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Mitochondrial and metabolic dysfunction in ageing and age-related diseases

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Abstract

Organismal ageing is accompanied by progressive loss of cellular function and systemic deterioration of multiple tissues, leading to impaired function and increased vulnerability to death. Mitochondria have become recognized not merely as being energy suppliers but also as having an essential role in the development of diseases associated with ageing, such as neurodegenerative and cardiovascular diseases. A growing body of evidence suggests that ageing and age-related diseases are tightly related to an energy supply and demand imbalance, which might be alleviated by a variety of interventions, including physical activity and calorie restriction, as well as naturally occurring molecules targeting conserved longevity pathways. Here, we review key historical advances and progress from the past few years in our understanding of the role of mitochondria in ageing and age-related metabolic diseases. We also highlight emerging scientific innovations using mitochondria-targeted therapeutic approaches.

Ageing is characterized by a gradual loss of normal physiological function, culminating in frailty, a lack of resilience and increased susceptibility to several diseases, including

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Competing interests

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cancer, as well as neurodegenerative, cardiovascular and metabolic diseases[1], culminating in death[2,3].

The increase in life expectancy observed since the beginning of the 20th century has been so rapid that the possibility that ageing is simply caused by genetic factors can be ruled out[4]. Moreover, individuals who survive to particularly old ages (>90 years of age), who often do not develop health problems, are predominantly in the so-called ‘blue zones’ of the world (for example, Ikaria in Greece and Okinawa in Japan)[5]. Remarkably, these populations are not genetically different from their neighbours, but their environment and lifestyles, including social networks, might have a role in their ability to stay healthy for longer than people in other areas[5]. Additional factors, such as diet, education, consistent physical activity[6], conditions during early life and parental health[7], have considerable effects on mortality. Although people from most nations are living longer than previously, their health span is not increasing[8]. Thus, preventing late-life morbidities should be an urgent goal in the field of gerontology.

Nine hallmarks of mammalian ageing have been described and are now generally accepted[2] (BOX 1). Notably, cellular metabolism interconnects these hallmarks of ageing[2]. Here, we review the role of mitochondria in metabolism and how mitochondria contribute to age-related metabolic diseases, which might guide us in designing therapies targeting metabolic disorders to extend human health span and lifespan.

Mitochondria

Mitochondria are essential for life, as they have a pivotal role in most fundamental energy transformation processes within a cell. Mitochondrial dysfunction can lead to severe impairment of cellular energy conversion, especially in tissues that rely heavily on chemical energy generated by mitochondria throughout life[9]. Hence, regulation of energy metabolism by environmental variations and excessive levels of the by-product reactive oxygen species (ROS) might be associated with age-related metabolic diseases through constitutive changes in mitochondrial oxidative phosphorylation (OXPHOS), that ultimately lead to mitochondrial dysfunction.

Oxidative phosphorylation

(OXPHOS). A metabolic pathway in which nutrients are oxidized, releasing energy in the form of ATP resulting from electron transfer from NADH and $FADH_2$ to O_2.

Crosstalk with longevity pathways

In the past few years, advances in ageing research have indicated that interventions to treat diseases affecting multiple organs could be developed, thus increasing health span and delaying the onset of later-life morbidities. The effects of these novel interventions can be, to some extent, interpreted within the realm of mitochondrial physiology. Ageing and age-related diseases are tightly related to an imbalance in energy supply and demand. This imbalance might be alleviated by various interventions, including physical activity, calorie restriction and agents such as metformin, resveratrol and rapamycin[10].

Although the mechanisms behind calorie restriction-driven longevity extension are still poorly understood, mitochondrial physiology is involved in the beneficial effects of calorie restriction in budding yeast[11] and *Caenorhabditis elegans*[12], as well as in mammals[13]. When carbohydrates and, consequently, calories are in excess, OXPHOS and antioxidant defences are downregulated, and the electron transport chain (ETC) remains in a chronically reduced state. This environment favours ROS production, mitochondrial DNA (mtDNA) mutagenesis and, consequently, premature cell death. By reducing the excess of ingested calories, calorie restriction interventions move the ETC away from a reduced state, lower ROS production, induce OXPHOS and antioxidant defences, prevent age-related diseases and increase lifespan[14].

Electron transport chain

(ETC). A series of electron transporters (complexes I–IV) embedded in the inner mitochondrial membrane that transport electrons from NADH and $FADH_2$ to O_2, in which O_2 is reduced to water. In parallel, a proton efflux is driven from the mitochondrial matrix towards the intermembrane space via proton pumps in complexes I, III and IV. The movement of protons back into the matrix occurs through ATP synthase (also known as complex V or FoF1 ATPase).

Evidence for reciprocal and multilevel interactions between longevity pathways, together with the intricate regulation of mitochondrial physiology under calorie restriction interventions[15], has opened the door to the hypothesis that mitochondria might be involved in the lifespan modulation of these pathways. Altogether, this system is multifactorial and highly dynamic, and controls the ageing process (FIG. 1).

IIS

Suppression of the insulin–insulin-like growth factor 1 (IGF1) signalling (IIS) pathway dramatically extends lifespan in multiple organisms, which suggests that it is an evolutionarily conserved mechanism[16].

Extensive overlap in gene expression has been found between the *C. elegans* insulin receptor DAF-2 mutant and long-lived mitochondrial mutant strains[17], with the DAF-2 mutant showing an overall decrease in mitochondrial protein turnover[18]. Moreover, DAF-2 mutants have increased abundance of proteins involved in OXPHOS, increased respiratory capacity and increased membrane potential[19]. The detected increase in respiratory capacity in DAF-2 mutants, contrary to what was observed in adult wild-type N2 worms, is in agreement with findings showing increased mitochondrial ROS production[19]. Importantly, this increase in ROS production was observed without any detectable adverse effect in mtDNA integrity and oxidative protein damage[19]. In addition, increased ROS levels induced expression of antioxidant enzymes, followed by a decrease in ROS levels[20]. Thus, these results suggest that inhibition of IIS improves mitochondrial bioenergetics together with a ROS-mediated adaptive response, contributing to the lifespan extension effects observed in *C. elegans*.

Mitochondrial morphology and dynamics, which undergo several changes in the neurons of *C. elegans* during ageing[21], are also affected by IIS. In DAF-2 mutants, age-related changes

in mitochondrial morphology and density are mitigated, and mitochondrial trafficking is maintained at under normal levels in adulthood[21]. Moreover, mitophagy is enhanced in DAF-2 mutants[22], and exposure to mitophagy modulators increases lifespan in worms[23,24]. Manipulation of a gene involved in mitophagy, *dct-1*, a nematode orthologue of the mammalian receptors NIX and BNIP3L[22], also shortens lifespan in DAF-2 mutants[22].

Mitophagy

A process that selectively degrades damaged mitochondria following damage or stress.

Work in mammals has also shown an important connection between IIS and mitochondrial physiology. In the long-lived Ames dwarf mice and growth hormone receptor knockout (GHR-KO) mice, which develop defects in the GH–IGF1 signalling pathway, mitochondrial bioenergetics is markedly improved. This improvement was demonstrated by the metabolic shift to β-oxidation, increased oxygen consumption rate and decreased respiratory quotient[25], accompanied by increased expression of peroxisome proliferator-activated receptor-γ coactivator 1α (PGC1α) as well as proteins involved in OXPHOS[26]. Likewise, fat-specific insulin receptor knockout (FIRKO) mice have increased lifespan compared with wild-type mice despite having a leaner phenotype from a young age (3 months of age) and normal food intake throughout life[27]. Expression of genes with protein products involved in key metabolic processes, such as glycolysis, the TCA cycle, β-oxidation and OXPHOS were increased in 2.5–3.0-year-old FIRKO mice compared with age-matched control mice[28]. Interestingly, the same phenotype was not observed when the insulin receptor was disrupted in liver, adipose and muscle tissues in adult mice[29], denoting the complex role of the mammalian IIS pathway.

β-Oxidation

A metabolic process involving multiple steps of fatty acid molecule breakdown to produce energy.

Respiratory quotient

The ratio used to measure basal metabolic rate by estimating the volume of carbon dioxide released in relation to the volume of oxygen produced during respiration.

TCA cycle

A series of chemical reactions taking place in the mitochondrial matrix that uses acetyl-CoA derived from carbohydrates, fat and proteins to provide electrons to the electron transport chain.

mTOR

Mammalian TOR (mTOR) is a ubiquitously expressed serine–threonine protein kinase, belonging to the PI3K family, that has a vital role in regulating cytoplasmic nutritional and

growth factor status, as well as cellular energy levels and overall cellular function[30]. mTOR is present in two complexes, mTORC1 (associated with ‘raptor’) and mTORC2 (associated with ‘rictor’), accounting for the diversity, specificity and selectivity of rapamycin-mediated inhibition of TOR signalling, which was discovered after it was shown that rapamycin is able to inhibit mTORC1 via binding to the protein FKBP12 (REFS[31,32]).

Despite the fact that a definite mechanism has not yet been elucidated, the inhibition of mTORC1 and consequent increase in autophagy[33] has gained some attention from the mitochondrial field. Decreased clearance of accumulating damaged organelles, such as mitochondria, has been highly associated with ageing and age-associated diseases[34] and might explain some of the beneficial effects that mTOR inhibition has on lifespan extension. Nevertheless, the mTORC1 complex is required for PGC1α binding to the transcription factor Ying Yang 1, which induces expression of nuclear-encoded mitochondrial genes[35]. Moreover, modulation of mTOR activity results in decreased mitochondrial bioenergetic capacity. Deletion of the mTORC1 component raptor in mouse skeletal muscle affects PGC1α expression, reducing oxidative capacity[36], and consequently inducing premature ageing.

In the context of mitochondrial diseases, inhibition of mTOR using rapamycin improves mitochondrial function and alleviates symptoms of Leigh syndrome[37], a disease caused by mtDNA deletions, in a mouse model of the syndrome. Additionally, a low dose of rapamycin extended lifespan in a mouse model of mtDNA depletion syndrome[38]. Therefore, mitochondria might be the critical mediator for the effects of mTOR inhibition on longevity, and might represent a notable therapeutic target in ageing and age-related diseases. However, additional research is necessary to understand the implications of mTOR inhibition in the context of both health and disease.

AMPK

AMPK is considered the primary sensor of cellular AMP to ATP and ADP to ATP ratios and a key regulator of cellular energy homeostasis and metabolism[39].

Due to the central role of AMPK in the regulation of cellular metabolism, AMPK activity is closely linked to mitochondrial physiology. Experiments in mice with a reduced ATP to AMP ratio in skeletal muscle showed an increase of mitochondrial biogenesis via AMPK induction of PGC1α expression[40]. Subsequent studies also elucidated this signalling pathway and indicated that activated AMPK phosphorylates PGC1α, which consequently induces mitochondrial gene expression[41]. Additionally, mitochondrial quality control (BOX 2) is also under the control of AMPK. AMPK is believed to regulate the autophagic turnover of dysfunctional mitochondria through initiation of mitophagy by phosphorylation and activation of the pro-mitophagic factor UNC51-like kinase 1 (ULK1)[42]. Under mitochondrial stress, fission is activated by AMPK-mediated phosphorylation of mitochondrial fission factor, facilitating the removal of damaged mitochondria[43]. Moreover, work on *C. elegans* showed that deficient mitochondrial dynamics are sufficient to block AMPK-mediated and calorie restriction-mediated increases in longevity[44], demonstrating the close relationship between mitochondrial dynamics and the role of AMPK in regulating

longevity. Therefore, decreased AMPK activity might be a contributor to the reduced mitochondrial function and dynamics observed during ageing[45].

Mitochondrial biogenesis

A process in which the mitochondrial mass of cells is increased.

Sirtuins

Another indicator of nutrient deficiency is a rise in the NAD^+ to NADH ratio. Sirtuins, a conserved family of deacetylase and deacylase proteins, require NAD^+ for their function. In addition, increased AMPK activity also correlates with conditions of nutrient depletion (that is, an increased NAD^+ to NADH ratio).

In response to calorie restriction, the yeast NAD biosynthetic gene that catalyses the first and rate-limiting step in NAD^+ biosynthesis, *PNC1*, is induced[46]. The resulting availability of NAD^+ increases SIR2 activity, which boosts mitochondrial respiration and suppresses recombinant DNA recombination[11,46], a known cause of ageing in yeast[47]. Interestingly, increased expression of mitochondrial biogenesis transcription driver HAP4 resulted in lifespan extension under normal caloric conditions in yeast (*Saccharomyces cerevisiae*), with calorie restriction failing to further extend this longevity benefit[11].

In rodents, the age-dependent decline in NAD^+ levels decreases SIRT1 activity, ultimately affecting mitochondrial homeostasis[48]. Moreover, SIRT1 regulates mitochondrial physiology by deacetylating and activating PGC1α and FOXO[49,50], allowing control over a broad range of mitochondrial physiology.

Mitochondrial sirtuins also control mitochondrial physiology[51]. Notably, SIRT3 regulates ATP levels in multiple tissues[52], as well as AMPK activation[53]. SIRT4 interacts with adenine nucleotide translocator, possibly regulating the ADP to ATP ratio and mitochondrial physiology[54]. Interestingly, SIRT3 has been suggested to be the mediator of some of the longevity benefits of calorie restriction through its targeting and deacetylation of mitochondrial proteins[55].

In summary, the regulation of mitochondrial physiology by pathways associated with longevity has relevelled the complex network of interactions between those pathways and mitochondrial function during ageing (FIG. 1). Failure to regulate energy homeostasis by these pathways is highly linked with mitochondrial dysfunction, which ultimately leads to age-related metabolic diseases.

Mitochondrial dysfunction in ageing

Different theories have been postulated to explain the evolutionary basis of ageing. The ‘antagonistic pleiotropy’ hypothesis provides a meaningful context for understanding the role of mitochondria in ageing.

Antagonistic pleiotropy

A theory that describes opposing effects, both harmful and beneficial, on an organism, in which a given gene or process controls more than one trait, which are maintained in the population due to a decline in the force of natural selection. At least one of these traits is beneficial to the organism's fitness early on in life, and at least one is detrimental to the organism's fitness later in life.

The antagonistic effects of mitochondria have long been appreciated in organismal ageing and has led many researchers to view the organelle as more than just an energy supplier, but also seeing mitochondria as having an essential role in ageing and age-related diseases. The accumulation of mitochondrial oxidative damage, which is the basis for Harman's 'free radical theory of ageing'[56], has been suggested as a driver of mitochondrial dysfunction and the observed age-related decline in organ function during ageing. However, while several studies have supported the free radical theory of ageing[57,58], others have shown that increased ROS production does not always shorten lifespan[59]. Research findings in multiple species question the role of oxidative stress on mtDNA mutagenesis[60]. These findings have helped guide the field to hypothesize that ROS production might not be the leading cause of the phenotypes correlated with ageing and that other factors might contribute.

mtDNA mutations

In 1972, Harman expanded the free radical theory of ageing with the 'mitochondrial theory of ageing', which included the possibility that mtDNA mutations might in fact be the drivers behind the mitochondrial dysfunction observed during ageing[61]. Inherited mtDNA and nuclear DNA determine the energetic capacity of an individual during the individual's lifetime[62]. The accumulation of somatic mtDNA mutations that occurs with ageing leads to a loss of mitochondrial function. The resultant decline in energy capacity, increase in oxidative damage and ultimately increase in apoptosis lead to cellular loss, which results in organ failure. Closely linked to mitochondrial ROS production, the number of mtDNA mutations is known to increase with age[63]. However, while some reports favour the idea that mitochondrial mutations contribute to ageing and age-related diseases[64], others have questioned this hypothesis due to the level of mutations observed[65].

Evidence for a contribution of mtDNA mutations to the mammalian ageing phenotypes has come from the mtDNA mutator mouse, which carries a mutated, proofreading-deficient form of the nuclear-encoded mtDNA polymerase (POLGγ). The mtDNA mutator mouse was shown to accumulate considerable levels of mtDNA mutations and deletions and to recapitulate several signs of premature ageing, such as reduced lifespan, reduced fertility, anaemia, sarcopenia, osteoporosis, hair greying, hair loss[66], hearing loss[67] and elevated lactate levels[68]. Moreover, it has been demonstrated that ageing phenotypes (such as spine curvature, reduced body weight and hair loss) and lifespan can be influenced by mtDNA mutation levels in mtDNA mutator mice[69] and surprisingly also in mice with maternally-inherited mtDNA mutations but an otherwise wild-type nuclear genome[70]. However, the role of mtDNA mutations as a primary driver of the ageing process is still debated, in part because the number of mutations observed in normal aged tissues is seemingly below the

threshold required to affect mitochondrial function[71]. That said, patients with HIV, who also present with phenotypes of premature ageing[72] and premature age-associated cardiovascular disease[73], have mitochondrial mutations and OXPHOS dysfunction[74], possibly caused by anti-retroviral drugs that inhibit POLGγ[75].

Genomic instability

Genomic instability in the nucleus can impact mitochondrial physiology during ageing via what is known as anterograde signalling[76]. Supporting this hypothesis, DNA repair-deficient premature ageing diseases, such as ataxia–telangiectasia (ATM), Cockayne syndrome group A and xeroderma pigmentosum group A, have been linked to changes in mitochondrial physiology, which are indicative of physiological responses to anterograde stimuli[77]. Moreover, most of the mitochondrial proteins are encoded by nuclear DNA, and mutations on those genes are typically associated with mitochondrial diseases[78].

Genomic instability induced by single-strand breaks or double-strand breaks triggering the DNA damage response (DDR) is a hallmark of ageing[2], and can arise from both exogenous and endogenous sources[79]. Fortunately, organisms have evolved a variety of nuclear DNA repair mechanisms to ensure that the alterations caused by agents in the nuclear genome are properly repaired[80]. Nuclear DNA repair is energy-demanding[80], and persistent DNA damage can lead to mitochondrial dysfunction and metabolic deficits[76].

In addition to its critical role in organismal health and mitochondrial physiology, NAD^+ is a rate-limiting metabolite for the poly(ADP-ribose) polymerases (PARPs)[81], which are key DDR proteins. When DNA breaks occur, PARP1 senses the breaks and initiates DNA repair signalling. Activated PARP1 will consume NAD^+, generating PAR through a process called PARylation[82].

An interplay between PARP1 and mitochondria might explain aspects of age-related functional decline. Indeed, deletion of the *Parp1* gene in mice increases NAD^+ levels, SIRT1 activity and mitochondrial biogenesis[83]. In nematodes, pharmacological inhibition of PARP1 increases mitochondrial biogenesis and extends lifespan[84]. Additionally, studies conducted in animal models and cells derived from people affected by xeroderma pigmentosum and Cockayne syndrome found decreased SIRT1 activity and persistent PARP activation due to unrepaired DNA damage. Pharmacological NAD^+ supplementation or inhibition of PARP1 was able to re-establish SIRT1 activity and recover mitochondrial homeostasis[85,86].

Additional evidence for the link between PARP1 and mitochondrial physiology has come from work done in ATM, which is caused by a defect in the *ATM* gene, which encodes one of the DNA repair proteins recruited by PARP1 to the DNA break site. Analysis of an ATM mouse model showed dysfunctional mitochondria with increased ROS levels and the accumulation of damaged mitochondria[87]. Similarly, $Atm^{-/-}$ mice also have mitochondrial defects and interventions based on NAD^+ supplementation[88] attenuate their health decline.

Cellular senescence

Cellular senescence is characterized by an irreversible cell cycle arrest, changes in gene expression, tumour suppression activation, altered chromatin, resistance to apoptosis and increased protein synthesis[89]. The build-up of senescent cells is considered a driver of the ageing process[90]. No universal marker has been discovered for senescent cells but cell cycle arrest, DDR, β-galactosidase activity and the pro-inflammatory senescence-associated secretory phenotype are common features of cells undergoing senescence[90].

Senescent cells exhibit numerous changes to mitochondria, including their structure, dynamics and function. For example, defects in mitochondrial OXPHOS are observed in the early stages of cellular senescence[91–93]. Cellular senescence leads to decreased mitochondrial membrane potential, increased proton leakage and increased ROS production[94], thus enhancing DNA damage and DDR[95]. Moreover, cells undergoing senescence display altered mitochondrial metabolite homeostasis[96], as well as decreased fatty acid oxidation, leading to accumulation of lipids[97]. In addition to functional alterations, the mitochondria in senescent cells show dramatic changes in morphology and dynamics. During senescence, mitochondrial biogenesis is upregulated, leading to an unhealthy mitochondrial network. This network is crucial to cells meeting their metabolic demands through mitochondrial fission and fusion events[95,98]. Furthermore, pathogenic mtDNA has also been reported to be involved in cellular senescence[99]. Nevertheless, the contribution of mitochondria to the development of the senescent phenotype, as well as the underlying mechanism driving mitochondrial dysfunction in ageing and age-related diseases, require further elucidation, as this knowledge might contribute to the discovery of new targets for possible anti-ageing interventions.

Epigenetic alterations

Whether epigenetic changes in mtDNA are relevant to health has been debated for years, especially questions about the extent of mtDNA methylation and the relative percentages of the methylated (5-mC) cytosine content in mtDNA and nuclear DNA. Additionally, the absence of CpG islands and the organization of mtDNA into so-called nucleoids has contributed to the controversy. However, in 2011, Shock and colleagues showed that both 5-mC and hydroxymethylated (5-hmC) cytosine residues, along with DNA methyltransferase 1 (DNMT1), are present in mtDNA from human and mouse cell lines[100]. Both 5-mC and 5-hmC have been found in mtDNA, as well as DNMT3A and B DNA methyltransferases[101–103]. Interestingly, it has been hypothesized that transcription from the heavy-strand promoter, which is regulated by the mitochondrial transcription factor A (TFAM), could be modulated by epigenetic markers on mtDNA; methylation levels could influence where TFAM binds and affects the transcriptional response[104].

CpG islands

Regions with a high frequency of CpG sites, which correspond to a cytosine nucleotide followed by a guanine nucleotide, in the 5′ to 3′ direction.

Furthermore, mtDNA methylation is disturbed by environmental and other factors, such as traffic-derived elemental carbon[105], maternal smoking[106], endogenous metabolites[107], cell senescence[108] and ageing[109]. Human diseases, including cardiovascular diseases[110], colorectal cancer[111], non-alcoholic steatohepatitis[112], obesity-induced insulin resistance[113] and Down syndrome[114], have also been associated with changes in mtDNA methylation patterns.

In age-related diseases, such as neurodegenerative diseases, perturbations to the mtDNA epigenome have been linked to human diseases. For example, there is a decrease in the methylation of the mtDNA D-loop region in patients carrying the amyotrophic lateral sclerosis-linked superoxide dismutase 1 (*SOD1*) mutations[115]. Patients with Parkinson disease have decreased methylation of the D-loop[116] and patients with Alzheimer disease-related pathology have reduced levels of *mt-ND1* methylation[116]. Additional research is necessary to elucidate the functional role of epigenetic alterations in mtDNA in the context of ageing and age-related diseases.

D-loop region

A non-coding region of 1,124 base pairs, containing essential transcription and replication elements, that acts as a promoter for both heavy and light strands in mtDNA.

Age-related metabolic disorders

Maintenance of healthy mitochondria and metabolic regulation is key to long-term health during ageing. Due to the high responsiveness of mitochondria to environmental variations, it is now acknowledged that several factors might contribute significantly to mitochondrial and metabolic dysfunction during ageing, ultimately leading to age-related metabolic disease (FIG. 2).

Metabolic syndrome

The metabolic syndrome, which occurs increasingly with age, is associated with obesity, hyperglycaemia, dyslipidaemia, insulin resistance and cardiovascular diseases[117,118]. An excess in total body mass, especially visceral adipose tissue, is associated with increased morbidity[119] as well as metabolic inflexibility[120], a phenomenon caused by the inability to transition between fatty acid and glucose metabolism. Consequently, individuals with obesity tend to oxidize lipids instead of metabolizing glucose under insulin-stimulated conditions[121]. Indeed, an association between dyslipidaemia and oxidized LDL has been observed in individuals with prediabetes[122].

Prediabetes

A condition characterized by moderately elevated fasting or postprandial blood levels of glucose.

In one study, insulin resistance in older patients (mean age 70 ± 2 years) was associated with decreased mitochondrial ATP production and modifications in mitochondrial OXPHOS[123].

Changes in energy metabolism, especially in tissues dependent on insulin-stimulated glucose disposal[124], lead to mitochondrial deregulation and increased ROS generation. Consequently, an observed decrease in fatty acid oxidation and an increase in cytosolic levels of free fatty acids ultimately lead to pathologies such as type 2 diabetes mellitus (T2DM), muscle dysfunction and insulin resistance[125].

Interestingly, mtDNA polymorphisms have been associated with an increased risk of metabolic diseases. Analysis of both European[126] and Asian populations[127] showed that the 16189T>C substitution in the D-loop region of mtDNA is associated with obesity and T2DM. Additionally, mtDNA analysis showed that the haplogroup N9A is likely to be protective against the metabolic syndrome in Japanese women[128], as well as being protective against T2DM in Japanese and Korean populations[129]. Furthermore, in a white family, the T4291C mtDNA mutation has been associated with the metabolic syndrome, and the T16189C mtDNA variant has been reported to be associated with abnormalities in body mass, insulin resistance and increased cardiomyopathy, as well as increased risk of the metabolic syndrome[130]. In addition, the A8890G mtDNA mutation was found in a juvenile patient with the metabolic syndrome phenotype[131]. Apart from variations in mtDNA, patients with the metabolic syndrome show a reduced number of mtDNA copies in peripheral blood and adipose tissue[132], a physiological change typically observed before the development of T2DM[133].

Haplogroup

A genetic population comprising a group of people who share a common ancestor on the patriline or the matriline.

Type 2 diabetes mellitus

T2DM is a chronic disease characterized by dysregulated blood levels of glucose, ultimately impairing insulin action and insulin secretion. Mitochondrial dysfunction and the associated impairment in fuel oxidation are considered to be notable contributors to the development of insulin resistance, non-alcoholic fatty liver disease (NAFLD) and T2DM. Mitochondrial dynamics are crucial to T2DM and related vascular complications, given the central role of mitochondria in ATP production, insulin secretion and ROS formation[134]. Chronic elevation of glucose levels stimulates ROS production and induces oxidative stress, which causes tissue damage. Individuals with prediabetes and T2DM have an increased risk of presenting with hyperglycaemia associated with a progressive rise in oxidative stress and altered mitochondrial morphology[135]. Decreased mitochondrial oxidative capacity and mitochondrial content, reduced oxidative capacity, defective lipid metabolism and increased lipid peroxidation are also seen in skeletal muscle from patients with obesity and insulin resistance compared with healthy and lean individuals[136,137].

Data published in 2017 regarding ^{31}P-magnetic resonance spectroscopy from individuals without diabetes mellitus in the Baltimore Longitudinal Study of Aging (BLSA) showed that a higher impairment of mitochondrial capacity is associated with greater insulin resistance and a higher likelihood of prediabetes than in participants classified as being ‘normal’

according to the American Diabetes Association criteria using fasting plasma glucose levels and/or glucose levels 2 h after oral glucose tolerance testing[138]. Furthermore, decreased mitochondrial respiration was found in first-degree relatives of individuals with T2DM[139]. The association between mitochondrial dysfunction and T2DM is also supported by the downregulation of genes involved in oxidative metabolism and under the control of PGC1α in the skeletal muscle of patients with T2DM[140].

Insulin resistance and β-cells

β-cells respond to increased metabolic demands by increasing their mass and insulin synthesizing and secretory activity. Long-term and chronic exposure to hyperglycaemia can cause desensitization and even depletion of β-cells, making them unable to secrete insulin due to the depletion of intracellular reserves[141]. Both desensitization and depletion of β-cells are reversible states; however, if the hyperglycaemic situation persists, it can lead to the irreversible loss of insulin production and, ultimately, the death of β-cells[142].

Mitochondrial dysfunction is increasingly thought to be a central contributor to β-cell failure in the evolution of T2DM, with ROS production induced by metabolic stress as a key event leading to β-cell failure. In islets from patients with T2DM, the ATP content, and the increase in the ATP to ADP ratio stimulated by glucose, was lower than in islets from control individuals[143]. Impairment in β-cell metabolism observed in islets from donors with T2DM and in β-cells from a mouse model of human neonatal diabetes upon exposure to hyperglycaemia support the notion that inhibition of mitochondrial metabolism is central to β-cell dysfunction[143,144]. While there is no evidence for mitochondrial depletion in β-cells from patients with T2DM, the mitochondrial density volume is considerably higher in islets from patients with T2DM than in islets from those without T2DM, with both morphological and functional abnormalities that have not been observed in β-cells from control individuals[143]. In agreement with reduced insulin secretion, PGC1α expression was found to be reduced by 90% in islets from patients with T2DM. In addition, PGC1α silencing in healthy human islets resulted in reduced insulin secretion[145], a phenomenon also found in patients with a common mtDNA variant in mitochondrial transcription factor B1 (TFB1M)[146].

Additionally, analysis of insulin-secreting pancreatic β-cells and islets from patients with T2DM showed alterations in mitochondrial physiology, such as hyperpolarization of the mitochondrial membrane and decreased activities of the enzymes glycerol phosphate dehydrogenase, pyruvate carboxylase and succinyl-CoA–3-ketoacid-CoA transferase[147,148]. Moreover, increased expression of uncoupling protein 2 (UCP2), complex I and ATP synthase have been observed in islets from patients with T2DM[143]. Increased ROS levels due to hyperglycaemia activate UCP2, resulting in proton leak across the mitochondrial inner membrane, which leads to reduced ATP synthesis and oxidative damage to the membrane, cytochrome *c* release and the induction of apoptosis[149].

Proton leak

The dissipation of proton motive force (Δp), where protons migrate into the mitochondrial matrix without producing ATP.

Cytochrome *c*

A haem protein localized in the mitochondrial intermembrane space that functions as an electron transporter between complex III and complex IV of the electron transport chain.

Non-alcoholic fatty liver disease

NAFLD is the most common form of chronic liver disease[150]. Epidemiological studies have suggested that the presence of NAFLD might be a precursor to the development of other conditions, such as insulin resistance and T2DM[151].

Age-associated liver diseases, such as NAFLD, have been described as hepatic examples of the metabolic syndrome[152], where insulin resistance is also a key pathological feature. Patients with NAFLD present with decreased ETC complex activity[153,154] and a fatty liver-associated decrease in SIRT3 activity, which leads to hyperacetylation of mitochondrial proteins and mitochondrial dysfunction[155]. In white adipose tissue, the uncoupling of OXPHOS has been shown to decrease fatty acid synthesis[156], leading to increased energy expenditure and reduced lipogenesis, which provides an important energy source to cells through oxidation of fatty acids[157]. Lipid accumulation in hepatocytes has been linked to insulin resistance and the development of T2DM[158]. It is likely that the lack of oxidative capacity to overcome the excess fatty acids that are transported to the liver during conditions such as obesity might have a vital role in the progression of NAFLD accompanied by insulin resistance[159].

Studies of obesity in rats and a T2DM model known as the OLETF obese hyperphagic rat showed that reduced hepatic mitochondrial content and function precede the development of NAFLD and insulin resistance, supporting a crucial role for mitochondria in the pathophysiology of NAFLD[160]. Decreased ATP content, impaired mitochondrial bioenergetics and increased ROS generation have all been found in patients with non-alcoholic steatohepatitis, and in fatty liver models in the rat[161–163]. These biochemical changes are associated with ultrastructural abnormalities that indicate an impaired OXPHOS. Ultrastructural changes include the presence of enlarged mitochondria, loss of cristae organization and crystalline inclusion bodies in the matrix in patients with NAFLD[164]. Thus, uncoupling of OXPHOS and increased ROS generation, with oxidative damage to ETC complexes and mtDNA, further compromise mitochondrial function and perpetuate ROS formation, thus destabilizing the redox environment of the cell. Interestingly, in work performed in humans with NAFLD, a rapid elevation of liver levels of triglycerides was observed and is associated with stimulation of mitochondrial oxidative function[165], suggesting a compensatory stimulation of mitochondrial β-oxidation. In line with these arguments, both hepatic insulin resistance and simple steatosis were already

established in mice fed a high-fat diet for 8 weeks, well before changes in mitochondrial bioenergetics became evident[166,167].

Vascular diseases

Vascular ageing is the major risk factor for vascular diseases and health status in the aged population[168], contributing to the age-dependent increased risk of pathological conditions of vascular dysfunction, such as hypertension and atherosclerosis, and are also characteristic of cardiovascular risk factors, such as obesity, smoking and a sedentary lifestyle[169].

Oxidative stress, a characteristic of T2DM, hypertension and atherosclerosis, is also the dominant feature of vascular ageing[170]. Indeed, elevated ROS levels lead to endothelial dysfunction[171], an early event in atherosclerosis pathogenesis that occurs mainly in atherosclerotic lesion formation[172,173]. Electron microscopy analysis has shown abnormal ultrastructural lesions in mitochondria from human aortic atherosclerotic lesions, compared with mitochondria from normal parts of the aortic intima[174]. Moreover, mtDNA mutations have been associated with the development of chronic human diseases, such as atherosclerosis, hypertension, T2DM, cardiovascular disease and obesity[175–178]. Finally, a reduction in mtDNA copy number has also been observed in cardiovascular risk factors independent of atherosclerosis, such as hyperlipidaemia[179].

Under conditions of an imbalanced redox state, increased mitochondrial ROS production might lead to modifications of the arterial walls[180], followed by an inflammatory environment[181]. Manganese-dependent superoxide dismutase (MnSOD) activity exhibits a negative feedback with atherogenesis, by modulating ROS levels and, consequently, increased LDL oxidation and endothelial dysfunction. In one study, 16-month-old $MnSOD^{+/-}$ mice consistently showed elevated ROS production and mtDNA damage, followed by the development of dramatic endothelial dysfunction[182]. Similarly, genetic ablation of mitochondrial GPX1 in mice triggered noticeable vascular dysfunction, coinciding with increased oxidative stress and impaired nitric oxide metabolism[183], thus linking perturbations in redox status to vascular ageing.

Closely related to ROS production, the mitochondrial Src homology–collagen (Shc) adaptor protein $p66^{Shc}$ might be the link between ageing and metabolic and cardiovascular diseases. Induction of Ser26 phosphorylation of $p66^{Shc}$ by protein kinase C-β (PKCβ) triggers the transport of $p66^{Shc}$ from the cytosol to mitochondria, where it promotes ROS accumulation by oxidizing cytochrome *c*[184], ultimately leading to apoptosis.

Mutant $p66^{Shc-/-}$ mice have been reported to be resistant to oxidative stress and to have an increased lifespan[185], as well as reduced age-dependent endothelial dysfunction[186]. Additional work has revealed that $p66^{Shc}$ activation is involved in adipogenesis, insulin resistance and cardiovascular complications associated with diabetes mellitus[187–189]. Moreover, vascular levels of $p66^{Shc}$ were increased in animal models of obesity and had a role in endothelial insulin resistance[189], an effect restored when $p66^{Shc}$ was silenced in vivo[189]. In humans, the first evidence for a role of $p66^{Shc}$ in diabetes mellitus came with the finding that patients with T2DM have increased expression of $p66^{Shc}$ in circulating peripheral blood mononuclear cells[190]. Consequently, work has shown that $p66^{Shc-/-}$ mice

are protected against streptozotocin-induced diabetic cardiomyopathy[191], diabetes mellitus-induced vascular endothelial dysfunction and oxidative stress[187]. Nevertheless, contradictory findings regarding the role of $p66^{Shc}$ in obesity have been reported. In one study using animal models and human samples from patients with obesity undergoing elective surgery, it was demonstrated that reduced $p66^{Shc}$ levels could protect from obesity but failed to prevent obesity-associated metabolic dysfunction[192], highlighting the need for further studies to dissect the role of $p66^{Shc}$ in metabolism.

Chronic inflammation

Mitochondrial dysfunction is a hallmark of many chronic inflammatory diseases[193], including those caused by the innate immune response[194]. Innate immunity has a central role in the inflammatory process and coordinates the three stages of inflammation: initiation, propagation and resolution[195]. Unlike the adaptive immune response, the innate immune response is rapid (<90 h) and non-specific, being activated by a broad range of molecules that comprise viruses and bacteria. Pathogen-associated molecular patterns (PAMPs) share conserved features among microbes not present in mammalian cells[196]. Once these PAMPs are recognized by specific receptors belonging to the pattern recognition receptor (PRR) family, an immune response is activated and escalates to induce inflammation and the release of numerous cytokines for recruitment of the more specific adaptive immune system[197]. Different classes of PRRs, including the membrane-bound Toll-like receptors (TLRs), the C-type lectin receptors and the cytosolic NOD (nucleotide-binding oligomerization domain)-like receptors, have been described elsewhere[198].

In addition to PAMPs, other endogenous molecules are released in the presence of cellular damage, known as damage-associated molecular patterns or alarmins. Alarmins bind to and activate PRRs, thus triggering an immune response and propagating the inflammation[199]. Several alarmins consist of mitochondrial molecules, and the ability of these molecules to activate PRRs is dependent on their similarity to bacterial components, reaffirming once again the prokaryotic origin of mitochondria[200]. One of the strongest alarmins released by stressed mitochondria is mtDNA, which has emerged as a strong activator of innate immunity, particularly of the interferon signalling pathway[201]. Several PRRs bind to mtDNA in the cytoplasm, triggering an innate immune signalling pathway. For example, TLR9 binds to mtDNA and activates the NF-κB signalling pathway, inducing the release of pro-inflammatory cytokines[202]. Similar to bacteria, mitochondrial protein translation initiates with an N-formylated methionine instead of a regular methionine as in eukaryotic cells. Thus, the leakage of N-formylated peptides outside the mitochondria activates a specific family of PRRs, the formyl peptide receptors, which are potent inducers of the innate immune system[203]. Another alarmin of mitochondrial origin is cardiolipin, a unique phospholipid abundant in prokaryotic membranes and a constituent of the mitochondrial inner membrane[204].

Other alarmins of mitochondrial origin, including ATP, succinate, TFAM and ROS, all induce and support inflammation by different means[205]. Extracellular ATP binds to purinergic P2 receptors that are present on several cell types, including macrophages, and promotes chemotaxis and phagocytosis[206]. Succinate, an intermediate of the TCA cycle,

is emerging as an important signalling molecule[207]. One of succinate's actions is the stabilization of HIF1α by inhibiting the prolyl hydroxylase domain enzyme activity in macrophages. This action promotes the secretion of pro-inflammatory chemokines, such as IL-1β[208]. TFAM is a DNA-binding protein belonging to the same family as high mobility group box 1 protein and is a very important alarmin involved in inflammation and autoimmune disorders[209]. Moreover, mitochondrially-derived ROS are a key component of the immune system. Hydrogen peroxide, a reaction product of superoxide radical ($O_2^{\bullet -}$) conversion by MnSOD, crosses the mitochondrial membranes into the cytoplasm, activating the inflammasome and redox-sensitive transcription factors, such as HIF1α and NF-κB[210–212].

Given the central role of mitochondria in regulating the inflammatory response, it is not surprising that altering their function and activity has beneficial and deleterious effects. An example of a beneficial response is the release of mitochondrial alarmins in the context of pathogen invasion or tissue damage to help fight infections. By contrast, an uncontrolled release of mitochondrial components into the cytoplasm or the extracellular space due to, for example, mitochondrial dysfunction, can induce chronic inflammation, a condition associated with several diseases, such as rheumatoid arthritis, atherosclerosis, liver injury, obesity and ageing, an effect known as 'inflammaging'.

Targeting mitochondria

Lifestyle interventions

Exercise is a well-known lifestyle intervention that prevents metabolic disorders, such as obesity and T2DM, whereas reduced physical activity and a sedentary lifestyle are key contributing factors[213]. In patients with T2DM, walking and ergometer cycling exercise increased mitochondrial muscle respiration and mitochondrial content and density, as well as the activity of oxidative enzymes[214,215]. Daily voluntary exercise also ameliorates progeroid mouse phenotypes, such as locomotor activity, alopecia and kyphosis, and the deregulated proteomic profiles in muscle and brain in mtDNA mutator mice[216].

Calorie restriction is another widely known lifestyle intervention that can prevent age-related metabolic disorders in multiple species[217]. Under calorie restriction, OXPHOS and antioxidant defences[14], as well as mitochondrial biogenesis, are induced[218]. Endothelial nitric oxide and PGC1α also have key roles. Decreased mitochondrial ROS production and macromolecular oxidative damage due, in part, to mitochondrial uncoupling might also explain the benefits of calorie restriction[219,220]. Moreover, calorie restriction stimulates mitochondrial turnover by boosting mitochondrial biogenesis and the clearance of damaged mitochondria via mitophagy[221,222].

Pharmacological interventions

Small molecules.—The ageing research field is driven by the goal of finding better medicines that can target multiple age-related diseases and prevent many others. Among them, several polyphenols that improve mitochondrial physiology have been described[223]. One of the most well-known polyphenols that improves mitochondrial physiology is

resveratrol[224,225]. Resveratrol enhances mitochondrial biogenesis and oxidative capacity in animal models of diet-induced obesity and ageing[226,227]. In humans, resveratrol improves mitochondrial function, reduces inflammation and decreases lipid levels in patients with obesity[228]. Moreover, resveratrol-dependent activation of SIRT1 has also been shown to trigger mitochondrial biogenesis both in vitro and in transgenic rats with human genes for renin and angiotensinogen by inducing mRNA expression of mitochondrial biogenesis markers, such as PGC1α[229]. Mitophagy can also be induced by resveratrol via FOXO3 activation, which triggers the PINK1–Parkin pathway[230]. Additionally, the non-naturally occurring sirtuin-activating compound, SRT1720, has enhanced insulin sensitivity and oxidative metabolism in animal models of obesity, in part, by improving mitochondrial oxidative capacity[231]. Notably, SRT2104, another non-naturally occurring sirtuin-activating compound, has been tested in the clinic but did not improve glucose homeostasis and insulin sensitivity in patients with T2DM[232]. Punicalagin, the most abundant ellagitannin in the pomegranate fruit, stimulates mitochondrial biogenesis in cardiomyocytes from obese rats[233]. Also, the administration of epicatechin-rich cocoa stimulated mitochondrial biogenesis in patients with T2DM[234]. Finally, oleuropein, a polyphenol present in the leaves of fruits and olives, promotes phosphorylation and consequent activation of mitophagy-related proteins by activation of the AMPK–ULK1 pathway in a diet-induced mouse model of obesity[235].

Activation of AMPK increases mitochondrial chemical energy generation by increasing the AMP to ATP ratio[236]. The AMPK agonist 5-aminoimidazole-4-carboxamide rescues mitochondrial dysfunction and improves exercise performance capacity in *Cox*-deficient mice[237]. Additionally, metformin, which is widely used to treat T2DM, activates AMPK, improves mitochondrial function, decreases age-associated inflammation and improves hyperglycaemia in people with obesity and prediabetes, as well as in HFD-fed mice[238,239]. Interestingly, a newly reported molecule, imeglimin, a tetrahydrotriazine-containing oral glucose-lowering agent, decreased ROS production and prevented hyperglycaemia-induced cell death via complex l by inhibiting reverse electron transfer-mediated ROS production in preclinical studies[240], and its efficacy and favourable safety and tolerability profile have been confirmed in patients with T2DM[241]. Similarly, chronic moderate inhibition of cytochrome *c* oxidase by TPP–thiazole improves mitochondrial bioenergetics and glucose homeostasis and reduces visceral adipose tissue in 14–18-month-old mice, limiting age-associated obesity and glucose irregularities[242].

In addition, inhibition of mTOR by rapamycin, which also extends the lifespan of model organisms (for example, yeast, nematodes, fruit flies and mice) and prevents age-related diseases, is also currently being tested in multiple clinical trials[243]. The health benefits provided by rapamycin, however, seem to be tissue-specific. Knocking out the Raptor complex in adipose tissue enhances mitochondrial respiration[244], but muscle-specific knockout causes muscular dystrophy in mice[36]. Adipose-specific Raptor knockout mice ($Raptor^{ad-/-}$) have a lean phenotype and are protected against diet-induced obesity, due to enhanced energy expenditure facilitated by UCP1-mediated mitochondrial uncoupling[244]. Interestingly, the phenotype of mice with knockout of a downstream target of mTOR, S6K1, mimics the lean phenotype of $Raptor^{ad-/-}$ mice, and the mice are protected against diet-induced obesity[245]. In *db*/*db* mice, rapamycin exhibits anti-obesity properties by

improving whole-body insulin sensitivity and increasing markers of fatty acid oxidation and mitochondrial biogenesis in white adipose tissue[246]. Finally, leucine, which is sensed by mTOR[247], increases NAD^+ levels, mitochondrial biogenesis, insulin sensitivity and lipid disposal when given to obese mice[248]. However, leucine supplementation has not improved muscle physiology, glycaemic control or lipidaemia in older patients (aged 71 ± 1 years) with T2DM[249], highlighting the need for further studies to dissect amino acid supplementation as a therapy for age-associated metabolic diseases. Interestingly, a human clinical trial testing leucine–resveratrol combinations showed improved glucose homeostasis and insulin sensitivity in patients with prediabetes, suggesting that synergistic approaches might be a good alternative to treat age-associated metabolic diseases[250].

NAD^+-boosting molecules.—Systematic NAD^+ decline and decreased sirtuin activity are considered among the molecular events that occur during ageing, possibly underlying the increase in age-related diseases that limit organismal lifespan[251]. A large body of evidence from animal studies supports NAD^+-boosting therapies as a way to ameliorate a wide variety of age-related metabolic disease.

Supplementation of the precursor nicotinamide mononucleotide (NMN) re-established β-cell glucose-stimulated insulin secretion as well as hepatic and skeletal muscle insulin sensitivity in mouse models with impaired glucose homeostasis[252,253]. In 22-month-old mice, NMN restored NAD^+ levels and markers of mitochondrial decline during ageing[48], while reducing age-associated body weight gain and increasing energy metabolism and physical activity[254]. Moreover, NMN[252] and nicotinamide riboside[255], another NAD^+ precursor, increased mitochondrial function and improved glucose homeostasis in obese mice. Currently, several ongoing clinical trials are investigating the safety and tolerability of NMN and similar molecules[256]. In hyperglycaemic mice, nicotinamide riboside administration improved liver steatosis and glucose tolerance, and reduced weight gain[257]. To some extent, this effect can be explained by the elicited mitochondrial unfolded protein response mediated by nicotinamide riboside[258]. However, results from human studies testing nicotinamide riboside supplementation at 2,000 mg per day showed that although safe, nicotinamide riboside does not improve insulin sensitivity and whole-body glucose metabolism in patients with obesity and insulin resistance[259].

The upregulation of the NAD salvage pathway, which recycles NAD^+ from nicotinamide, was first shown to extend lifespan and mimic caloric restriction in yeast cells[46]. Interestingly, treatment of obese rats with nicotinamide induces mitochondrial biogenesis and improves insulin sensitivity in a SIRT1-dependent manner[260]. Additionally, inhibition of NAD^+-consuming enzymes has been shown to be a promising approach to treat mitochondrial dysfunction in age-related metabolic dysfunction[261]. Indeed, PARP inhibition in mice with NAFLD restores NAD^+ levels, thereby improving mitochondrial function and insulin sensitivity, and decreasing hepatic lipid accumulation[261].

Mitochondrial uncouplers.—Mitochondrial uncoupling is defined as a process that uncouples nutrient oxidation from ATP production[262]. One of the first molecules to be described as having potential therapeutic benefits in obesity and metabolic diseases is the mitochondrial uncoupler 2,4-dinitrophenol (DNP)[263]. Mice fed a high-

fat diet have improved metabolic parameters, such as reduced body weight, improved glucose homeostasis, decreased serum levels of insulin and decreased liver levels of triglycerides[264,265]. However, the potential adverse effects of DNP led to the development of derivatives of DNP, for example a liver-targeted methyl ether derivative of DNP (DNP methyl ether; DNPME) and a controlled-release mitochondrial uncoupler (controlled release mitochondrial protonophore; CRMP). Both DNPME and CRMP decreased triglyceride accumulation in liver and improved insulin sensitivity in rat models of T2DM and NAFLD[266,267]. Importantly, in two different nonhuman primate models of the metabolic syndrome, high-fat, fructose-fed cynomolgus macaques and spontaneously obese dysmetabolic rhesus macaques, CRMP was shown to reduce hepatic steatosis and insulin resistance and increase the rates of β-oxidation[268].

FDA-approved drugs that have uncoupling activity have also been tested in animal models. Niclosamide ethanolamine reversed hyperglycaemia, hyperinsulinaemia and hepatic steatosis in mice fed a high-fat diet and delayed hyperglycaemia in *db*/*db* mice[269]. Another promising candidate is 1,3-bis(dichlorophenyl)urea, which reduces body weight gain and body adipose tissue mass, improving insulin sensitivity and glucose homeostasis, and decreasing hepatic lipid content[270]. CZ5, an orally bioavailable mitochondrial uncoupler[271], also reduces body weight, total adipose tissue mass, fasting glucose levels and insulin levels[271].

Two studies published in the past few years have investigated additional potential candidates for the treatment of age-related metabolic diseases. Kanemoto and colleagues[272] described a liver-localized mitochondrial uncoupler, OPC-163493, which was shown to have various benefits in multiple rodent models of the metabolic syndrome, such as decreasing hepatic steatosis and lowering fasting levels of glucose and HbA_{1c}, as well as increasing nitric oxide bioavailability and reducing oxidative stress in blood vessels. These findings demonstrate the cardiometabolic benefits of OPC-163493. Alexopoulus and colleagues[273] found that the orally available BAM15, a pH-dependent self-limiting uncoupler, improved insulin sensitivity and decreased oxidative stress, body adipose tissue mass, hepatic triglyceride accumulation and inflammatory lipids in diet-induced obese mice. In an attempt to optimize BAM15 limitations, such as tissue distribution and uncoupling effects, 10b and 12i (SHS4121705) derivatives are being tested and are being shown to be efficacious in the STAM mouse model of non-alcoholic steatohepatitis, by decreasing hepatic levels of triglycerides, improving serum levels of alanine transaminase and improving NAFLD activity score[274,275]. Another compound that has been synthesized to oppose the possible adverse effects of uncontrolled mitochondrial uncoupling (for example, when mitochondrial uncoupling exceeds the ability of mitochondria to produce ATP), is the dual-action compound 6j, which has both mitochondrial uncoupling activity and pyruvate dehydrogenase activation effects[276]. Studies performed in diabetic mice have shown that compound 6j is able to improve insulin sensitivity, glucose homeostasis and liver steatosis[276].

Collectively, these observations suggest that this class of drugs might hold the potential to treat obesity and metabolic diseases. However, because the use of mitochondrial uncouplers in humans has led to severe adverse effects in the past, safety studies will be particularly

important. In 2021, positive results from a phase I clinical trial testing mitochondrial uncoupler HU6 were presented. HU6 was well tolerated without serious adverse effects, and provided additional improvements in resting energy expenditure and dose-dependent weight loss[277], supporting the potential for the development of mitochondrial uncouplers for the treatment of metabolic dysfunction in humans.

Mitochondrially-targeted antioxidants.—Due to the central role of mitochondrial ROS and mitochondrial oxidative damage in disease, mitochondrial-targeted antioxidant therapies have been explored as potential treatments[278]. Traditional exogenous antioxidants (for example, vitamins E, C and D), as well as chemical compounds with described antioxidants properties (for example, coenzyme Q, α-lipoic acid and *N*-acetylcysteine), have been used to reduce oxidative stress[279,280], but their cellular uptake is limited and they do not accumulate where needed[281]. Mitochondrially-targeted coenzyme Q (MitoQ) or mitochondrially-targeted vitamin E (MitoVitE) have been shown to target mitochondrial dysfunction[282,283]. MitoQ, one of the best-characterized mitochondrially-targeted antioxidants, has been shown to protect against oxidative stress[284] and offers neuroprotection[285] in preclinical studies. In humans, MitoQ improves vascular ageing in middle-aged and older adults (aged 60–79 years)[286]. MitoVitE decreases hepatic oxidative stress and inhibits hepatic lipid deposition in mice[282]. Interestingly, MitoQ also alleviated Alzheimer disease-like pathology in the 3×Tg-AD mouse model[287] by improving cognition, decreasing oxidative stress and increasing survival. These findings support the idea that targeting mitochondria to relieve symptomology in patients with Alzheimer disease is worthwhile. Another promising mitochondrial target antioxidant is elamipretide (SS-31), a cell-permeable tetrapeptide that localizes to the inner mitochondrial membrane, where it associates with and stabilizes cardiolipin. Clinical studies using elamipretide are more advanced for hereditary mitochondrial diseases than for metabolic diseases. Nevertheless, concerning T2DM, studies using animal models and human islet and leukocyte samples support the beneficial effects of elamipretide in improving mitochondrial function[288,289], restoring glucose levels[290], decreasing oxidative stress[291] and preventing atherosclerosis[292]. Interestingly, one clinical study published in 2021 showed that elamipretide reverses mitochondrial dysfunction in ageing human muscle, paving the way to test this molecule against age-associated metabolic diseases[293].

Conclusions

One of the most important discoveries in the field of ageing is that the rate of ageing is coordinated, at least in part, by conserved genetic and biochemical pathways, many of which control mitochondrial physiology. The modulation of these pathways could be the key to improving human health and preventing major age-related diseases, especially those associated with metabolic dysfunction. That said, how important mitochondria are to the ageing process is still under debate. Nevertheless, the maintenance of healthy mitochondria and metabolic regulation are key to long-term health.

A summary of different approaches targeting mitochondria in ageing and age-associated metabolic disease, as well as their clinical stage, can be found in TABLE 1. Despite continuing progress over the past two decades in the field of ageing, combatting

mitochondrial dysfunction in age-associated metabolic diseases has proven challenging. To some extent, this can be explained by the ubiquitous nature of mitochondria and the heterogeneous clinical presentations associated with multi-organ involvement in age-associated metabolic diseases. In the preclinical context, one of the main challenges in the field of ageing is adequate disease modelling when considering biological age. Moreover, it has been challenging to focus on specific pathologies in the clinical context because of the functional decay of multiple organs during ageing. Therefore, new digital health technologies, such as artificial intelligence and big data, could pave the way to understanding the complex nature of these diseases, providing associations between specific biological or clinical phenotypes and corresponding biomarkers, which could help future preclinical research as well as clinical trial design and change the health span panorama in the aged population.

In conclusion, of all the processes that affect ageing, those controlled by mitochondria are the most responsive to environmental variations, making the mitochondria an excellent model for elucidating the effects of environment on longevity and disease. In the long term, a fundamental understanding of the role of mitochondria in human health and ageing will be needed if there are to be breakthroughs in combatting frailty and age-related diseases.

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References

1. Zenin A et al. Identification of 12 genetic loci associated with human healthspan. Commun. Biol 2, 41 (2019). [PubMed: 30729179]
2. López-Otín C, Blasco MA, Partridge L, Serrano M & Kroemer G The hallmarks of aging. Cell 153, 1194–1217 (2013). [PubMed: 23746838]
3. Kennedy BK et al. Geroscience: linking aging to chronic disease. Cell 159, 709–713 (2014). [PubMed: 25417146]
4. Burger O, Baudisch A & Vaupel JW Human mortality improvement in evolutionary context. Proc. Natl Acad. Sci. USA 109, 18210–18214 (2012). [PubMed: 23071331]
5. Poulain M, Herm A & Pes G The Blue Zones: areas of exceptional longevity around the world. Vienna Yearb. Popul. Res 11, 87–108 (2013).
6. Cooper R, Strand BH, Hardy R, V Patel K & Kuh D Physical capability in mid-life and survival over 13 years of follow-up: British Birth Cohort Study. BMJ 348, g2219 (2014). [PubMed: 24787359]
7. Fogel RW & Costa DL A theory of technophysio evolution, with some implications for forecasting population, health care costs, and pension costs. Demography 34, 49–66 (1997). [PubMed: 9074831]
8. Crimmins EM Lifespan and healthspan: past, present, and promise. Gerontologist 55, 901–911 (2015). [PubMed: 26561272]
9. Chinnery PF Mitochondrial disease in adults: what's old and what's new? EMBO Mol. Med 7, 1503–1512 (2015). [PubMed: 26612854]

10. Gonzalez-Freire M et al. The road ahead for health and lifespan interventions. Ageing Res. Rev 59, 101037 (2020). [PubMed: 32109604]
11. Lin S et al. Calorie restriction extends *Saccharomyces cerevisiae* lifespan by increasing respiration. Nature 418, 344–348 (2002). [PubMed: 12124627]
12. Schulz TJ et al. Glucose restriction extends *Caenorhabditis elegans* life span by inducing mitochondrial respiration and increasing oxidative stress. Cell Metab 6, 280–293 (2007). [PubMed: 17908557]
13. Nisoli E et al. Calorie restriction promotes mitochondrial biogenesis by inducing the expression of eNOS. Science 310, 314–317 (2005). [PubMed: 16224023]
14. Wallace DC A mitochondrial paradigm of metabolic and degenerative diseases, aging, and cancer: a dawn for evolutionary medicine. Annu. Rev. Genet 39, 359–407 (2005). [PubMed: 16285865]
15. López-Lluch G & Navas P Calorie restriction as an intervention in ageing. J. Physiol 594, 2043–2060 (2016). [PubMed: 26607973]
16. Fontana L, Partridge L & Longo V Extending healthy life span–from yeast to humans. Science 328, 321–326 (2010). [PubMed: 20395504]
17. Senchuk MM et al. Activation of DAF-16/FOXO by reactive oxygen species contributes to longevity in long-lived mitochondrial mutants in *Caenorhabditis elegans*. PLoS Genet 14, e1007268 (2018). [PubMed: 29522556]
18. Dhondt I et al. FOXO/DAF-16 activation slows down turnover of the majority of proteins in *C. elegans*. Cell Rep 16, 3028–3040 (2016). [PubMed: 27626670]
19. Brys K, Castelein N, Matthijssens F, Vanfleteren JR & Braeckman BP Disruption of insulin signalling preserves bioenergetic competence of mitochondria in ageing *Caenorhabditis elegans*. BMC Biol 8, 91 (2010). [PubMed: 20584279]
20. Zarse K et al. Impaired insulin/IGF1 signaling extends life span by promoting mitochondrial L-proline catabolism to induce a transient ROS signal. Cell Metab 15, 451–465 (2012). [PubMed: 22482728]
21. Morsci NS, Hall DH, Driscoll M & Sheng ZH Age-related phasic patterns of mitochondrial maintenance in adult *Caenorhabditis elegans* neurons. J. Neurosci 36, 1373–1385 (2016). [PubMed: 26818523]
22. Palikaras K, Lionaki E & Tavernarakis N Coordination of mitophagy and mitochondrial biogenesis during ageing in *C. elegans*. Nature 521, 525–528 (2015). [PubMed: 25896323]
23. Fang EF et al. Tomatidine enhances lifespan and healthspan in *C. elegans* through mitophagy induction via the SKN-1/Nrf2 pathway. Sci. Rep 7, 46208 (2017). [PubMed: 28397803]
24. Ryu D et al. Urolithin A induces mitophagy and prolongs lifespan in *C. elegans* and increases muscle function in rodents. Nat. Med 22, 879–888 (2016). [PubMed: 27400265]
25. Westbrook R, Bonkowski MS, Strader AD, & Bartke A Alterations in oxygen consumption respiratory quotient and heat production in long-lived GHRKO and Ames dwarf mice and short-lived bGH transgenic mice. J. Gerontol. A. Biol. Sci. Med. Sci 64, 443–451 (2009). [PubMed: 19286975]
26. Brown-Borg HM, Johnson WT & Rakoczy SG Expression of oxidative phosphorylation components in mitochondria of long-living Ames dwarf mice. Age 34, 43–57 (2012). [PubMed: 21327718]
27. Blüher M, Kahn B & Kahn C Extended longevity in mice lacking the insulin receptor in adipose tissue. Science 299, 572–574 (2003). [PubMed: 12543978]
28. Katic M et al. Mitochondrial gene expression and increased oxidative metabolism: role in increased lifespan of fat-specific insulin receptor knock-out mice. Aging Cell 6, 827–839 (2007). [PubMed: 18001293]
29. Merry TL et al. Impairment of insulin signalling in peripheral tissue fails to extend murine lifespan. Aging Cell 16, 761–772 (2017). [PubMed: 28544360]
30. Saxton RA & Sabatini DM mTOR signaling in growth, metabolism, and disease. Cell 168, 960–976 (2017). [PubMed: 28283069]
31. Loewith R et al. Two TOR complexes only one of which is rapamycin sensitive have distinct roles in cell growth control. Mol. Cell 10, 457–468 (2002). [PubMed: 12408816]

32. Bai X et al. Rheb activates mTOR by antagonizing its endogenous inhibitor, FKBP38. Science 318, 977–980 (2007). [PubMed: 17991864]
33. Hosokawa N et al. Nutrient-dependent mTORC1 association with the ULK1-Atg13-FIP200 complex required for autophagy. Mol. Biol. Cell 20, 1981–1991 (2009). [PubMed: 19211835]
34. Guo C, Kroemer G & Kepp O Mitophagy: an emerging role in aging and age-associated diseases. Front. Cell Dev. Biol 8, 200 (2020). [PubMed: 32274386]
35. Cunningham JT et al. mTOR controls mitochondrial oxidative function through a YY1-PGC-1α transcriptional complex. Nature 450, 736–740 (2007). [PubMed: 18046414]
36. Bentzinger CF et al. Skeletal muscle-specific ablation of raptor, but not of rictor, causes metabolic changes and results in muscle dystrophy. Cell Metab 8, 411–424 (2008). [PubMed: 19046572]
37. Johnson S et al. mTOR inhibition alleviates mitochondrial disease in a mouse model of Leigh syndrome. Science 342, 1524–1529 (2013). [PubMed: 24231806]
38. Siegmund SE et al. Low-dose rapamycin extends lifespan in a mouse model of mtDNA depletion syndrome. Hum. Mol. Genet 26, 4588–4605 (2017). [PubMed: 28973153]
39. Herzig S & Shaw RJ AMPK: guardian of metabolism and mitochondrial homeostasis. Nat. Rev. Mol. Cell Biol 19, 121–135 (2018). [PubMed: 28974774]
40. Zong H et al. AMP kinase is required for mitochondrial biogenesis in skeletal muscle in response to chronic energy deprivation. Proc. Natl Acad. Sci. USA 99, 15983–15987 (2002). [PubMed: 12444247]
41. Jäger S, Handschin C, St-Pierre J & Spiegelman B AMP-activated protein kinase (AMPK) action in skeletal muscle via direct phosphorylation of PGC-1α. Proc. Natl Acad. Sci. USA 104, 12017–12022 (2007). [PubMed: 17609368]
42. Egan DF et al. Phosphorylation of ULK1 (hATG1) by AMP-activated protein kinase connects energy sensing to mitophagy. Science 331, 456–461 (2011). [PubMed: 21205641]
43. Toyama E et al. Metabolism. AMP-activated protein kinase mediates mitochondrial fission in response to energy stress. Science 351, 275–281 (2016). [PubMed: 26816379]
44. Weir HJ et al. Dietary restriction and AMPK increase lifespan via mitochondrial network and peroxisome remodeling. Cell Metab 26, 884–896.e5 (2017). [PubMed: 29107506]
45. Reznick RM et al. Aging-associated reductions in AMP-activated protein kinase activity and mitochondrial biogenesis. Cell Metab 5, 151–156 (2007). [PubMed: 17276357]
46. Anderson RM, Bitterman KJ, Wood JG, Medvedik O & Sinclair DA Nicotinamide and Pnc1 govern lifespan extension by calorie restriction and stress. Nature 423, 181–185 (2003). [PubMed: 12736687]
47. Sinclair DA & Guarente L Extrachromosomal rDNA circles–a cause of aging in yeast. Cell 91, 1033–1042 (1997). [PubMed: 9428525]
48. Gomes A et al. Declining NAD(+) induces a pseudohypoxic state disrupting nuclear-mitochondrial communication during aging. Cell 155, 1624–1638 (2013). [PubMed: 24360282]
49. Brunet A et al. Stress-dependent regulation of FOXO transcription factors by the SIRT1 deacetylase. Science 303, 2011–2015 (2004). [PubMed: 14976264]
50. Rodgers JT et al. Nutrient control of glucose homeostasis through a complex of PGC-1α and SIRT1. Nature 434, 113–118 (2005). [PubMed: 15744310]
51. Verdin E, Hirschey MD, Finley LWS & Haigis MC Sirtuin regulation of mitochondria: energy production, apoptosis, and signaling. Trends Biochem. Sci 35, 669–675 (2010). [PubMed: 20863707]
52. Ahn BH et al. A role for the mitochondrial deacetylase Sirt3 in regulating energy homeostasis. Proc. Natl Acad. Sci. USA 105, 14447–14452 (2008). [PubMed: 18794531]
53. Palacios OM et al. Diet and exercise signals regulate SIRT3 and activate AMPK and PGC-1α in skeletal muscle. Aging 1, 771–783 (2009). [PubMed: 20157566]
54. Ahuja N et al. Regulation of insulin secretion by SIRT4, a mitochondrial ADP-ribosyltransferase. J. Biol. Chem 282, 33583–33592 (2007). [PubMed: 17715127]
55. Someya S et al. Sirt3 mediates reduction of oxidative damage and prevention of age-related hearing loss under caloric restriction. Cell 143, 802–812 (2010). [PubMed: 21094524]

56. Harman D Aging: a theory based on free radical and radiation chemistry. J. Gerontol 11, 298–300 (1956). [PubMed: 13332224]
57. Melov S et al. Lifespan extension and rescue of spongiform encephalopathy in superoxide dismutase 2 nullizygous mice treated with superoxide dismutase-catalase mimetics. J. Neurosci 21, 8348–8353 (2001). [PubMed: 11606622]
58. Kirby K, Hu J, Hilliker AJ & Phillips JP RNA interference-mediated silencing of Sod2 in *Drosophila* leads to early adult-onset mortality and elevated endogenous oxidative stress. Proc. Natl Acad. Sci. USA 99, 16162–16167 (2002). [PubMed: 12456885]
59. Van Raamsdonk JM & Hekimi S Superoxide dismutase is dispensable for normal animal lifespan. Proc. Natl Acad. Sci. USA 109, 5785–5790 (2012). [PubMed: 22451939]
60. Kauppila TES, Kauppila JHK & Larsson NG Mammalian mitochondria and aging: an update. Cell Metab 25, 57–71 (2017). [PubMed: 28094012]
61. Harman D The biologic clock: the mitochondria? J. Am. Geriatr. Soc 20, 145–147 (1972). [PubMed: 5016631]
62. Wallace DC The human mitochondrion and pathophysiology of aging and age-related diseases. in Molecular Biology of Aging 1st edn (eds. Guarente L, Partridge L & Wallace DC) 1–38 (Cold Spring Harbor Laboratory Press, 2008).
63. Cortopassi GA & Arnheim N Detection of a specific mitochondrial DNA deletion in tissues of older humans. Nucleic Acids Res 18, 6927–6933 (1990). [PubMed: 2263455]
64. Linnane AW, Ozawa T, Marzuki S & Tanaka M Mitochondrial DNA mutations as an important contributor to ageing and degenerative diseases. Lancet 1, 642–645 (1989). [PubMed: 2564461]
65. Khrapko K & Vijg J Mitochondrial DNA mutations and aging: devils in the details? Trends Genet 25, 91–98 (2009). [PubMed: 19110336]
66. Trifunovic A et al. Premature ageing in mice expressing defective mitochondrial DNA polymerase. Nature 429, 417–423 (2004). [PubMed: 15164064]
67. Niu X, Trifunovic A, Larsson NG & Canlon B Somatic mtDNA mutations cause progressive hearing loss in the mouse. Exp. Cell Res 313, 3924–3934 (2007). [PubMed: 17662273]
68. Ross JM et al. High brain lactate is a hallmark of aging and caused by a shift in the lactate dehydrogenase A/B ratio. Proc. Natl Acad. Sci. USA 107, 20087–20092 (2010). [PubMed: 21041631]
69. Ross JM et al. Germline mitochondrial DNA mutations aggravate ageing and can impair brain development. Nature 501, 412–415 (2013). [PubMed: 23965628]
70. Ross JM, Coppotelli G, Hoffer BJ & Olson L Maternally transmitted mitochondrial DNA mutations can reduce lifespan. Sci. Rep 4, 6569 (2014). [PubMed: 25299268]
71. Vermulst M et al. Mitochondrial point mutations do not limit the natural lifespan of mice. Nat. Genet 39, 540–543 (2007). [PubMed: 17334366]
72. Desquilbet L et al. HIV-1 infection is associated with an earlier occurrence of a phenotype related to frailty. J. Gerontol. A Biol. Sci. Med. Sci 62, 1279–1286 (2007). [PubMed: 18000149]
73. Guaraldi G et al. Coronary aging in HIV-infected patients. Clin. Infect. Dis 49, 1756–1762 (2009). [PubMed: 19886793]
74. Payne BAI et al. Mitochondrial aging is accelerated by anti-retroviral therapy through the clonal expansion of mtDNA mutations. Nat. Genet 43, 806–810 (2011). [PubMed: 21706004]
75. Sohl CD et al. Probing the structural and molecular basis of nucleotide selectivity by human mitochondrial DNA polymerase γ. Proc. Natl Acad. Sci. USA 112, 8596–8601 (2015). [PubMed: 26124101]
76. Fang EF et al. Nuclear DNA damage signalling to mitochondria in ageing. Nat. Rev. Mol. Cell Biol 17, 308–321 (2016). [PubMed: 26956196]
77. Scheibye-Knudsen M, Fang EF, Croteau DL & Bohr VA Contribution of defective mitophagy to the neurodegeneration in DNA repair-deficient disorders. Autophagy 10, 1468–1469 (2014). [PubMed: 24991831]
78. Wilkins HM et al. in The Molecular and Cellular Basis of Neurodegenerative Diseases: Underlying Mechanisms 369–414 (Elsevier, 2018).

79. Hoeijmakers JHJ DNA damage, aging, and cancer. N. Engl. J. Med 361, 1475–1485 (2009). [PubMed: 19812404]
80. Chaudhuri AR & Nussenzweig A The multifaceted roles of PARP1 in DNA repair and chromatin remodelling. Nat. Rev. Mol. Cell Biol 18, 610–621 (2017). [PubMed: 28676700]
81. Chambon P, Weill JD & Mandel P Nicotinamide mononucleotide activation of new DNA-dependent polyadenylic acid synthesizing nuclear enzyme. Biochem. Biophys. Res. Commun 11, 39–43 (1963). [PubMed: 14019961]
82. Gibson BA & Kraus WL New insights into the molecular and cellular functions of poly(ADP-ribose) and PARPs. Nat. Rev. Mol. Cell Biol 13, 411–424 (2012). [PubMed: 22713970]
83. Bai P et al. PARP-1 inhibition increases mitochondrial metabolism through SIRT1 activation. Cell Metab 13, 461–468 (2011). [PubMed: 21459330]
84. Mouchiroud L et al. The NAD+/sirtuin pathway modulates longevity through activation of mitochondrial UPR and FOXO signaling. Cell 154, 430–441 (2013). [PubMed: 23870130]
85. Fang EF et al. Defective mitophagy in XPA via PARP-1 hyperactivation and NAD+/SIRT1 reduction. Cell 157, 882–896 (2014). [PubMed: 24813611]
86. Scheibye-Knudsen M et al. A high-fat diet and NAD+ activate sirt1 to rescue premature aging in Cockayne syndrome. Cell Metab 20, 840–855 (2014). [PubMed: 25440059]
87. Valentin-vega YA et al. Mitochondrial dysfunction in ataxia-telangiectasia. Blood 119, 1490–1500 (2012). [PubMed: 22144182]
88. Fang EF et al. NAD+ replenishment improves lifespan and healthspan in ataxia telangiectasia models via mitophagy and DNA repair. Cell Metab 24, 566–581 (2016). [PubMed: 27732836]
89. Tchkonia T, Zhu Y, Deursen JV, Campisi J & Kirkland JL Cellular senescence and the senescent secretory phenotype: therapeutic opportunities. J. Clin. Invest 123, 966–972 (2013). [PubMed: 23454759]
90. Campisi J & D'Adda Di Fagagna F Cellular senescence: when bad things happen to good cells. Nat. Rev. Mol. Cell Biol 8, 729–740 (2007). [PubMed: 17667954]
91. Yoon YS, Byun HO, Cho H, Kim BK & Yoon G Complex II defect via down-regulation of iron-sulfur subunit induces mitochondrial dysfunction and cell cycle delay in iron chelation-induced senescence-associated growth arrest. J. Biol. Chem 278, 51577–51586 (2003). [PubMed: 14512425]
92. Byun HO et al. GSK3 inactivation is involved in mitochondrial complex IV defect in transforming growth factor (TGF) β1-induced senescence. Exp. Cell Res 318, 1808–1819 (2012). [PubMed: 22652454]
93. Lafargue A et al. Ionizing radiation induces long-term senescence in endothelial cells through mitochondrial respiratory complex II dysfunction and superoxide generation. Free. Radic. Biol. Med 108, 750–759 (2017). [PubMed: 28431961]
94. Passos JF et al. Mitochondrial dysfunction accounts for the stochastic heterogeneity in telomere-dependent senescence. PLoS Biol 5, e110 (2007). [PubMed: 17472436]
95. Passos JF et al. Feedback between p21 and reactive oxygen production is necessary for cell senescence. Mol. Syst. Biol 6, 347 (2010). [PubMed: 20160708]
96. Correia-Melo C & Passos JF Mitochondria: are they causal players in cellular senescence? Biochim. Biophys. Acta 1847, 1373–1379 (2015). [PubMed: 26028303]
97. Ogrodnik M et al. Cellular senescence drives age-dependent hepatic steatosis. Nat. Commun 8, 15691 (2017). [PubMed: 28608850]
98. Mai S, Klinkenberg M, Auburger G, Bereiter-Hahn J & Jendrach M Decreased expression of Drp1 and Fis1 mediates mitochondrial elongation in senescent cells and enhances resistance to oxidative stress through PINK1. J. Cell Sci 123, 917–926 (2010). [PubMed: 20179104]
99. Kwon SM, Hong SM, Lee Y & Min S Metabolic features and regulation in cell senescence. BMB Rep 52, 5–12 (2019). [PubMed: 30526768]
100. Shock LS, Thakkar PV, Peterson EJ, Moran RG & Taylor SM DNA methyltransferase 1, cytosine methylation, and cytosine hydroxymethylation in mammalian mitochondria. Proc. Natl Acad. Sci. USA 108, 3630–3635 (2011). [PubMed: 21321201]

101. Chestnut BA et al. Epigenetic regulation of motor neuron cell death through DNA methylation. J. Neurosci 31, 16619–16636 (2011). [PubMed: 22090490]
102. Bellizzi D et al. The control region of mitochondrial DNA shows an unusual CpG and non-CpG methylation pattern. DNA Res 20, 537–547 (2013). [PubMed: 23804556]
103. Wong M et al. Mitochondrial DNMT3A and DNA methylation in skeletal muscle and CNS of transgenic mouse models of ALS. Front. Cell Neurosci 7, 279 (2013). [PubMed: 24399935]
104. Lodeiro MF et al. Transcription from the second heavy-strand promoter of human mtDNA is repressed by transcription factor A in vitro. Proc. Natl Acad. Sci. USA 109, 6513–6518 (2012). [PubMed: 22493245]
105. Janssen BG et al. Placental mitochondrial methylation and exposure to airborne particulate matter in the early life environment: an ENVIRONAGE birth cohort study. Epigenetics 10, 536–544 (2015). [PubMed: 25996590]
106. Armstrong DA et al. Maternal smoking during pregnancy is associated with mitochondrial DNA methylation. Environ. Epigenetics 2, dvw020 (2016).
107. Mishra M & Kowluru RA Epigenetic modification of mitochondrial DNA in the development of diabetic retinopathy. Investig. Ophthalmol. Vis. Sci 56, 5133–5142 (2015). [PubMed: 26241401]
108. Yu D et al. Mitochondrial DNA hypomethylation is a biomarker associated with induced senescence in human fetal heart mesenchymal stem cells. Stem Cell Int 2017, 14–16 (2017).
109. Mawlood SK, Dennany L, Watson N, Dempster J & Pickard BS Quantification of global mitochondrial DNA methylation levels and inverse correlation with age at two CpG sites. Aging 8, 636–641 (2016). [PubMed: 26887692]
110. Baccarelli AA & Byun H Platelet mitochondrial DNA methylation: a potential new marker of cardiovascular disease. Clin. Epigenetics 7, 44 (2015). [PubMed: 25901189]
111. Feng SHI, Xiong L, Ji Z, Cheng WEI & Yang H Correlation between increased ND2 expression and demethylated displacement loop of mtDNA in colorectal cancer. Mol. Med. Rep 6, 125–130 (2012). [PubMed: 22505229]
112. Pirola C et al. Epigenetic modification of liver mitochondrial DNA is associated with histological severity of nonalcoholic fatty liver disease. Gut 62, 1356–1263 (2013). [PubMed: 22879518]
113. Zheng LD et al. Insulin resistance is associated with epigenetic and genetic regulation of mitochondrial DNA in obese humans. Clin. Epigenetics 7, 60 (2015). [PubMed: 26110043]
114. Infantino V et al. Impairment of methyl cycle affects mitochondrial methyl availability and glutathione level in Down's syndrome. Mol. Genet. Metab 102, 378–382 (2011). [PubMed: 21195648]
115. Stoccoro A et al. Mitochondrial DNA copy number and D-loop region methylation in carriers of amyotrophic lateral sclerosis gene mutations. Epigenomics 10, 1431–1443 (2018). [PubMed: 30088417]
116. Blanch M, Mosquera JL, Ansoleaga B, Ferrer I & Barrachina M Altered mitochondrial DNA methylation pattern in Alzheimer disease-related pathology and in Parkinson disease. Am. J. Pathol 186, 385–397 (2016). [PubMed: 26776077]
117. Haslam DW & James WPT Obesity. Lancet 366, 1197–1209 (2005). [PubMed: 16198769]
118. Bhatti GK, Bhadada SK, Vijayvergiya R, Mastana SS & Bhatti JS Metabolic syndrome and risk of major coronary events among the urban diabetic patients: North Indian Diabetes and Cardiovascular Disease Study–NIDCVD-2. J. Diabetes Complicat 30, 72–78 (2016).
119. Matsumoto AM Andropause: clinical implications of the decline in serum testosterone levels with aging in men. J. Gerontol. A. Biol. Sci. Med. Sci 57, M76–M99 (2002). [PubMed: 11818427]
120. Griffin TM, Humphries KM, Kinter M, Lim HY & Szweda LI Nutrient sensing and utilization: getting to the heart of metabolic flexibility. Biochimie 124, 74–83 (2015). [PubMed: 26476002]
121. Storlien L, Oakes ND & Kelley DE Metabolic flexibility. Proc. Nutr. Soc 63, 363–368 (2004). [PubMed: 15294056]
122. Kopprasch S et al. In vivo evidence for increased oxidation of circulating LDL in impaired glucose tolerance. Diabetes 51, 3102–3106 (2002). [PubMed: 12351454]
123. Petersen KF et al. Mitochondrial dysfunction in the elderly: possible role in insulin resistance. Science 300, 1140–1142 (2003). [PubMed: 12750520]

124. Litvinova LS, Kirienkova EV, Mazunin IO, Vasilenko MA & Fattakhov NS Pathogenesis of insulin resistance in metabolic obesity. Biochem. Suppl. B Biomed. Chem 8, 192–202 (2014).
125. Pagel-Langenickel I, Bao J, Pang L & Sack MN The role of mitochondria in the pathophysiology of skeletal muscle insulin resistance. Endocr. Rev 31, 25–51 (2010). [PubMed: 19861693]
126. Poulton J et al. The presence of a common mitochondrial DNA variant is associated with fasting insulin levels in Europeans in Auckland. Diabet. Med 19, 969–971 (2002). [PubMed: 12421439]
127. Kim JH et al. The prevalence of the mitochondrial DNA 16189 variant in non-diabetic Korean adults and its association with higher fasting glucose and body mass index. Diabet. Med 19, 681–684 (2002). [PubMed: 12147150]
128. Tanaka M et al. Women with mitochondrial haplogroup N9a are protected against metabolic syndrome. Diabetes 56, 518–521 (2007). [PubMed: 17259400]
129. Fuku N et al. Mitochondrial haplogroup N9a confers resistance against type 2 diabetes in Asians. Am. J. Hum. Genet 80, 407–415 (2007). [PubMed: 17273962]
130. Palmieri VO et al. T16189C mitochondrial DNA variant is associated with metabolic syndrome in Caucasian subjects. Nutrition 27, 773–777 (2011). [PubMed: 21146361]
131. Ye W, Chen S, Jin S & Lu J A novel heteroplasmic mitochondrial DNA mutation, A8890G, in a patient with juvenile-onset metabolic syndrome: a case report. Mol. Med. Rep 8, 1060–1066 (2013). [PubMed: 23921547]
132. Mozhey OI et al. Evaluating the number of mitochondrial DNA copies in leukocytes and adipocytes from metabolic syndrome patients: pilot study. Mol. Biol 48, 590–593 (2014).
133. Lee HK et al. Decreased mitochondrial DNA content in peripheral blood precedes the development of non-insulin-dependent diabetes mellitus. Diabetes Res. Clin. Pract 42, 161–167 (1998). [PubMed: 9925346]
134. Kytövuori L et al. A novel mutation m.8561C>G in MT-ATP6/8 causing a mitochondrial syndrome with ataxia, peripheral neuropathy, diabetes mellitus, and hypergonadotropic hypogonadism. J. Neurol 263, 2188–2195 (2016). [PubMed: 27502083]
135. Bhansali S, Bhansali A, Walia R, Saikia UN & Dhawan V Alterations in mitochondrial oxidative stress and mitophagy in subjects with prediabetes and type 2 diabetes mellitus. Front. Endocrinol 8, 347 (2017).
136. Kelley DE, He J, Menshikova EV & Ritov VB Dysfunction of mitochondria in human skeletal muscle in type 2 diabetes. Diabetes 51, 2944–2950 (2002). [PubMed: 12351431]
137. Schrauwen P, Schrauwen-Hinderling V, Hoeks J & Hesselink MKC Mitochondrial dysfunction and lipotoxicity. Biochim. Biophys. Acta Biophys. Acta 1801, 266–271 (2010).
138. Fabbri E et al. Insulin resistance is associated with reduced mitochondrial oxidative capacity measured by 31P-magnetic resonance spectroscopy in participants without diabetes from the Baltimore Longitudinal Study of Aging. Diabetes 66, 170–176 (2017). [PubMed: 27737951]
139. Phielix E et al. Lower intrinsic ADP-stimulated mitochondrial respiration underlies in vivo mitochondrial dysfunction in muscle of male type 2 diabetic patients. Diabetes 57, 2943–2949 (2008). [PubMed: 18678616]
140. Mootha VK et al. PGC-1α-responsive genes involved in oxidative phosphorylation are coordinately downregulated in human diabetes. Nat. Genet 34, 267–273 (2003). [PubMed: 12808457]
141. Robertson R, Harmon J, Tran P, Tanaka Y & Takahashi H Glucose toxicity in β-cells: type 2 diabetes, good radicals gone bad, and the glutathione connection. Diabetes 52, 581–587 (2003). [PubMed: 12606496]
142. DeFronzo RA & Abdul-Ghani MA Preservation of β-cell function: the key to diabetes prevention. J. Clin. Endocrinol. Metab 96, 2354–2366 (2011). [PubMed: 21697254]
143. Anello M et al. Functional and morphological alterations of mitochondria in pancreatic beta cells from type 2 diabetic patients. Diabetologia 48, 282–289 (2005). [PubMed: 15654602]
144. Brereton MF et al. Hyperglycaemia induces metabolic dysfunction and glycogen accumulation in pancreatic β-cells. Nat. Commun 7, 13496 (2016). [PubMed: 27882918]
145. Ling C et al. Epigenetic regulation of PPARGC1A in human type 2 diabetic islets and effect on insulin secretion. Diabetologia 51, 615–622 (2008). [PubMed: 18270681]

146. Koeck T et al. A common variant in TFB1M is associated with reduced insulin secretion and increased future risk of type 2 diabetes. Cell Metab 13, 80–91 (2011). [PubMed: 21195351]

147. Goehring I et al. Plasma membrane potential oscillations in insulin secreting Ins-1 832/13 cells do not require glycolysis and are not initiated by fluctuations in mitochondrial bioenergetics. J. Biol. Chem 287, 15706–15717 (2012). [PubMed: 22418435]

148. MacDonald MJ et al. Decreased levels of metabolic enzymes in pancreatic islets of patients with type 2 diabetes. Diabetologia 52, 1087–1091 (2009). [PubMed: 19296078]

149. Butler A et al. β-cell deficit and increased β-cell apoptosis in humans with type 2 diabetes. Diabetes 102, 1295–1297 (2003).

150. Abd El-Kader SM & El-Den Ashmawy EMS Non-alcoholic fatty liver disease: the diagnosis and management. World J. Hepatol 7, 846–858 (2015). [PubMed: 25937862]

151. Valenti L, Bugianesi E, Pajvani U & Targher G Nonalcoholic fatty liver disease: cause or consequence of type 2 diabetes? Liver Int 36, 1563–1579 (2016). [PubMed: 27276701]

152. Paschos P & Paletas K Non-alcoholic fatty liver disease and the metabolic syndrome. Hippokratia 13, 9–19 (2009). [PubMed: 19240815]

153. Koliaki C et al. Adaptation of hepatic mitochondrial function in humans with non-alcoholic fatty liver is lost in steatohepatitis. Cell Metab 21, 739–746 (2015). [PubMed: 25955209]

154. Pérez-Carreras M et al. Defective hepatic mitochondrial respiratory chain in patients with nonalcoholic steatohepatitis. Hepatology 38, 999–1007 (2003). [PubMed: 14512887]

155. Kendrick AA et al. Fatty liver is associated with reduced SIRT3 activity and mitochondrial protein hyperacetylation. Biochem. J 433, 505–514 (2011). [PubMed: 21044047]

156. Rossmeisl M et al. Decreased fatty acid synthesis due to mitochondrial uncoupling in adipose tissue. FASEB J 14, 1793–1800 (2000). [PubMed: 10973929]

157. Serra D, Mera P, Malandrino MI, Mir JF & Herrero L Mitochondrial fatty acid oxidation in obesity. Antioxid. Redox Signal 19, 269–284 (2013). [PubMed: 22900819]

158. Kotronen A, Seppälä-Lindroos A, Bergholm R & Yki-Järvinen H Tissue specificity of insulin resistance in humans: fat in the liver rather than muscle is associated with features of the metabolic syndrome. Diabetologia 51, 130–138 (2008). [PubMed: 18008059]

159. Pessayre D & Fromenty B NASH: a mitochondrial disease. J. Hepatol 42, 928–940 (2005). [PubMed: 15885365]

160. Rector RS et al. Mitochondrial dysfunction precedes insulin resistance and hepatic steatosis and contributes to the natural history of non-alcoholic fatty liver disease in an obese rodent model. J. Hepatol 52, 727–736 (2010). [PubMed: 20347174]

161. Cortez-Pinto H et al. Alterations in liver ATP homeostasis in human nonalcoholic steatohepatitis: a pilot study. JAMA 282, 1659–1664 (1999). [PubMed: 10553793]

162. Teodoro J, Rolo AP, Oliveira PJ & Palmeira CM Decreased ANT content in Zucker fatty rats: relevance for altered hepatic mitochondrial bioenergetics in steatosis. FEBS Lett 580, 2153–2157 (2006). [PubMed: 16554051]

163. Teodoro JS, Rolo AP, Duarte FV, Simões AM & Palmeira CM Differential alterations in mitochondrial function induced by a choline-deficient diet: understanding fatty liver disease progression. Mitochondrion 8, 367–376 (2008). [PubMed: 18765303]

164. Shami GJ et al. Three-dimensional ultrastructure of giant mitochondria in human non-alcoholic fatty liver disease. Sci. Rep 11, 3319 (2021). [PubMed: 33558594]

165. Sunny NE, Parks EJ, Browning JD & Burgess SC Excessive hepatic mitochondrial TCA cycle and gluconeogenesis in humans with nonalcoholic fatty liver disease. Cell Metab 14, 804–810 (2011). [PubMed: 22152305]

166. Patterson RE et al. Lipotoxicity in steatohepatitis occurs despite an increase in tricarboxylic acid cycle activity. Am. J. Physiol. Endocrinol. Metab 310, E484–E494 (2016). [PubMed: 26814015]

167. Satapati S et al. Elevated TCA cycle function in the pathology of diet-induced hepatic insulin resistance and fatty liver. J. Lipid Res 53, 1080–1092 (2012). [PubMed: 22493093]

168. El Assar M, Angulo J & Rodríguez-Mañas L Oxidative stress and vascular inflammation in aging. Free Radic. Biol. Med 65, 380–401 (2013). [PubMed: 23851032]

169. Jani B & Rajkumar C Ageing and vascular ageing. Postgrad. Med. J 82, 357–362 (2006). [PubMed: 16754702]
170. Ding YN, Tang X, Chen HZ & Liu DP Epigenetic regulation of vascular aging and age-related vascular diseases. Adv. Exp. Med. Biol 1086, 55–75 (2018). [PubMed: 30232752]
171. Thomas SR, Witting PK & Drummond GR Redox control of endothelial function and dysfunction: molecular mechanisms and therapeutic opportunities. Antioxid. Redox Signal 10, 1713–1765 (2008). [PubMed: 18707220]
172. Abe J & Berk BC Reactive oxygen species as mediators of signal transduction in cardiovascular disease. Trends Cardiovasc. Med 8, 59–64 (1998). [PubMed: 21235913]
173. Grootaert MOJ, Roth L, Schrijvers DM, De Meyer GRY & Martinet W Defective autophagy in atherosclerosis: to die or to senesce? Oxid. Med. Cell. Longev 2018, 7687083 (2018). [PubMed: 29682164]
174. Sobenin IA, Sazonova MA, Postnov AY, Bobryshev YV & Orekhov AN Changes of mitochondria in atherosclerosis: possible determinant in the pathogenesis of the disease. Atherosclerosis 227, 283–288 (2013). [PubMed: 23395464]
175. Nomiyama T et al. Accumulation of somatic mutation in mitochondrial DNA and atherosclerosis in diabetic patients. Ann. N. Y. Acad. Sci 1011, 193–204 (2004). [PubMed: 15126297]
176. Bhopal RS & Rafnsson SB Could mitochondrial efficiency explain the susceptibility to adiposity, metabolic syndrome, diabetes and cardiovascular diseases in South Asian populations? Int. J. Epidemiol 38, 1072–1081 (2009). [PubMed: 19423659]
177. Corral-Debrinski M et al. Hypoxemia is associated with mitochondrial DNA damage and gene induction: implications for cardiac disease. JAMA 266, 1812–1816 (1991). [PubMed: 1890710]
178. Ding Y, Xia B, Yu J, Leng J & Huang J Mitochondrial DNA mutations and essential hypertension (review). Int. J. Mol. Med 32, 768–774 (2013). [PubMed: 23900616]
179. Liu CS et al. Alteration of the copy number of mitochondrial DNA in leukocytes of patients with hyperlipidemia. Ann. N. Y. Acad. Sci 1042, 70–75 (2005). [PubMed: 15965047]
180. Naik E & Dixit VM Mitochondrial reactive oxygen species drive proinflammatory cytokine production. J. Exp. Med 208, 417–420 (2011). [PubMed: 21357740]
181. Davies JMS et al. The Oxygen Paradox, the French Paradox, and age-related diseases. GeroScience 39, 499–550 (2017). [PubMed: 29270905]
182. Wenzel P et al. Manganese superoxide dismutase and aldehyde dehydrogenase deficiency increase mitochondrial oxidative stress and aggravate age-dependent vascular dysfunction. Cardiovasc. Res 80, 280–289 (2008). [PubMed: 18596060]
183. Oelze M et al. Glutathione peroxidase-1 deficiency potentiates dysregulatory modifications of endothelial nitric oxide synthase and vascular dysfunction in aging. Hypertension 63, 390–396 (2014). [PubMed: 24296279]
184. Giorgio M et al. Electron transfer between cytochrome c and p66Shc generates reactive oxygen species that trigger mitochondrial apoptosis. Cell 122, 221–233 (2005). [PubMed: 16051147]
185. Migliaccio E et al. The p66^{Shc} adaptor protein controls oxidative stress response and life span in mammals. Nature 402, 309–313 (1999). [PubMed: 10580504]
186. Francia P et al. Deletion of p66^{Shc} gene protects against age-related endothelial dysfunction. Circulation 110, 2889–2895 (2004). [PubMed: 15505103]
187. Camici GG et al. Genetic deletion of p66^{Shc} adaptor protein prevents hyperglycemia-induced endothelial dysfunction and oxidative stress. Proc. Natl Acad. Sci. USA 104, 5217–5222 (2007). [PubMed: 17360381]
188. Berniakovich I et al. p66^{Shc}-generated oxidative signal promotes fat accumulation. J. Biol. Chem 283, 34283–34293 (2008). [PubMed: 18838380]
189. Paneni F, Costantino S & Cosentino F p66^{Shc}-induced redox changes drive endothelial insulin resistance. Atherosclerosis 236, 426–429 (2014). [PubMed: 25150941]
190. Pagnin E et al. Diabetes induces p66^{shc} gene expression in human peripheral blood mononuclear cells: relationship to oxidative stress. J. Clin. Endocrinol. Metab 90, 1130–1136 (2005). [PubMed: 15562031]

191. Rota M et al. Diabetes promotes cardiac stem cell aging and heart failure, which are prevented by deletion of the p66^{shc} gene. Circ. Res 99, 42–52 (2006). [PubMed: 16763167]
192. Ciciliot S et al. *p66Shc* deletion or deficiency protects from obesity but not metabolic dysfunction in mice and humans. Diabetologia 58, 2352–2360 (2015). [PubMed: 26122877]
193. Dela Cruz CS & Kang MJ Mitochondrial dysfunction and damage associated molecular patterns (DAMPs) in chronic inflammatory diseases. Mitochondrion 41, 37–44 (2018). [PubMed: 29221810]
194. Chaplin DD Overview of the immune response. J. Allergy Clin. Immunol 125, S3–S23 (2010). [PubMed: 20176265]
195. Sugimoto MA, Sousa LP, Pinho V, Perretti M & Teixeira MM Resolution of inflammation: what controls its onset? Front. Immunol 7, 160 (2016). [PubMed: 27199985]
196. Akira S, Uematsu S & Takeuchi O Pathogen recognition and innate immunity. Cell 124, 783–801 (2006). [PubMed: 16497588]
197. Jain A & Pasare C Innate control of adaptive immunity: beyond the three-signal paradigm. J. Immunol 198, 3791–3800 (2017). [PubMed: 28483987]
198. Brubaker SW, Bonham KS, Zanoni I & Kagan JC Innate immune pattern recognition: a cell biological perspective. Annu. Rev. Immunol 33, 257–290 (2015). [PubMed: 25581309]
199. Gong T, Liu L, Jiang W & Zhou R DAMP-sensing receptors in sterile inflammation and inflammatory diseases. Nat. Rev. Immunol 20, 95–112 (2020). [PubMed: 31558839]
200. Roger AJ, Muñoz-Gómez SA & Kamikawa R The origin and diversification of mitochondria. Curr. Biol 27, R1177–R1192 (2017). [PubMed: 29112874]
201. West AP & Shadel GS Mitochondrial DNA in innate immune responses and inflammatory pathology. Nat. Rev. Immunol 17, 363–375 (2017). [PubMed: 28393922]
202. Garcia-Martinez I et al. Hepatocyte mitochondrial DNA drives nonalcoholic steatohepatitis by activation of TLR9. J. Clin. Invest 126, 859–864 (2016). [PubMed: 26808498]
203. Bufe B et al. Recognition of bacterial signal peptides by mammalian formyl peptide receptors: a new mechanism for sensing pathogens. J. Biol. Chem 290, 7369–7387 (2015). [PubMed: 25605714]
204. Oemer G et al. Molecular structural diversity of mitochondrial cardiolipins. Proc. Natl Acad. Sci. USA 115, 4158–4163 (2018). [PubMed: 29618609]
205. Chandel NS Evolution of mitochondria as signaling organelles. Cell Metab 22, 204–206 (2015). [PubMed: 26073494]
206. Elliott MR et al. Nucleotides released by apoptotic cells act as a find-me signal to promote phagocytic clearance. Nature 461, 282–286 (2009). [PubMed: 19741708]
207. Mills E & O'Neill LAJ Succinate: a metabolic signal in inflammation. Trends Cell Biol 24, 313–320 (2014). [PubMed: 24361092]
208. Tannahill GM et al. Succinate is an inflammatory signal that induces IL-1β through HIF-1α. Nature 496, 238–242 (2013). [PubMed: 23535595]
209. Harris HE, Andersson U & Pisetsky DS HMGB1: a multifunctional alarmin driving autoimmune and inflammatory disease. Nat. Rev. Rheumatol 8, 195–202 (2012). [PubMed: 22293756]
210. Wang D, Malo D & Hekimi S Elevated mitochondrial reactive oxygen species generation affects the immune response via hypoxia-inducible factor-1α in long-lived *Mclk1*+/− mouse mutants. J. Immunol 184, 582–590 (2010). [PubMed: 20007531]
211. Mittal M, Siddiqui MR, Tran K, Reddy SP & Malik AB Reactive oxygen species in inflammation and tissue injury. Antioxid. Redox Signal 20, 1126–1167 (2014). [PubMed: 23991888]
212. Chandel NS, Trzyna WC, McClintock DS & Schumacker PT Role of oxidants in NF-κB activation and TNF-α gene transcription induced by hypoxia and endotoxin. J. Immunol 165, 1013–1021 (2000). [PubMed: 10878378]
213. Knowler WC et al. Reduction in the incidence of type 2 diabetes with lifestyle intervention or metformin. N. Engl. J. Med 346, 393–403 (2002). [PubMed: 11832527]
214. Toledo FGS et al. Effects of physical activity and weight loss on skeletal muscle mitochondria and relationship with glucose control in type 2 diabetes. Diabetes 56, 2142–2147 (2007). [PubMed: 17536063]

215. Phielix E, Meex R, Moonen-Kornips E, Hesselink MKC & Schrauwen P Exercise training increases mitochondrial content and ex vivo mitochondrial function similarly in patients with type 2 diabetes and in control individuals. Diabetologia 53, 1714–1721 (2010). [PubMed: 20422397]
216. Ross JM et al. Voluntary exercise normalizes the proteomic landscape in muscle and brain and improves the phenotype of progeroid mice. Aging Cell 18, e13029 (2019). [PubMed: 31489782]
217. Omodei D & Fontana L Calorie restriction and prevention of age-associated chronic disease. FEBS Lett 585, 1537–1542 (2011). [PubMed: 21402069]
218. Civitarese AE et al. Calorie restriction increases muscle mitochondrial biogenesis in healthy humans. PLoS Med 4, e76 (2007). [PubMed: 17341128]
219. Brand MD Uncoupling to survive? The role of mitochondrial inefficiency in ageing. Exp. Gerontol 35, 811–820 (2000). [PubMed: 11053672]
220. Barja G Endogenous oxidative stress: relationship to aging, longevity and caloric restriction. Ageing Res. Rev 1, 397–411 (2002). [PubMed: 12067594]
221. Kume S et al. Calorie restriction enhances cell adaptation to hypoxia through Sirt1-dependent mitochondrial autophagy in mouse aged kidney. J. Clin. Invest 120, 1043–1055 (2010). [PubMed: 20335657]
222. Pardo R et al. Calorie restriction prevents diet-induced insulin resistance independently of PGC-1-driven mitochondrial biogenesis in white adipose tissue. FASEB J 33, 2343–2358 (2019). [PubMed: 30277821]
223. Davinelli S, De Stefani D, De Vivo I & Scapagnini G Polyphenols as caloric restriction mimetics regulating mitochondrial biogenesis and mitophagy. Trends Endocrinol. Metab 31, 536–550 (2020). [PubMed: 32521237]
224. Price NL et al. SIRT1 is required for AMPK activation and the beneficial effects of resveratrol on mitochondrial function. Cell Metab 15, 675–690 (2012). [PubMed: 22560220]
225. Hubbard BP et al. Evidence for a common mechanism of SIRT1 regulation by allosteric activators. Science 339, 1216–1219 (2013). [PubMed: 23471411]
226. Baur JA et al. Resveratrol improves health and survival of mice on a high-calorie diet. Nature 444, 337–342 (2006). [PubMed: 17086191]
227. Lagouge M et al. Resveratrol improves mitochondrial function and protects against metabolic disease by activating SIRT1 and PGC-1α. Cell 127, 1109–1122 (2006). [PubMed: 17112576]
228. Timmers S et al. Calorie restriction-like effects of 30 days of resveratrol supplementation on energy metabolism and metabolic profile in obese humans. Cell Metab 14, 612–622 (2011). [PubMed: 22055504]
229. Biala A et al. Resveratrol induces mitochondrial biogenesis and ameliorates Ang II-induced cardiac remodeling in transgenic rats harboring human renin and angiotensinogen genes. Blood Press 19, 196–205 (2010). [PubMed: 20429690]
230. Kuno A et al. Resveratrol ameliorates mitophagy disturbance and improves cardiac pathophysiology of dystrophin-deficient *mdx* mice. Sci. Rep 8, 15555 (2018). [PubMed: 30348945]
231. Milne JC et al. Small molecule activators of SIRT1 as therapeutics for the treatment of type 2 diabetes. Nature 450, 712–716 (2007). [PubMed: 18046409]
232. Baksi A et al. A phase II, randomized, placebo-controlled, double-blind, multi-dose study of SRT2104, a SIRT1 activator, in subjects with type 2 diabetes. Br. J. Clin. Pharmacol 78, 69–77 (2014). [PubMed: 24446723]
233. Cao K et al. Punicalagin, an active component in pomegranate, ameliorates cardiac mitochondrial impairment in obese rats via AMPK activation. Sci. Rep 5, 14014 (2015). [PubMed: 26369619]
234. Taub PR et al. Alterations in skeletal muscle indicators of mitochondrial structure and biogenesis in patients with type 2 diabetes and heart failure: effects of epicatechin rich cocoa. Clin. Transl. Sci 5, 43–47 (2012). [PubMed: 22376256]
235. Porcu C et al. Oleuropein induces AMPK-dependent autophagy in NAFLD mice, regardless of the gender. Int. J. Mol. Sci 19, E3948 (2018). [PubMed: 30544824]
236. Canto C & Auwerx J AMP-activated protein kinase and its downstream transcriptional pathways. Cell. Mol. Life Sci 67, 3407–3423 (2010). [PubMed: 20640476]

237. Viscomi C et al. In vivo correction of COX deficiency by activation of the AMPK/PGC-1α axis. Cell Metab 14, 80–90 (2011). [PubMed: 21723506]
238. Bharath LP et al. Metformin enhances autophagy and normalizes mitochondrial function to alleviate aging-associated inflammation. Cell Metab 32, 44–55 (2020). [PubMed: 32402267]
239. Wang Y et al. Metformin improves mitochondrial respiratory activity through activation of AMPK. Cell Rep 29, 1511–1523 (2019). [PubMed: 31693892]
240. Detaille D et al. Imeglimin prevents human endothelial cell death by inhibiting mitochondrial permeability transition without inhibiting mitochondrial respiration. Cell Death Discov 2, 15072 (2016). [PubMed: 27551496]
241. Hallakou-Bozec S et al. Mechanism of action of imeglimin: a novel therapeutic agent for type 2 diabetes. Diabetes Obes. Metab 23, 664–673 (2021). [PubMed: 33269554]
242. Tavallaie M et al. Moderation of mitochondrial respiration mitigates metabolic syndrome of aging. Proc. Natl Acad. Sci. USA 117, 9840–9850 (2020). [PubMed: 32303655]
243. Garay RP Investigational drugs and nutrients for human longevity. Recent clinical trials registered in ClinicalTrials.gov and clinicaltrialsregister.eu. Expert Opin. Investig. Drugs 30, 749–758 (2021).
244. Polak P et al. Adipose-specific knockout of raptor results in lean mice with enhanced mitochondrial respiration. Cell Metab 8, 399–410 (2008). [PubMed: 19046571]
245. Binsch C et al. Absence of the kinase S6k1 mimics the effect of chronic endurance exercise on glucose tolerance and muscle oxidative stress. Mol. Metab 6, 1443–1453 (2017). [PubMed: 29107291]
246. Deepaa SS et al. Rapamycin modulates markers of mitochondrial biogenesis and fatty acid oxidation in the adipose tissue of db/db mice. J. Biochem. Pharmacol. Res 1, 114–123 (2013). [PubMed: 24010023]
247. Gran P & Cameron-Smith D The actions of exogenous leucine on mTOR signalling and amino acid transporters in human myotubes. BMC Physiol 11, 10 (2011). [PubMed: 21702994]
248. Li H, Xu M, Lee J, He C & Xie Z Leucine supplementation increases SIRT1 expression and prevents mitochondrial dysfunction and metabolic disorders in high-fat diet-induced obese mice. Am. J. Physiol. Endocrinol. Metab 303, E1234–E1244 (2012). [PubMed: 22967499]
249. Leenders M et al. Prolonged leucine supplementation does not augment muscle mass or affect glycemic control in elderly type 2 diabetic men. J. Nutr 141, 1070–1076 (2011). [PubMed: 21525248]
250. Zemel MB Modulation of energy sensing by leucine synergy with natural sirtuin activators: effects on health span. J. Med. Food 23, 1129–1135 (2020). [PubMed: 32758058]
251. Rajman L, Chwalek K & Sinclair DA Therapeutic potential of NAD-boosting molecules: the in vivo evidence. Cell Metab 27, 529–547 (2018). [PubMed: 29514064]
252. Yoshino J, Mills KF, Yoon MJ & Imai S Nicotinamide mononucleotide, a key NAD(+) intermediate, treats the pathophysiology of diet- and age-induced diabetes in mice. Cell Metab 14, 528–536 (2011). [PubMed: 21982712]
253. Stromsdorfer KL et al. NAMPT-mediated NAD^+ biosynthesis in adipocytes regulates adipose tissue function and multi-organ insulin sensitivity in mice. Cell Rep 16, 1851–1860 (2016). [PubMed: 27498863]
254. Mills K et al. Long-term administration of nicotinamide mononucleotide mitigates age-associated physiological decline in mice. Cell Metab 24, 795–806 (2016). [PubMed: 28068222]
255. Canto C et al. The NAD^+ precursor nicotinamide riboside enhances oxidative metabolism and protects against high-fat diet-induced obesity. Cell Metab 15, 838–847 (2012). [PubMed: 22682224]
256. Hong W, Mo F, Zhang Z, Huang M & Wei X Nicotinamide mononucleotide: a promising molecule for therapy of diverse diseases by targeting NAD+ metabolism. Front. Cell Dev. Biol 8, 246 (2020). [PubMed: 32411700]
257. Trammell SAJ et al. Nicotinamide riboside opposes type 2 diabetes and neuropathy in mice. Sci. Rep 6, 26933 (2016). [PubMed: 27230286]

258. Gariani K et al. Eliciting the mitochondrial unfolded protein response by nicotinamide adenine dinucleotide repletion reverses fatty liver disease in mice. Hepatology 63, 1190–1204 (2016). [PubMed: 26404765]

259. Dollerup OL et al. A randomized placebo-controlled clinical trial of nicotinamide riboside in obese men: safety, insulin-sensitivity, and lipid-mobilizing effects. Am. J. Clin. Nutr 108, 343–353 (2018). [PubMed: 29992272]

260. Yang SJ et al. Nicotinamide improves glucose metabolism and affects the hepatic NAD–sirtuin pathway in a rodent model of obesity and type 2 diabetes. J. Nutr. Biochem 25, 66–72 (2014). [PubMed: 24314867]

261. Gariani K et al. Inhibiting poly ADP-ribosylation increases fatty acid oxidation and protects against fatty liver disease. J. Hepatol 66, 132–141 (2017). [PubMed: 27663419]

262. Weinbach EC & Garbus J Mechanism of action of reagents that uncouple oxidative phosphorylation. Nature 221, 1016–1018 (1969). [PubMed: 4180173]

263. Tainter ML, Stockton AB & Cutting WC Use of dinitrophenol in obesity and related conditions. J. Am. Med. Assoc 101, 1472–1475 (1933).

264. Caldeira Da Silva CC, Cerqueira FM, Barbosa LF, Medeiros MHG & Kowaltowski AJ Mild mitochondrial uncoupling in mice affects energy metabolism, redox balance and longevity. Aging Cell 7, 552–560 (2008). [PubMed: 18505478]

265. Goldgof M et al. The chemical uncoupler 2,4-dinitrophenol (DNP) protects against diet-induced obesity and improves energy homeostasis in mice at thermoneutrality. J. Biol. Chem 289, 19341–19350 (2014). [PubMed: 24872412]

266. Perry RJ, Zhang D, Zhang XM, Boyer JL & Shulman GI Controlled-release mitochondrial protonophore reverses diabetes and protonophore reverses diabetes and steatohepatitis in rats. Science 347, 1253–1256 (2015). [PubMed: 25721504]

267. Perry RJ et al. Reversal of hypertriglyceridemia, fatty liver disease, and insulin resistance by a liver-targeted mitochondrial uncoupler. Cell Metab 18, 740–748 (2013). [PubMed: 24206666]

268. Goedeke L et al. Controlled-release mitochondrial protonophore (CRMP) reverses dyslipidemia and hepatic steatosis in dysmetabolic nonhuman primates. Sci. Transl. Med 11, eaay0284 (2019). [PubMed: 31578240]

269. Tao H, Zhang Y, Zeng X, Shulman GI & Jin S Niclosamide ethanolamine-induced mild mitochondrial uncoupling improves diabetic symptoms in mice. Nat. Med 20, 1263–1269 (2014). [PubMed: 25282357]

270. Figarola JL et al. COH-SR4 reduces body weight, improves glycemic control and prevents hepatic steatosis in high fat diet-induced obese mice. PLoS ONE 8, e83801 (2013). [PubMed: 24376752]

271. Fu YY et al. A novel chemical uncoupler ameliorates obesity and related phenotypes in mice with diet-induced obesity by modulating energy expenditure and food intake. Diabetologia 56, 2297–2307 (2013). [PubMed: 23912111]

272. Kanemoto N et al. Antidiabetic and cardiovascular beneficial effects of a liver-localized mitochondrial uncoupler. Nat. Commun 10, 2172 (2019). [PubMed: 31092829]

273. Alexopoulos SJ et al. Mitochondrial uncoupler BAM15 reverses diet-induced obesity and insulin resistance in mice. Nat. Commun 11, 2397 (2020). [PubMed: 32409697]

274. Childress ES et al. [1,2,5]Oxadiazolo[3,4-*b*]pyrazine-5,6-diamine derivatives as mitochondrial uncouplers for the potential treatment of nonalcoholic steatohepatitis. J. Med. Chem 63, 2511–2526 (2020). [PubMed: 32017849]

275. Salamoun JM et al. 6-Amino[1,2,5]oxadiazolo[3,4-*b*] pyrazin-5-ol derivatives as efficacious mitochondrial uncouplers in STAM mouse model of nonalcoholic steatohepatitis. J. Med. Chem 63, 6203–6224 (2020). [PubMed: 32392051]

276. Jiang H et al. Mitochondrial uncoupling coordinated with PDH activation safely ameliorates hyperglycemia via promoting glucose oxidation. Diabetes 68, 2197–2209 (2019). [PubMed: 31471292]

277. Rivus Pharmaceuticals. Rivus Pharmaceuticals announces positive results from phase 1 trial of lead candidate HU6, demonstrating safety, efficacy in key targets for multiple cardio-metabolic diseases https://www.rivuspharma.com. (2021).

278. Silva FS, Simoes RF, Couto R & Oliveira PJ Targeting mitochondria in cardiovascular diseases. Curr. Pharm. Des 22, 5698–5717 (2016). [PubMed: 27549376]

279. Safarpour P et al. Vitamin D supplementation improves SIRT1, irisin, and glucose indices in overweight or obese type 2 diabetic patients: a double-blind randomized placebo-controlled clinical trial. BMC Fam. Pract 21, 26 (2020). [PubMed: 32033527]

280. Yi X & Maeda N α-Lipoic acid prevents the increase in atherosclerosis induced by diabetes in apolipoprotein E-deficient mice fed high-fat/low-cholesterol diet. Diabetes 55, 2238–2244 (2006). [PubMed: 16873686]

281. Eskurza I, Monahan KD, Robinson JA & Seals DR Effect of acute and chronic ascorbic acid on flow-mediated dilatation with sedentary and physically active human ageing. J. Physiol 556, 315–324 (2004). [PubMed: 14754992]

282. Mao G et al. A mitochondria-targeted vitamin E derivative decreases hepatic oxidative stress and inhibits fat deposition in mice. J. Nutr 140, 1425–1431 (2010). [PubMed: 20554905]

283. Jauslin ML, Meier T, Smith RAJ & Murphy PM Mitochondria-targeted antioxidants protect Friedreich ataxia fibroblasts from endogenous oxidative stress more effectively than untargeted antioxidants. FASEB J 17, 1972–1974 (2003). [PubMed: 12923074]

284. James AM et al. Interaction of the mitochondria-targeted antioxidant MitoQ with phospholipid bilayers and ubiquinone oxidoreductases. J. Biol. Chem 282, 14708–14718 (2007). [PubMed: 17369262]

285. Zhou J et al. Mitochondrial-targeted antioxidant MitoQ provides neuroprotection and reduces neuronal apoptosis in experimental traumatic brain injury possibly via the Nrf2-ARE pathway. Am. J. Transl. Res 10, 1887–1899 (2018). [PubMed: 30018728]

286. Rossman MJ et al. Chronic supplementation with a mitochondrial antioxidant (MitoQ) improves vascular function in healthy older adults. Hypertension 71, 1056–1063 (2018). [PubMed: 29661838]

287. Young ML & Franklin JL The mitochondria-targeted antioxidant MitoQ inhibits memory loss, neuropathology, and extends lifespan in aged 3xTg-AD mice. Mol. Cell. Neurosci 101, 103409 (2019). [PubMed: 31521745]

288. Bhatti JS et al. Protective effects of a mitochondria-targeted small peptide SS31 against hyperglycemia-induced mitochondrial abnormalities in the liver tissues of diabetic mice, Tallyho/JngJ mice. Mitochondrion 58, 49–58 (2021). [PubMed: 33639273]

289. Bhatti JS et al. Mitochondria-targeted small peptide, SS31 ameliorates diabetes induced mitochondrial dynamics in male TallyHO/JngJ mice. Mol. Neurobiol 58, 795–808 (2021). [PubMed: 33025510]

290. Thomas DA et al. Mitochondrial targeting with antioxidant peptide SS-31 prevents mitochondrial depolarization, reduces islet cell apoptosis, increases islet cell yield, and improves posttransplantation function. J. Am. Soc. Nephrol 18, 213–222 (2007). [PubMed: 17151329]

291. Escribano-Lopez I et al. The mitochondrial antioxidant SS-31 increases SIRT1 levels and ameliorates inflammation, oxidative stress and leukocyte-endothelium interactions in type 2 diabetes. Sci. Rep 8, 15862 (2018). [PubMed: 30367115]

292. Zhang M et al. Chronic administration of mitochondrion-targeted peptide SS-31 prevents atherosclerotic development in ApoE knockout mice fed Western diet. PLoS ONE 12, e0185688 (2017). [PubMed: 28961281]

293. Roshanravan B et al. In vivo mitochondrial ATP production is improved in older adult skeletal muscle after a single dose of elamipretide in a randomized trial. PLoS ONE 16, e0253849 (2021). [PubMed: 34264994]

294. Wahab A et al. Significance of resveratrol in clinical management of chronic diseases. Molecules 22, 1329 (2017).

295. Si H, Lai C-Q & Liu D Dietary epicatechin, a novel anti-aging bioactive small molecule. Curr. Med. Chem 28, 3–18 (2021). [PubMed: 31886745]

296. Castejón ML, Montoya T, Alarcón-de-la-lastra C & Sánchez-hidalgo M Potential protective role exerted by secoiridoids from *Olea europaea* L. in cancer, cardiovascular, neurodegenerative, aging-related, and immunoinflammatory diseases. Antioxidants 9, 149 (2020).

297. Lv Z & Guo Y Metformin and its benefits for various diseases. Front. Endocrinol 11, 191 (2020).

298. Custodero C et al. Nicotinamide riboside–a missing piece in the puzzle of exercise therapy for older adults? Exp. Gerontol 137, 110972 (2020). [PubMed: 32450270]

299. Fontana L & Partridge L Promoting health and longevity through diet: from model organisms to humans. Cell 161, 106–118 (2015). [PubMed: 25815989]

300. Hipp MS, Kasturi P & Hartl FU The proteostasis network and its decline in ageing. Nat. Rev. Mol. Cell Biol 20, 421–435 (2019). [PubMed: 30733602]

301. Sedlackova L & Korolchuk VI Mitochondrial quality control as a key determinant of cell survival. Biochim. Biophys. Acta Mol. Cell. Res 1866, 575–587 (2019). [PubMed: 30594496]

302. Nagashima S, Tokuyama T, Yonashiro R, Inatome R & Yanagi S Roles of mitochondrial ubiquitin ligase MITOL/MARCH5 in mitochondrial dynamics and diseases. J. Biochem 155, 273–279 (2014). [PubMed: 24616159]

303. Peng J, Ren K, Di, Yang J & Luo XJ Mitochondrial E3 ubiquitin ligase 1: a key enzyme in regulation of mitochondrial dynamics and functions. Mitochondrion 28, 49–53 (2016). [PubMed: 27034206]

304. Voos W Chaperone-protease networks in mitochondrial protein homeostasis. Biochim. Biophys. Acta 1833, 388–399 (2013). [PubMed: 22705353]

305. Hamon MP, Bulteau AL & Friguet B Mitochondrial proteases and protein quality control in ageing and longevity. Ageing Res. Rev 23, 56–66 (2015). [PubMed: 25578288]

306. Yang J et al. Lon in maintaining mitochondrial and endoplasmic reticulum homeostasis. Arch. Toxicol 92, 1913–1923 (2018). [PubMed: 29721585]

307. Kang SG, Dimitrova MN, Ortega J, Ginsburg A & Maurizi MR Human mitochondrial ClpP is a stable heptamer that assembles into a tetradecamer in the presence of ClpX. J. Biol. Chem 280, 35424–35432 (2005). [PubMed: 16115876]

308. Gispert S et al. Loss of mitochondrial peptidase Clpp leads to infertility, hearing loss plus growth retardation via accumulation of CLPX, mtDNA and inflammatory factors. Hum. Mol. Genet 22, 4871–4887 (2013). [PubMed: 23851121]

309. Torres-Odio S et al. Loss of mitochondrial protease CLPP activates type I IFN responses through the mitochondrial DNA–cGAS–STING signaling axis. J. Immunol 206, 1890–1900 (2021). [PubMed: 33731338]

Key points

- The rate of ageing is coordinated, at least in part, by conserved genetic and biochemical pathways.
- A complex network of interactions between longevity pathways reveals an intricate regulation of mitochondrial physiology during ageing.
- Cellular metabolism interconnects the nine hallmarks of ageing, and deregulation of energy metabolism by environmental variations is an essential process leading to mitochondrial dysfunction during ageing.
- A better understanding of mitochondrial dysfunction during ageing and age-related metabolic diseases will provide fundamental knowledge to develop therapies to combat late-life morbidities.
- Human longitudinal studies will be essential to understand individuals' risk of diseases much earlier in life, and will inform health choices and medical care options.

Box 1 |

The hallmarks of ageing

In a seminal review in 2013, López-Otín and colleagues took on the task of providing some order to the multitude of biological pathways that are involved in the ageing process by proposing that all factors implicated in ageing are organized into nine categories, or hallmarks. Criteria for being hallmarks are that the process has to be present during normal ageing and affects the pace of ageing, if experimentally manipulated. The list includes primary hallmarks, whose effects are undoubtedly negative, such as: genomic instability, telomere loss, epigenetic alteration and loss of proteostasis. Antagonistic hallmarks, whose effects can be beneficial or deleterious depending on their intensity, are deregulated nutrient sensing, mitochondrial dysfunction and cellular senescence. Last are the integrative hallmarks that affect the homeostasis and functionality of the tissue and include stem cell exhaustion and altered intercellular communication[2].

Notably, even though several interventions that target one of the hallmarks have dramatic benefits on the ageing process in different organisms, such as improving nutrient sensing pathways or proteostasis[299,300], not all hallmarks completely fulfil the last criterion, which consists of slowing down the ageing process by ameliorating the hallmark. This latter requirement remains the most difficult to achieve, mainly due to the pervasive interconnectedness among the ageing hallmarks. The challenge is to improve our understanding of the exact causal network and the interdependence among the hallmarks to develop targeted therapies to improve their function and slow down the ageing process.

Box 2 |

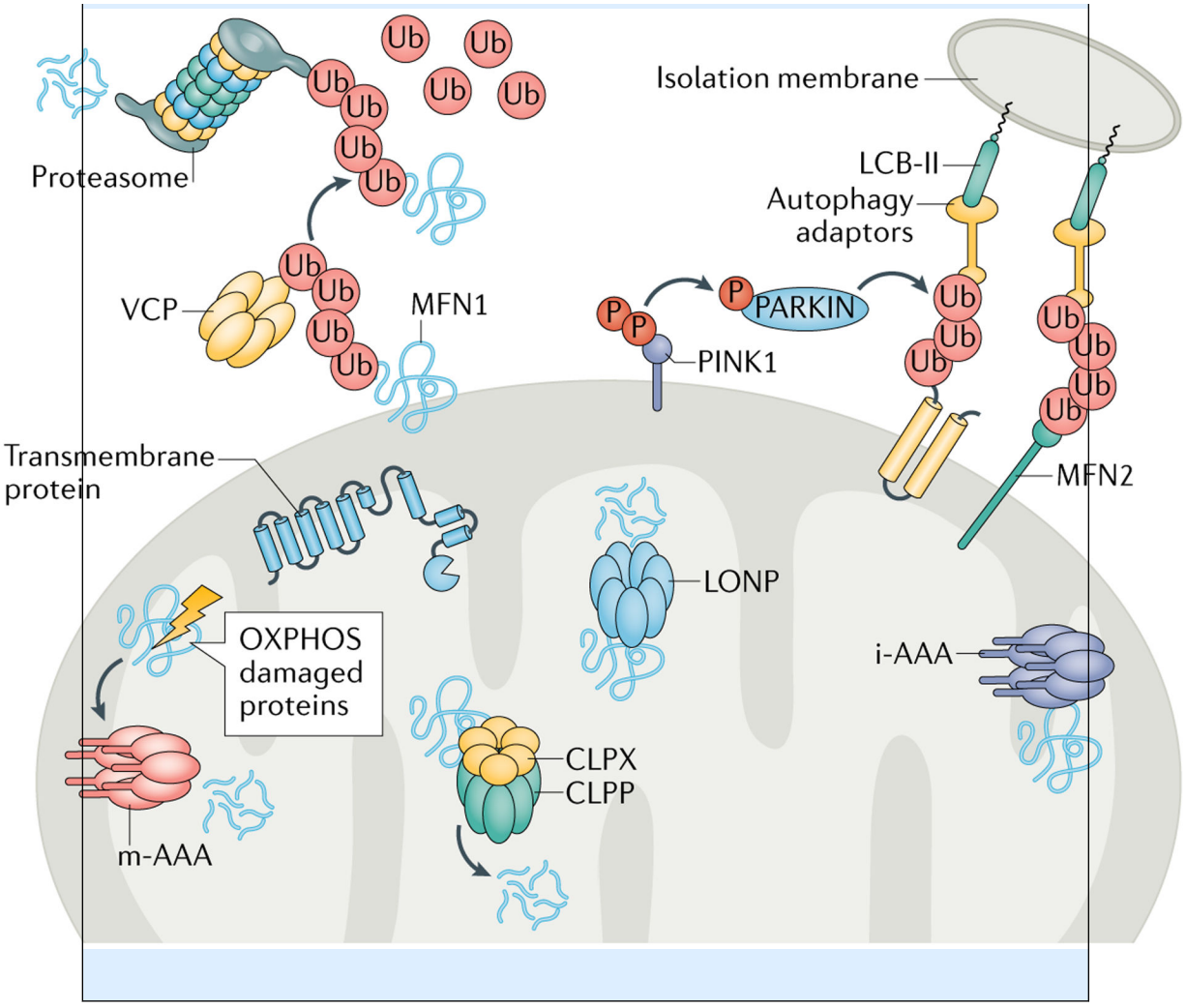
Mitochondrial quality control

To ensure a healthy mitochondrial network, cells have quality control mechanisms that preserve mitochondrial integrity (see the figure) by refolding misfolded proteins, removing those that are not recoverable, and eliminating dysfunctional complexes[301].

The ubiquitin proteasome system (UPS) exerts a first level of surveillance on nuclear-encoded mitochondrial proteins immediately after their translation, ensuring that misfolded or mis-localized proteins are removed before they become a threat to proteostasis. Outer membrane proteins facing the cytoplasm can also be ubiquitinated and removed by the UPS in coordination with an ATpase complex, valosin-containing protein (VCP) in mammals or CDC48 in yeast. in addition to the removal of damaged proteins, UPS also regulates mitochondrial dynamics by degrading mediators of the fission and fusion processes through MITOL-mediated and MUL1-mediated ubiquitination[302,303].

Misfolded proteins within the mitochondrial compartments are processed by a set of chaperones and proteases that are found exclusively in mitochondria for either refolding or degradation[304]. When chaperones fail in refolding and restoring the function of compromised proteins, a set of proteases are present within the mitochondrial matrix, as well as on both sides of the inner membrane, to eliminate dysfunctional proteins before they threaten the functionality of the organelle[305]. Soluble chambered proteases that form ring-shaped structures have their active domains inside the barrel-shaped oligomer; thus, proteins need to be unfolded and inserted inside the chamber for degradation through an ATP-dependent reaction. This chaperone-like activity can be an intrinsic property of the complex, as it is for the two mitochondrial AAA proteases, i-AAA and m-AAA, and the Lon proteases[306], or provided by a second enzyme, such as in the CLPX–CLPP complex[307]. The key role of the CLPX–CLPP complex in maintaining mitochondrial homeostasis emerged from studying the CLPP-knockout mouse[308,309].

When the mitochondrial quality control mechanisms fail to resolve protein aggregation, and dysfunctional proteins accumulate at levels that are incompatible with the functionality of the organelle, terminally damaged mitochondria can be removed and degraded by a selective form of macroautophagy, termed mitophagy.



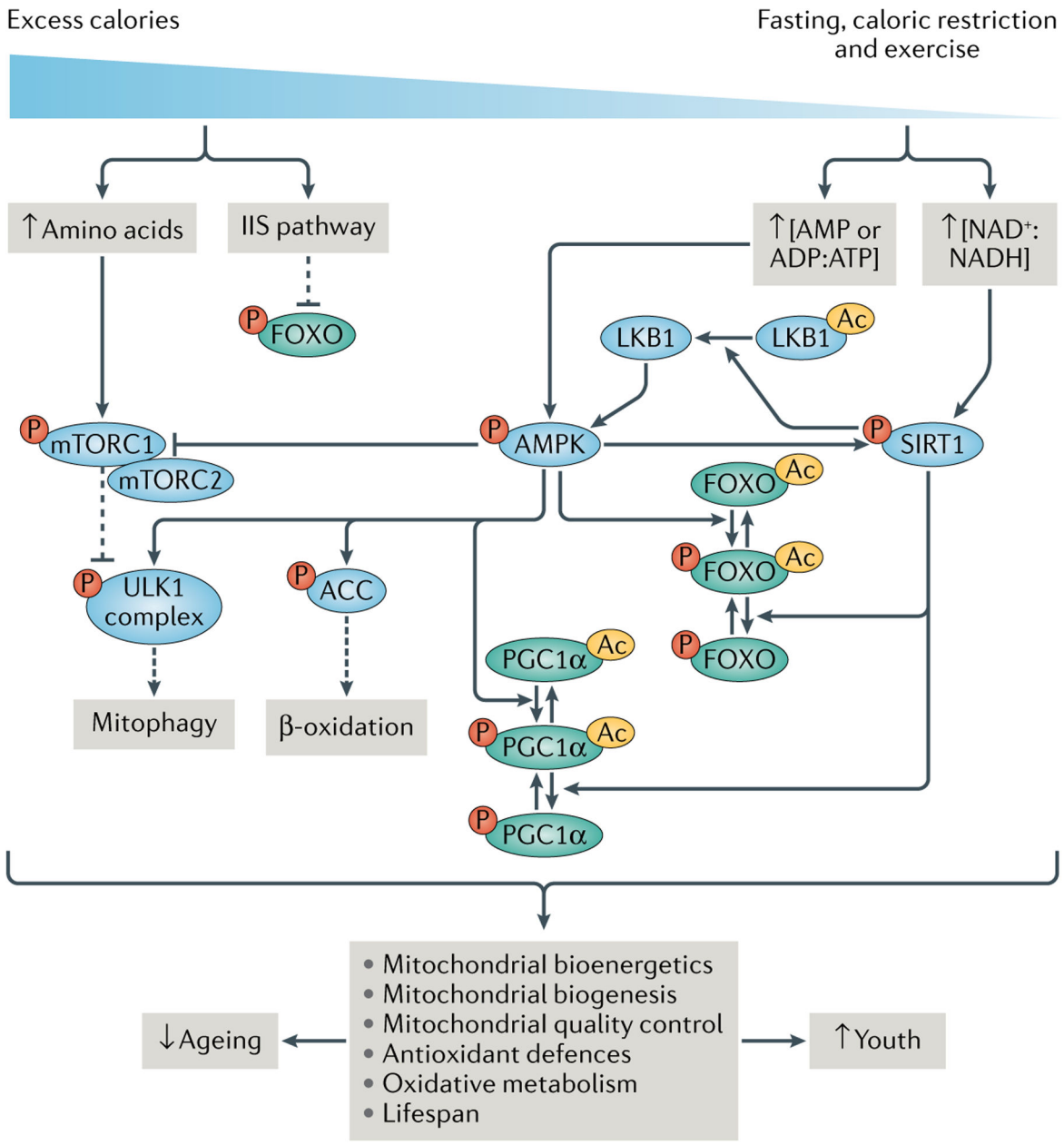


Fig. 1 |. Longevity signalling pathways control mitochondrial health.

Caloric restriction reduces the concentrations of glucose, amino acids and lipids, while raising the concentrations of key metabolites, such as NAD^+ and AMP. These metabolites modulate the activity of metabolic sensors, such as the sirtuins (SIRTs), AMP kinase (AMPK), the target of rapamycin (TOR) and insulin–IGF1 signalling (IIS). Downstream to metabolic sensors, transcription factors, such as forkhead box O (FOXO) and the peroxisome proliferator-activated receptor-γ coactivator 1α (PGC1α), orchestrate mitochondrial physiology and homeostasis. Dysregulation of this multilevel regulatory system reduces mitochondrial homeostasis, resulting in frailty and disease. Ac, acetyl group; ACC, acetyl-CoA carboxylase; LKB1, liver kinase B1; ULK1, UNC51-like kinase 1.

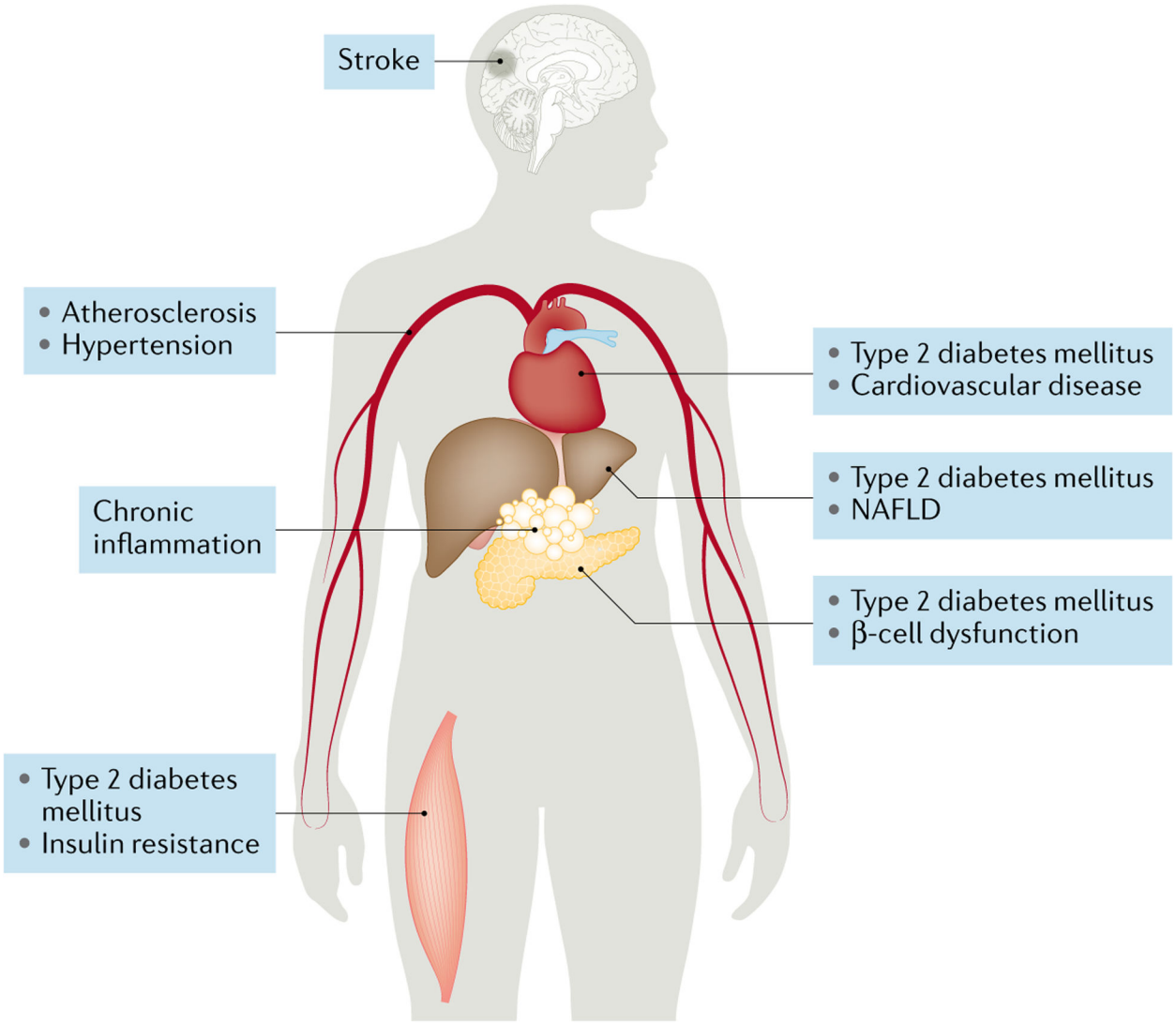


Fig. 2 |. Mitochondrial dysfunction in age-related metabolic diseases.
Mitochondrial dysfunction during ageing predisposes the elderly to age-related metabolic diseases. Diseases associated with metabolic deregulation can occur in various tissues, especially organs that have high energy demands. NAFLD, non-alcoholic fatty liver disease.

Table 1 |

Current clinical status of therapies targeting mitochondria in age-associated metabolic dysfunction

Drug type	Drug name	Most advanced stage	Refs
Small molecule	Resveratrol	Clinical	294
	SRT1720	Preclinical	231
	SRT2104	Clinical	232
	Punicalagin	Preclinical	233
	Epicatechin	Clinical	295
	Oleuropein	Preclinical	296
	Metformin	Clinical	297
	Imeglimin	Clinical	241
	TPP–thiazole	Preclinical	242
	Rapamycin	Clinical	243
	Leucine	Clinical	249
NAD^+-boosting molecules	Nicotinamide mononucleotide	Clinical	256
	Nicotinamide riboside	Clinical	298
	Nicotinamide	Preclinical	260
Mitochondrial uncouplers	DNP methyl ether	Preclinical	267
	Controlled release mitochondrial protonophore	Preclinical	268
	Niclosamide ethanolamine	Preclinical	269
	1,3-bis(dichlorophenyl)urea	Preclinical	270
	CZ5	Preclinical	271
	OPC-163493	Preclinical	272
	BAM15 and derivates	Preclinical	273–275
	Compound 6j	Preclinical	276
	HU6	Clinical	277
Mitochondrially-targeted antioxidants	Mitochondrially-targeted coenzyme Q	Clinical	286
	Mitochondrially-targeted vitamin E	Preclinical	282
	Elamipretide	Clinical	293