying the high demand for energy in the heart. However, there is about a 35% drop of creatine kinase activity in heart failure, following a drop of related substrate creatine [104]. These alterations lead to impaired energy metabolism and energy insufficiency. The important energy parameter, Phosphocreatine/ATP ratio decreases which impairs the systolic and diastolic function of the heart [105]. Another major pathway for ATP production is the metabolism of glucose and fatty acids. Studies discover the alterations and impairments in metabolism of these metabolites. The oxidation of glucose is reported to be impaired during heart failure, which promotes the glycolysis and produces lactate [106]. The activity of pyruvate dehydrogenase is reported to be decreased in heart failure [107], which impairs the oxidation of glucose. At later stages of heart failure, the glycolysis pathway is declined due to decompensation of heart failure, leading to further energy insufficiency. Fatty acids are another type of energy source of the heart. Fatty acids can cross the membrane and converted to acetyl-CoA, enters the TCA cycle and produces ATP. Alteration of fatty acid and lipids has a major role in heart failure. Patients with heart failure are found to have elevated levels of C18:1 acylcarnitines in plasma [108]. However, the level of acylcarnitines in myocardial decreases, suggesting the impaired mitochondrial function [109]. Consistently, oxidation of fatty acids is reported to be downregulated at sever stages of heart failure [110].

Neurodegenerative diseases

Alzheimer's disease

Alzheimer's disease, an age-related neurodegenerative disorder, is characterized by impaired cognitive functions and structural changes in the brain. Metabolism plays a pivotal role in AD pathology, with alterations in mitochondrial metabolism and TCA cycle intermediates evident in AD mouse models [111]. The metabolic landscape of AD brains, encompassing fatty acids, amino acids, and lipids, undergoes significant alterations [112,113]. Lipid metabolism, in particular, holds a crucial position in AD pathology, as ample clinical evidence indicates alterations in lipids in AD patients. Brain cholesterol, constituting about 25% of total cholesterol in the body, is vital for brain function and pathology [114]. Studies on AD brains reveal a correlation between cholesterol levels and AD severity [115], along with an elevation of cholesterol in plaques within AD brains [116]. Reports suggest that cholesterol levels are elevated in AD brains compared to healthy controls [117], although some studies find no such change [118]. The diverse stages of AD pathology and variations in brain regions may account for these seemingly contradictory results. Furthermore, research confirms the protective effects of lowering cholesterol levels on AD by knocking down SREBF2, which disrupts cholesterol synthesis [119]. Inhibition of cholesterol synthesis by ApoE-mediated miRNA elevates the expression of genes related to memory [120]. Consistently, cholesterol is implicated in the formation of Aβ [119]. The core factors of AD, including γ-secretase, Aβ, and APP, also regulate lipid metabolism, influencing cholesterol synthesis and lipoprotein regulation [121–123].

Fatty acids also play pivotal roles in AD pathology. They are a crucial energy source in the brain, where β-oxidation of fatty acids in the mitochondria provides a substantial amount of ATP for biological activities [124]. However, β-oxidation of fatty acids produces ROS, and neurons have poor antioxidant systems [124]. Oxidative stress is a significant factor in AD pathology. Beyond neurons, the oxidation of fatty acids in astrocytes also contributes to AD. A recent study demonstrates that disrupted oxidative degradation of fatty acids in astrocytes leads to dysregulation of lipids in the AD brain [125]. Additionally, ApoE4 is involved in the disruption of neuron-astrocyte interaction in fatty acid metabolism [125]. Reports suggest that fatty acid synthesis is closely linked to AD, as the level of fatty acid synthase is upregulated near plaques [126].

Lipid droplets, which store lipids and cholesterols, are present in cells undergoing aging and senescence. These droplets are also reported to accumulate in various cells in the nervous system during AD pathology [127]. Furthermore, the transcriptional landscape of lipid droplets accumulating in microglia overlaps with that of microglia in AD, relating to the proinflammatory state [128].

Parkinson's disease

Parkinson's disease, another age-related neurodegenerative disorder, is intimately linked to metabolism. PD involves impairment of the dopamine system and related metabolic pathways. For instance, dopamine release is impaired during PD pathology [129], along with disturbances in dopamine biosynthesis and loss of dopaminergic neurons [130]. Enhancing dopamine release helps alleviate PD pathology [131]. Dopamine and its related metabolic system are key factors in PD pathology. A study reports the diagnostic value of imaging dopamine transporters [132], illustrating the connection between PD pathology and dopamine metabolism. Other studies reveal impaired lipid metabolism and alterations in lipids, organic acids, amino acids, and steroids in PD patients [133,134]. Sphingolipid

metabolism is reported to be altered in PD and contributes to its pathology. Altered sphingolipid levels are observed in PD patients and are related to neuroinflammation [135]. A recent study further emphasizes the crucial role of ceramide and sphingomyelin in PD pathology by showing elevated levels of these two sphingolipids in cerebrospinal fluid (CSF) samples of PD patients [136]. This study suggests the potential of sphingolipids as biomarkers of PD. An untargeted mass spectrometry (MS) analysis of serum samples from a clinical cohort also indicates distinguishable differences in sphingomyelin and ceramide, along with other lipids including acylcarnitine, phosphatidylcholine, and triacylglycerol between PD patients and healthy controls [137]. Glycosphingolipids are reported to change during aging [138], and aging is a common risk factor for PD. The catabolism enzymes for glycosphingolipids are downregulated in brain regions closely related to PD during aging, and their levels are further reduced in PD patients [139]. Consequently, PD patients exhibit higher levels of GlcCer in the substantia nigra, while gangliosides are significantly lower in PD patients and also downregulated with age [139].

Fatty acids are also involved in the metabolic alterations of PD pathology. Clinical studies indicate significant changes in fatty acids and the metabolome in PD patients. A recent study discovered the dysregulation of fatty acid metabolism in PD patients through GC-MS analysis of plasma samples [140]. The study reveals alterations in long-chain and short-chain fatty acids, including oleic acid, tetradecanoic acid, pentanoic acid, and propanoic acid. Among the altered fatty acids, propanoic acid, along with 2,3,4-trihydroxybutyric acid, can serve as biomarkers for PD. Another study illustrates the perturbation of unsaturated free fatty acids, such as AA and linoleic acid, in PD patients [134]. Unsaturated free fatty acids are reported to facilitate the assembly of α-synuclein [141], further strengthening the link between fatty acid metabolism and PD pathology.

Impairment of glucose metabolism is reported in PD patients [142]. A portion of PD patients suffers from diabetes [143], suggesting a link between impaired glucose metabolism and PD. Recent studies suggest that the elevated risk of PD is related to prediabetes [144,145], increased fasting glucose variability [146], and elevated fasting glucose [147]. Hyperglycemia is reported to inhibit the synthesis and release of dopamine in dopaminergic neurons [148] through the disturbance of a range of transporters and channels [149]. Additionally, hyperglycemia promotes the aggregation of α-synuclein and mediates neuronal loss [150], along with the impairment of mitochondrial function [151]. Another study suggests that the duration of diabetes is related to PD risk [152]. Furthermore, PD progression is also linked with diabetes [153]. Glucose hypermetabolism is reported in PD patients at early stages in frontal regions of the brain, illustrating the dysfunction of glucose metabolism in PD pathology [154]. The key enzyme in glycolysis, phosphoglycerate kinase 1, is reported to reduce dopaminergic neuron loss and PD risk [155,156]. Consistently, elevation of phosphoglycerate kinase 1 activity helps ameliorate PD symptoms [157]. GAPDH, which catalyzes the conversion of glyceraldehyde triphosphate to 1,3-diphosphoglycerate, interacts with α-synuclein and impairs glycolysis [158]. These results suggest the role of glucose metabolism in PD pathogenesis and progression.

Other metabolic molecules, including amino acids (such as histidine, valine, phenylalanine, glycine, isoleucine, tyrosine, alanine, taurine, and GABA), ketones (such as acetoacetate), carbohydrates (such as fucose), along with other metabolites, are reported to be elevated in the saliva of PD patients according to a recent NMR metabolomics study [159]. Sebum metabolism has also been correlated with PD phenotypes, providing insights into the metabolic mechanisms underlying PD pathology [160]. This study offers potential for easy and quick detection and diagnosis of PD.

Diabetes

Diabetes, another common age-related disease, is characterized by impaired insulin sensitivity and secretion along with abnormal blood glucose levels. Mitochondrial dysfunction is implicated in diabetes pathogenesis, as dysfunctional mitochondria fail to sustain normal oxidation processes, contributing to insulin resistance [161]. Metabolic changes, including impaired lipid metabolism and reduced mitochondrial oxidative capacity, are observed in patients with diabetes [162–164]. ROS production, as a result of mitochondrial dysfunction, leads to β-cell dysfunction, which regulates blood glucose levels, is critical in diabetes development [165,166]. Insulin signaling is rather high in energy consumption, relying on mitochondrial energy metabolism. Mitochondrial dysfunction in adipose tissue contributes to insulin resistant and diabetes. Mitochondria functional proteins are reported to be lower in subcutaneous adipose tissue of insulin-resistant subjects [167]. Dysfunctional mitochondrial failed to sustain the secretion of endocrine factors, leading to change in whole-body level insulin sensitivity [168]. Impaired insulin signaling leads to failure in glycogen storage in muscle [169], further weaken nonoxidative metabolism of glucose [170]. A study of patients also suggests the critical role of hepatic glycogen metabolism in type 2 diabetes [171]. Patients with type 2 diabetes have lower postprandial hepatic glycogen synthesis, along with endogenous glucose production.

Adipose tissue and lipid metabolism is also involved in insulin resistance and contributes to the pathology of diabetes. Lipolysis and lipogenesis along with mitochondrial functional alterations in adipose tissue are key factors in establishment of insulin resistance. Lipolysis is crucial in the establishment of insulin resistance. Lipolysis is a catabolism of triacylglycerols, releasing fatty acids and glycerol from adipose tissue. Enhanced lipolysis in adipose tissue links obesity and insulin resistance. Immune cells infiltrate adipose tissue in obesity, and establishes an inflammatory environment which leads to the activation of lipolysis in adipocytes [172]. Lipolysis increases the level of circulation lipids and induces insulin resistance in distant organs [173]. Nicotinic acid inhibits lipolysis in adipose tissue and promotes *de novo* lipogenesis, regulating the energy fluxes and improve insulin sensitivity in a study of obese men [174]. Lipogenesis is activated by insulin which increases the expression of fatty acid synthesis leading to *de novo* lipogenesis and glucose uptake. Impaired *de novo* lipogenesis in adipose tissue is critical in the pathogenesis of whole-body insulin resistance [175], which is a feature of diabetes. Increased *de novo* lipogenesis in adipose tissue improves insulin sensitivity and promotes insulin signaling [176]. Moreover, fatty acid esters generated by *de novo* lipogenesis mediates the cross talk between adipose and tissues with insulin sensitivity, regulating the energy metabolism and insulin signaling [177]. Palmitoleate, a product in *de novo* lipogenesis, is identified as a signal molecule which enhances insulin signaling in muscle cells [176].

Osteoporosis

Osteoporosis is an age-related disease tightly linked with metabolism. Osteoporosis is characterized by loss and deterioration of bone tissue as a result of imbalance of osteoclasts and osteoblasts, leading to risks in bone fracture. Phospholipids are reported to be upregulated in plasm of osteoporosis mouse model. Two common phospholipids, lysophosphatidylcholine and phosphatidylcholine, indicate oxidative stress and may involves in the pathogenesis of osteoporosis [178]. Dysregulation of sphingolipid metabolism is important in pathology of osteoporosis as well. The sphingomyelin is found to be downregulated in osteoporosis while glycosphingolipids are significantly upregulated [178]. Oxidized lipids, as a product of oxidative stress, inhibit the differentiation of mesenchymal stem cells to osteocytes and impair the balance of osteoclasts and osteoblasts by activating the PPARγ [179] and cAMP-PKA signal pathway [180]. Cholesterol has the similar effects as oxidized lipids and leads to inhibited bone formation and risk of osteoporosis [181]. Additionally, mitochondrial metabolism and redox metabolism are involved in the pathology of osteoporosis as well. Some osteocyte specific knockdown studies suggest the crucial role of normal mitochondrial function in bone health. The knockdown of superoxide dismutase leads to premature osteoporosis and knockdown of mitochondrial transcription factor A leads to bone resorption [182].

Metabolomic studies further illustrates the role of metabolism in osteoporosis. A series of metabolites are identified to be associated with bone mineral density in patients with osteoporosis. Prolyl-hydroxyproline excretion in urine is specifically related with post-menopausal osteoporosis [183]. Lysine, a common amino acid, is reported to be inversely correlated with bone mineral density and may serve as a predictor of osteoporosis [184]. Valine is in positive relation with bone mineral density and reported to regulate mesenchymal stem cells in proliferation and gene expression [185]. Lipids, including sphingolipids, phospholipids and fatty acids, are altered in osteoporotic patients, suggesting the dysregulated lipid metabolism [186]. This study also identified hyocholic acids as potential markers for osteoporosis which may serve as a diagnostic strategy.

Altogether, common age-related diseases including cardiovascular diseases, neurodegenerative diseases, diabetes, and osteoporosis are tightly inked with metabolism. Lipids, amino acids, carbohydrates, and redox metabolism along with mitochondrial function are all involved in the pathogenesis of these common age-related diseases. These discoveries may lead to further development in strategies for diagnosis and therapeutics.

Metabolic interventions for aging and age-related diseases

Given the regulatory role of metabolism in senescence, aging, and age-related diseases, potential metabolic intervention methods for these conditions have been thoroughly investigated (Fig. 3). The pursuit of preventing aging and age-related diseases through metabolic modulation offers a novel avenue for both biological and clinical research. Over the past decades, a range of metabolic strategies have been reported to modulate aging to some extent.

Key metabolites and metabolism-related signaling pathways regulate aging

Direct intervention with endogenous metabolites also promotes lifespan extension and healthy aging. NAD^+ is a prime example of metabolic intervention for aging. In model animals and even humans, tissue NAD^+ levels are reported to decrease with age [187]. The loss of NAD^+ impairs nuclear-mitochondrial communication, leading to the loss of mitochondrial OXPHOS subunits, whereas

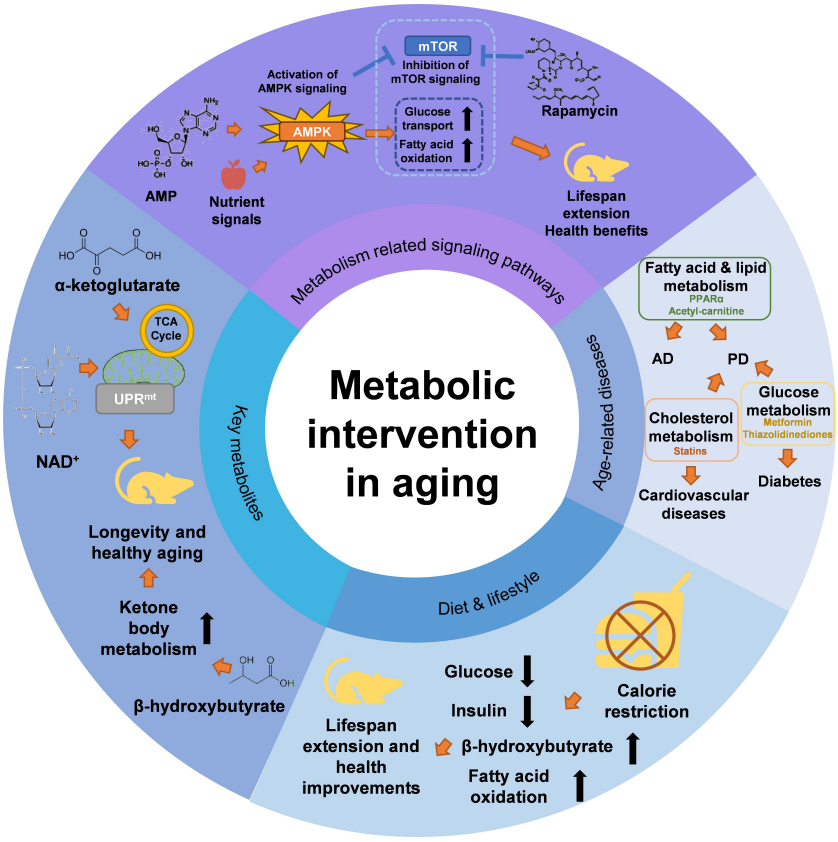

Fig. 3 The diagrams illustrate the role of metabolism and metabolic interventions in aging, focusing on metabolic regulation and metabolic interventions, whether through key metabolites or regulating metabolism-related signaling pathways, have been shown to promote lifespan extension and healthy aging.

NAD^+ supplementation restores mitochondrial function [188]. NAD^+ also promotes longevity through FOXO signaling and activation of the mitochondrial UPR [87]. Nicotinamide mononucleotide (NMN), a precursor of NAD^+, has been shown to treat age-induced diabetes, restore lipid profiles, and enhance glucose tolerance through SIRT1 activation [189]. Other metabolites, such as α-ketoglutarate and β-hydroxybutyrate, involved in the tricarboxylic acid (TCA) cycle and ketone body metabolism, respectively, promote healthy aging and alleviate age-related functional decline [89,90]. Recently, the effect of hypotaurine in lifespan extension has been reported, wherein hypotaurine supplementation extends lifespan through FOXO and NRF2 signaling in *C. elegans*, a classical model for aging research [190].

The AMPK signaling pathway, sensitive to intracellular AMP levels, functions as a nutrient sensing pathway and plays a pivotal role in balancing energy metabolism across multiple tissues [191]. AMPK activation in muscle enhances glucose transport and fatty acid oxidation, while in the hypothalamus, it promotes food intake. Consequently, AMPK activation boosts energy production in the body and is intricately linked to metabolic regulation. Studies on the AMPK pathway suggest that its activation promotes lifespan extension and health benefits. For instance, the application of AICAR, an AMPK agonist, enhances the running endurance of sedentary mice [192]. Metformin, a drug used for diabetes treatment, activates AMPK and extends lifespan in mice [193]. Studies also reveal the relationship between dysfunctional AMPK signaling and cardiovascular disorders [194]. These studies establish a causal link between AMPK activation and health benefits, though the underlying mechanisms remain to be fully elucidated. Future research may focus on elucidating the regulatory role of AMPK in metabolic regulation of aging using advanced metabolomic techniques.

The mTOR pathway, a conserved signaling cascade across multiple organisms, is closely associated with energy sensing and plays a crucial role in glucose and energy metabolism [195,196]. Abnormal activation of the mTOR pathway is implicated in aging, lifespan, AD, PD, diabetes, and other age-related diseases. Inhibition of the mTOR pathway has been shown to extend lifespan in nematodes, yeasts, fruit flies, and even vertebrates [195]. Moreover, inhibiting S6K, a downstream target of mTOR, also extends the lifespan of fruit flies and nematodes [195]. These findings underscore the

regulatory role of the mTOR pathway in aging and suggest potential intervention strategies involving manipulation of mTOR signaling. Indeed, rapamycin, which inhibits mTOR signaling, significantly promotes median and maximal lifespan extension in mice [197].

Metabolic effect and aging intervention of diet and lifestyle

Diet and lifestyle are also significant regulatory factors for aging and age-related diseases, as metabolism is closely tied to both. Caloric restriction (CR) has been reported to ameliorate or even delay age-related changes and extend lifespan [198]. CR also alleviates age-related impairments in hepatic function and adipose accumulation [199]. Considered a valuable research model for aging and age-related diseases, CR yields modulators of lifespan and aging. CR exerts a broad range of effects on the transcriptome, proteome, and metabolome [198] and reduces glucose and insulin levels along with multiple hormones, resembling a younger physiologic state. Animals under CR exhibit reduced reliance on carbohydrates and increased fatty acid oxidation [200]. These metabolic changes are associated with the beneficial effects of CR, such as reduced inflammation [198]. The underlying metabolic mechanism is likely related to the elevated β-hydroxybutyrate levels resulting from CR [201], which inhibits the inflammasome through reduction of apoptosis-associated speck-like protein containing a CARD (ASC) oligomerization and regulation of K^+ efflux.

Potential metabolic targets for therapy of age-related diseases

Given the strong connection between metabolism and age-related diseases, therapeutic strategies involving metabolic intervention have garnered significant scientific interest. As previously discussed, fatty acids and lipid metabolism play a pivotal role in AD pathology, making them promising targets for AD intervention. PPARα, a regulator of key enzymes in the fatty acid oxidation pathway, is primarily expressed in astrocytes in the brain [202]. Moreover, PPARα in neurons has been reported to regulate memory and learning [203]. Recent research has highlighted the potential of PPARα agonists in ameliorating memory deficits and related pathologies in AD mice [204]. Acetyl-carnitine, a derivative of the amino acid carnitine, serves as a precursor for Acetyl-CoA, fueling the TCA cycle and playing a crucial role in transporting fatty acids into mitochondria. It has been shown to prevent Aβ-induced changes in mitochondrial morphology and function in cultured neurons [205].

Cholesterol has also been implicated in PD pathology, including its interaction with α-synuclein, disruption of dopamine transport, and regulation of lipid rafts [206]. Statins, drugs designed to control cholesterol levels, are clinically used to prevent vascular incidents and have demonstrated therapeutic effects in reducing PD risks, including anti-inflammatory properties, amelioration of oxidative stress, and elevation of nitric oxide bioavailability [206]. Additional studies suggest statins may prevent motor deterioration [207] and reduce PD incidence [208]. However, further research is needed to elucidate the detailed mechanism and potential for clinical application. Statins and other lipid-lowering therapies are also utilized in the prevention of cardiovascular diseases [209], lowering the risk of cardiovascular incidents by either reducing cholesterol levels or targeting hepatic uptake of LDL-C. Other studies focusing on lipoproteins and lipid metabolism have revealed therapeutic targets for cardiovascular diseases as well, with lipoprotein(a) emerging as a target for aortic valve stenosis and atherosclerotic cardiovascular disease therapy [210]. Further exploration is necessary to expand these strategies through other metabolic pathways or targets and to dissect the underlying mechanisms.

Glucose metabolism is closely linked to diabetes and represents a therapeutic target for diabetes treatment. Targeting glucose metabolism has also been reported to improve symptoms of PD. Some antidiabetic drugs, such as GLP-1 receptor agonists, have shown promise in reducing the incidence of PD [211] and alleviating PD symptoms [212] by improving mitochondrial function [213]. Metformin, a long-used antidiabetic drug, has demonstrated efficacy in PD therapy beyond its diabetic effects [214,215]. Thiazolidinediones increase insulin sensitivity and regulate glucose metabolism, stabilizing glucose levels and controlling diabetes [212]. They also reduce the risk of developing PD in diabetic patients [216].

Collectively, metabolic intervention strategies represent alternative approaches in aging research, opening up new therapeutic possibilities for aging and age-related diseases. Specifically, intervention with endogenous metabolites offers a safer avenue for potential clinical applications.

Advanced metabolomics techniques in aging research

Advancements in analytical techniques have been pivotal in elucidating the metabolic mechanisms underlying aging and age-related diseases. Recent breakthroughs in metabolomics, particularly multiscale metabolomic profiling techniques, have significantly propelled the study of metabolism during aging (Table 2).

Table 2 Comparison of common advances in metabolomic techniques

Techniques	Advantages	Drawbacks	Potential applications
Single-cell metabolimics			
SCMS	*In situ* analysis; real time monitoring; compatible with other techniques including live-cell imaging and patch-clamp recording; capable of capturing multi-modal data when combined with other techniques	Low throughput: cells are sampled one-by-one by hand; destructive analysis: cells are no longer viable for further analysis; rather complex, especially when combined with other techniques	Single-cell combined analysis of metabolome with other cellular features, including electrical activity, cellular oxidative stress, and cellular senescence; multi-modal data mining
scCE-MS	*In situ* analysis; real time monitoring; non-destructive analysis; suitable for time series analysis of the same single cell; capturing big data	Rather low throughput; delicate equipment and experimental settings	Single-cell analysis of metabolome in live cells, especially suitable for sequent analysis of cellular senescence
MALDI-MSI	High throughput; automatic sampling and analysis; high spatial resolution; better detection and analysis of lipids; capturing big data	Expensive; time-consuming; destructive analysis, cells are no longer available for further analysis	High throughput analysis of single-cell metabolomics and lipidomics in cellular senescence; omic data mining
SIMS-MSI	High throughput; automatic sampling and analysis; ultra-high spatial resolution; analysis of sub-structures in single cells	Expensive; time-consuming; destructive analysis; complex equipment and techniques	High through analysis of metabolic changes and heterogeneity of structures inside cells during cellular senescence
MIMS-MSI	High throughput; ultra-high spatial resolution; lineage tracing of cells and subcellular structures with isotopes	Expensive; time-consuming; destructive analysis; complex equipment and techniques	High throughput analysis of metabolomic heterogeneity in cells and subcellular structures; tracing specific changes in subcellular structures during cellular senescence
Single-organelle metabolomics			
SLMS	*In situ* analysis; real time monitoring; capable of combining with other techniques including fluorescent imaging and patch-clamp recording; combined analysis of metabolism and lysosome function	Low throughput; very delicate experiment setup; difficult to master, needs systemic training before performing experiments	Dissecting metabolomic mechanism at single-organelle level in cellular senescence with big data mining
Raman microscopy	Real-time, live cell analysis	Detecting only lipids, the information of other metabolites is omitted	Lipidomic profiling of live cells in aging and senescence
Spatial/tissue metabolomics			
DESI	*In situ* analysis; fast analysis; non-destructive analysis; tissue sections are available for further analysis such as HE staining	Rather low spatial resolution; limited detection of metabolites; insensitive to non-polar metabolites	Analysis of metabolomic and distributional changes in a variety of tissues in aging and age-related diseases
MALDI	*In situ* analysis; high throughput; automatic sampling and analysis; high spatial resolution; capturing big data	Expensive; time-consuming; destructive analysis, tissues have to be treated before further analysis	Analysis of metabolomic changes in organs and tissues in aging and age-related diseases; big data mining
MOSR	Obtain high resolution images in a short time from low resolution techniques (such as DESI) with deep learning and transfer learning, allowing the combination of high spatial resolution and fast analysis. The tissues are also available for further analysis	Relies on training data set on the model training phase; building the model is rather time-consuming	Fast acquisition of high resolution MSI images for analysis, especially suitable for clinical samples that are difficult to acquire. The technique helps to yield high resolution MSI images and the samples are available for further analysis

Table 2 summarizes the advantages and drawbacks of metabolomic techniques and their potential application in study of aging and age-related diseases.

Single-organelle metabolomics

Organelles, as functional compartments within cells, are fundamental to cellular processes. Their heterogeneity, stemming from functional and spatial compartmentalization within the intracellular space, makes understanding their metabolic features at the single-organelle level crucial. Numerous studies have highlighted the crucial role of lysosomes in senescence and aging. However, due to technical limitations, the metabolomic signature of individual lysosomes in aging remained elusive for decades. Recently, a novel technique called SLMS, which combines nanoESI-MS and lysosomal patch-clamp, has been developed to profile the metabolomic features of single lysosomes in aging [79]. This technique enables the quantification of metabolites within lysosomes, leading to the discovery of metabolomic changes in aging lysosomes. The study reveals metabolic heterogeneity

among lysosomes during aging and identifies potential aging biomarkers. SLMS has identified five distinct subtypes of lysosomes with specific metabolomic signatures, highlighting the highly heterogeneous metabolic profile and the crucial role of organelle heterogeneity in aging. Another study utilizes micro-Raman assay to enable the analysis of lipidomics in single organelles [217]. This technique employs Raman microscopy and successfully quantifies cholesterol and sphingolipid levels, as well as cis/trans isomer ratios. The advantage of this method lies in its ability to perform real-time analysis on live cells without perturbing their natural state.

Single-cell metabolomics

Single-cell metabolomics, another emerging technique, captures metabolomic features at the single-cell level. Understanding metabolic changes at this level is essential for comprehending cellular senescence. Similar to SLMS, single cell mass spectrometry (SCMS), which combines single-cell patch-clamp, has been developed for metabolomic profiling at the single-cell level [218]. This technique can simultaneously capture *in situ* electrophysiological activity and metabolomic features. Metabolic profiling using SCMS has identified over 2000 metabolites in single cells. SCMS has confirmed the glutamine-glutamate-GABA cycle in single neurons using ^{13}C tracing and quantified common metabolites in various brain regions. This technique is well-suited for metabolomic research in aging, particularly brain aging, where functional deterioration can be captured through simultaneous electrical recording.

Other single-cell metabolomic techniques, such as capillary electrophoresis mass spectrometry (CE-MS), enable sequential sampling and analysis of single cells without compromising cellular viability [219]. This technique is applicable to studies of cellular senescence models, such as replicative senescence, where metabolomic features can be sequentially acquired during passages. Matrix-assisted laser desorption ionization mass spectrometry imaging (MALDI-MSI) has been used to profile single fibroblasts with distinct lipid signatures, leading to different differentiation fates [220]. MALDI-MSI offers high spatial resolution, enabling single-cell level analysis. Compared to other techniques, MALDI-MSI is highly throughput, allowing the profiling of a large number of single cells mounted on glass slides. Secondary ion mass spectrometry mass imaging (SIMS-MSI) boasts an ultimate high spatial resolution, capable of analyzing subcellular or even molecular structures. Using this technique, the heterogeneity of membrane lipid distribution in membrane fusion events has been uncovered [221]. Another study employing SIMS-MSI reveals the interactions between cholesterol and sphingolipid in membrane structures [222]. These high spatial resolution techniques are excellent for studying metabolic dynamics and molecular bases in aging.

Multi-isotope imaging mass spectrometry (MIMS), a modification of SIMS-MSI that introduces isotope tracing and detection, reveals the age mosaic phenotype of cells in aged tissues [223]. Mice were feed with isotope labeled foods and the isotopes were incorporated into a variety of metabolic pathways, resulting in a traceable marker in cells and subcellular components. Cells of different types exhibit varying "ages" due to heterogeneity in cell renewal. With its ultimate high spatial resolution, MIMS uncovers the chimera of subcellular structure components, including primary cilia. This study offers a comprehensive view of cellular heterogeneity in aging.

Spatial metabolomics

Spatial metabolomics enables *in situ* analysis of metabolites, depicting their abundance and distribution in tissue sections. These techniques are crucial in the study of aging and age-related diseases.

With modifications to desorption electrospray ionization mass imaging (DESI-MSI), nonpolar metabolites in wild-type and AD brain sections have been profiled, revealing alterations in cholesterol, spermidine, and putrescine in AD pathology [224]. DESI-MSI is a rapid analysis technique with a simple setup that can be easily integrated with other techniques. For example, the combination of compact postphotoionization and DESI-MSI establishes the DESI/PI technique, enabling the detection and visualization of nonpolar metabolites such as creatine, cholesterol, phosphatidylethanolamine (PE), HexCer, and GalCer lipids [225]. Another advantage of DESI-MSI is its non-destructive nature, preserving tissue section morphology and molecular features, allowing combined pathological analysis like HE staining and immunohistochemistry to simultaneously capture metabolic and pathological features.

SIMS-MSI, with its high spatial resolution, allows imaging of subcellular components. Time-of-flight SIMS has been employed in AD studies, revealing the interaction between Aβ plaques and liposomes, along with sulfatides and cholesterol [226]. This study provides insights into lipid-protein interactions in AD pathology. In another study using SIMS-MSI, cognitive deficits in cocaine-induced brain pathology were linked to changes in the lipid profile, including alterations in phosphatidylcholine (PC), PE, and phosphatidylinositol [227]. This study indicates a causal relationship between cognitive function and lipid metabolism, offering potential possibilities for metabolic intervention in age-related dementia and neurodegenerative diseases.

MALDI-MSI is another technique widely used in aging research. For instance, a study demonstrates the

interaction between gangliosides and Aβ plaques [228], as well as the perturbation of ganglioside metabolism in AD pathology. Another study discovered the downregulation of glycerophosphoinositol and sulfoglycosphingolipid in brain regions related to cognitive functions in an AD mouse model [229]. Thanks to the ultra-high spatial resolution of MALDI-MSI, the lipid profile of human AD brain samples at the single plaque level has been demonstrated [230]. The study revealed alterations in ceramide-1-phosphates (CerP), ceramide monohexosides (HexCer), ceramide phosphoethanolamine conjugates (PE-Cer), monosialo-gangliosides (GM), sulfatides (ST), along with phosphatidic acid (PA), phosphatidylethanolamines (PE), and phosphatidylinositols (PI). Furthermore, the localization of AA-containing PI and PE was also discovered.

Furthermore, advances in data processing algorithms have also facilitated metabolomic studies in aging and age-related diseases. A new algorithm called MSI from optical super-resolution (MOSR) enhances imaging resolution [231]. MOSR enables rapid imaging of high-resolution MSI images, crucial for metabolomic profiling. High-resolution imaging allows detailed analysis of ultrastructures and provides higher-quality data for further data mining. Another recent study has developed microscopy-directed imaging mass spectrometry, enabling rapid imaging of glomeruli tissue [232]. This technique also facilitates the clustering and identification of healthy and diseased glomeruli based on metabolic profiles within the tissue.

However, there are certain limitations to multi-scale metabolomic analysis in the field of gerontology. First, annotating and identifying metabolites poses a significant challenge. MS-based techniques generate a large volume of ion signals in a single analysis, but most of these signals are difficult to identify. A common approach to annotating m/z values involves searching observed values in metabolomic databases and comparing them with theoretical values. An annotation is then assigned to the m/z value within a certain error range (i.e., 15 ppm). However, this method is unable to distinguish between isoforms. While MS/MS analysis can distinguish some isoforms, it is ineffective for metabolites with very similar structures. This challenge restricts the application of metabolomic analysis in gerontology, as a significant number of metabolites remain unannotated. Secondly, analyzing metabolomic data are another challenge. Metabolomic profiling produces big data that is often complex and high-dimensional. In some cases, noise and background signals may be mixed in with the acquired data. Resolving the essence of the metabolome requires the development of sophisticated algorithms and data analysis techniques. Although there has been progress in data analysis and mining, which has aided in the discovery of secrets in metabolism, more remains to be explored with advanced techniques in data science. Finally, samples for metabolomic studies in gerontology are rare. While cellular and animal models of disease and aging help to dissect underlying mechanisms, differences exist when it comes to humans. Human samples are difficult to acquire due to ethical and clinical considerations, which restricts the application of metabolomic research in gerontology. However, technological advancements have been rapid in recent years. Despite these limitations, we remain confident that metabolomic research will propel the advancement of gerontology with further studies and developments.

In summary, advances in novel techniques have significantly advanced the study of metabolomes in aging and age-related diseases (Fig. 4). Further investigations are certain to provide deeper insights into the metabolic secrets of aging.

Conclusions

Senescence, a complex and heterogeneous biological process, encompasses numerous mechanisms. The accumulation of senescent cells represents a pivotal characteristic of aging, ultimately leading to tissue dysfunction and the development of age-related diseases. Metabolism, as a direct manifestation of cellular function and biological activity, plays a crucial role in regulating senescence, aging, and associated pathologies. The application of metabolomic profiling has granted us novel insights into the intricate metabolic alterations that occur during aging and age-related diseases, thereby facilitating the discovery of underlying mechanisms.

Recent advancements in multiscale metabolomics have further deepened our comprehension of the role of metabolism in aging, offering potential interventional strategies. Pivotal metabolites have been identified and demonstrated to modify the aging process, extending both lifespan and healthspan in diverse aging models. As such, future research focusing on metabolism in aging holds promising potential for delivering significant health benefits.

The future of applying metabolism and metabolomics research in clinical practice looks promising. So far, a series of metabolomic studies have focused on age-related diseases, revealing potential targets for metabolic intervention. Clinical studies have already confirmed the effectiveness of metabolic and metabolism-related intervention strategies on age-related diseases such as cardiovascular disease and PD. Metabolomic studies of these diseases also provide numerous potential biomarkers for diagnosis, which can aid in the development of diagnostic strategies. However, the direct application of these discoveries still requires further clinical studies to confirm the effectiveness and safety of these strategies in large populations or cohorts.

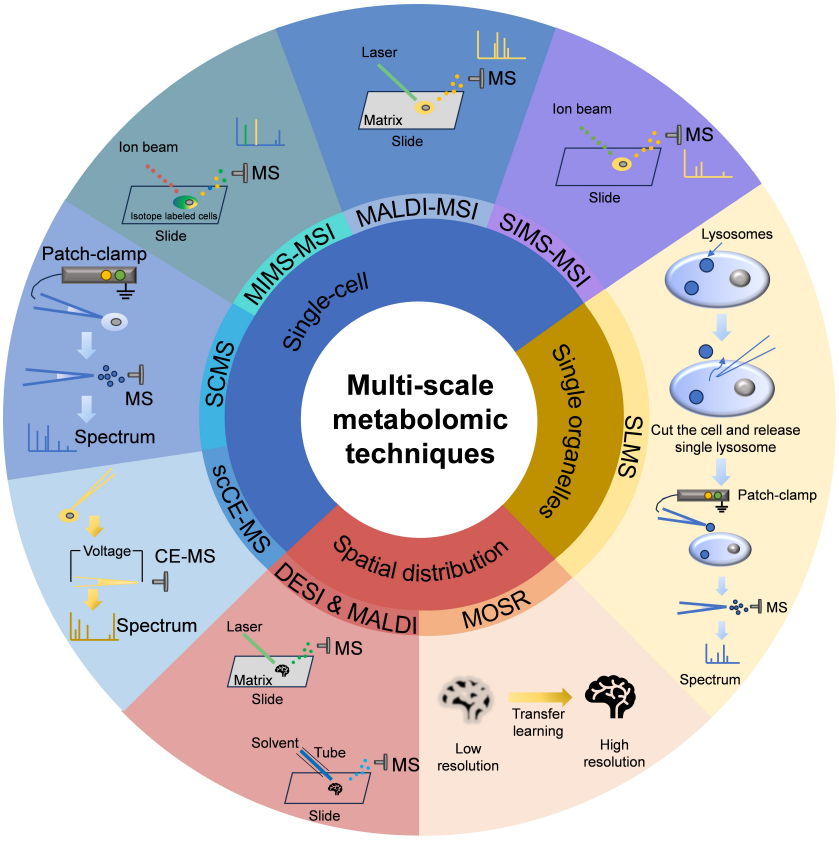

Fig. 4 New advances in multi-scale metabolomic techniques and algorithms involving single-organelle, single-cell, and spatial metabolomics. CE-MS, capillary electrophoresis mass spectrometry; DESI, desorption electrospray ionization; MALDI, matrix-assisted laser desorption/ionization; MALDI-MSI, matrix-assisted laser desorption/ionization mass imaging; MIMS-MSI, multi-isotope imaging mass spectrometry mass imaging; MOSR, MSI from optical super-resolution; MS, mass spectrometer; scCE-MS, single-cell capillary electrophoresis mass spectrometry; SCMS, single-cell mass spectrometry; SIMS-MSI, secondary ion mass spectrometry mass imaging; SLMS, single-lysosome mass spectrometry.

Nonetheless, we believe that metabolism and metabolomic research hold promise for advancing the diagnosis and therapy of age-related diseases with further studies and the development of new techniques.

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Compliance with ethics guidelines

Conflicts of interest Ziyi Wang, Hongying Zhu, and Wei Xiong declare no conflicts of interest.

This article is a review article and does not involve a research protocol requiring approval by a relevant institutional review board or ethics committee.

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