Tipping the metabolic scales towards increased longevity in mammals

Celine E. Riera and Andrew Dillin

A hallmark of ageing is dysfunction in nutrient signalling pathways that regulate glucose homeostasis, negatively affecting whole-body energy metabolism and ultimately increasing the organism's susceptibility to disease. Maintenance of insulin sensitivity depends on functional mitochondrial networks, but is compromised by alterations in mitochondrial energy metabolism during ageing. Here we discuss metabolic paradigms that influence mammalian longevity, and highlight recent advances in identifying fundamental signalling pathways that influence metabolic health and ageing through mitochondrial perturbations.

Life expectancy has been strikingly prolonged in developed countries as a positive outcome of medical progress and modern lifestyle. Nonetheless, with old age comes a wide range of age-associated diseases including neurodegenerative diseases, cardiovascular and metabolic disorders, and higher susceptibility to cancer.

Although the clinical manifestations of ageing are well understood, the genetic or environmental causes driving age-associated decline in healthspan remain difficult to isolate. By identifying genetic perturbations that can extend lifespan as well as retard the onset of physiological decline in invertebrate model organisms, ageing research has started to provide therapeutic strategies to prolong both healthspan and lifespan[1]. Research over the last decade using the multitude of spatial and temporal genetic tools available in mice has begun to identify points of convergence between mammals and invertebrates. In comparison to humans, mice are relatively short-lived mammals that suffer from clinically relevant pathologies including metabolic syndrome, osteoporosis, cancer, sarcopenia, cardiovascular dysfunction and neurodegeneration[2,3]. Molecular and cellular pathways have been identified that not only increase longevity of mammalian model organisms but also generally promote their healthspan by delaying the onset of metabolic decline. This delay is tightly coupled to an age-dependent deterioration of insulin metabolism and subsequent disruption of nutrient uptake by body tissues. Mitochondria play a key role in the utilization of nutrient substrates for energy production. Alterations in mitochondrial biogenesis, dynamics and integrity render an organism unable to maintain metabolic homeostasis or properly respond to metabolic demand observed during ageing.

In this Review, we provide an overview of the metabolic signalling pathways that modulate mammalian lifespan and discuss their potential as therapeutic targets to treat age-associated loss in glucose homeostasis and healthspan. Finally, we present key mitochondrial pathways that have been implicated in age-dependent malfunction of metabolic homeostasis.

Insulin action regulates metabolic health during ageing

The pathologies encountered in mouse longevity studies revealed a dominant role for glucose homeostasis in regulating metabolic health during ageing. In humans, ageing is also linked to increased visceral fat mass, loss in lean mass and a deterioration in insulin sensitivity resulting in glucose intolerance — all factors that favour the occurrence of metabolic syndrome and cardiovascular diseases[4,5]. Similarly, in mice, signs of physiological decline are characterized by an increase in fat mass and the progressive development of glucose intolerance, reflecting a similar impairment of insulin sensitivity[6,7]. However, mild insulin resistance has been observed in a variety of long-lived mice and is therefore not necessarily an indicator of poor health or shortened lifespan when paired with improved glucose tolerance[7–10]. Over the last decade, elevated energy expenditure has emerged as a putative longevity biomarker, as it has been associated with lifespan extension[11]. In old animals, a decrease in energy expenditure is associated with reduced activity and endurance linked to decreased oxygen consumption and lipid accumulation in non-adipose tissue. The respiratory exchange ratio (RER), obtained by indirect calorimetry, compares the volume of carbon dioxide an organism produces to the volume of oxygen consumed over a given time and varies inversely with lipid oxidation. In young and healthy mice, the RER displays a youthful circadian shift from night to day, reflecting the daily transition from carbohydrate to lipid metabolism. Old mice, however, develop a substrate preference towards lipids, losing the capacity to switch between fuel sources (Fig. 1). This is in accordance with a metabolic flexibility theory of ageing, which predicts that longevity depends on metabolic health, and lifespan extension is achieved by maintaining a youthful RER, thus

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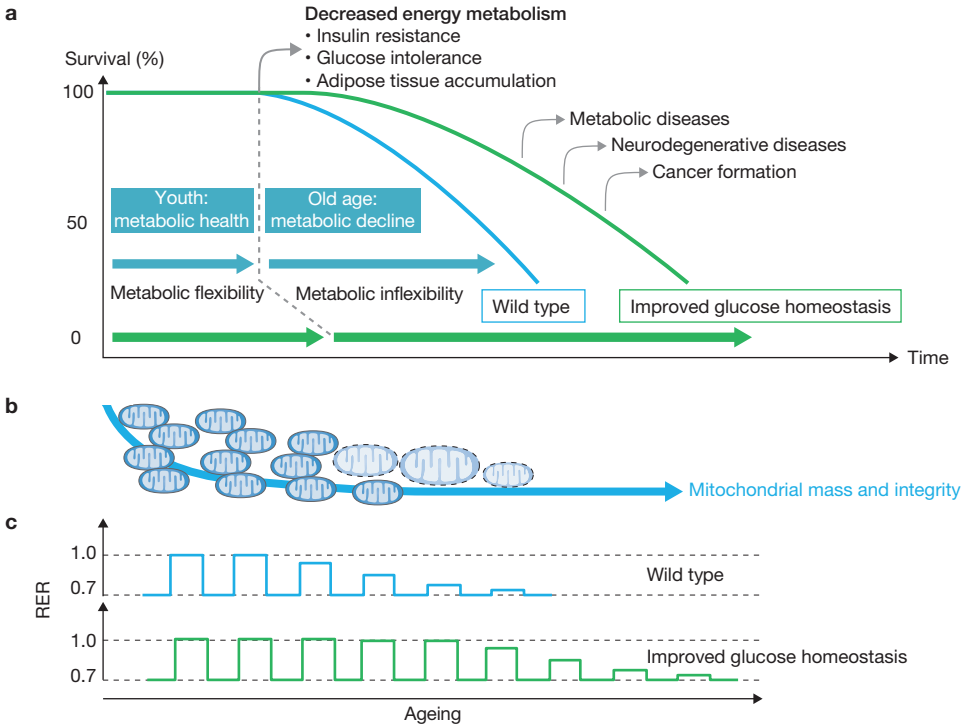

Figure 1 Metabolic flexibility controls healthspan and lifespan. (**a**) A schematic showing survival curves of a wild-type mouse (blue curve) compared to a long-lived genetic model of improved glucose homeostasis (green curve). The onset of metabolic decline precedes the appearance of morbid pathologies associated with insulin resistance and nutrient uptake. (**b**) Throughout lifespan, mitochondrial biogenesis, function and dynamics decline. Accumulation of oversized abnormal mitochondria which result from impaired degradation of dysfunctional mitochondria is observed after the onset of metabolic inflexibility. (**c**) Metabolic flexibility correlates with a healthy respiratory exchange ratio (RER) resulting from the circadian shift between carbohydrate (value of 1.0) to lipid metabolism (value of 0.7). Metabolic inflexibility observed at old age correlates with a substrate preference towards lipids, reflecting an inability to adapt fuel oxidation to fuel availability.

protecting the organism against systemic damage associated with fat metabolism and storage. In contrast, the onset of metabolic inflexibility corresponds to aberrant glucose homeostasis and increased susceptibility to age-onset diseases in response to an age-associated decline in mitochondrial energy metabolism[12]. Another mechanism that may contribute to the deterioration of metabolic health with age operates through the oscillations of the circadian clock. Modulating the activity of the major circadian regulators BMAL1 (also known as ARNTL; aryl hydrocarbon receptor nuclear translocator-like) and CLOCK (clock circadian regulator) results in premature ageing[13], whereas enhanced transcriptional activity of these factors protects against age-associated decline in adapting to new circadian periods[14]. Most physiological, endocrine and behavioural rhythms are governed by the molecular clock, and accumulating evidence indicates that disruption of circadian rhythms is associated with cancer[15]. Future research is required to shed light on how the rhythmicity of nutrient homeostatic processes affects metabolic flexibility and the ageing process.

Genetic manipulation of insulin metabolism in mouse lifespan and healthspan

To date, dietary restriction is the most robust intervention to increase healthspan and lifespan in multiple organisms including rodents and primates, delaying the onset of age-related diseases[2,16,17]. In mice, dietary restriction increases insulin sensitivity and induces a healthy transition of the RER, due to a more dramatic shift from carbohydrate oxidation and endogenous fatty acid synthesis in the feeding phase to fatty acid oxidation during resting[18]. Similarly to dietary restriction, multiple genetic manipulations in mice improve metabolic flexibility and longevity. Alterations of components in the insulin and insulin-like growth factor signalling (IIS) pathway that dampen insulin signalling promote longevity associated with improved insulin sensitivity. Some of these long-lived mice include fat-specific insulin receptor knockout (FIRKO) mice, hypomorphic PI3K mice, and mice overexpressing the liver hormone FGF21 (fibroblast growth factor 21), which antagonizes growth hormone (GH) and insulin-like growth factor 1 (IGF-1)[19–21]. Reduction of circulating IGF-1 positively correlates with increased longevity in inbred mouse strains[22], but is also linked to a reduced body size and even dwarfism[23].

Although Ames (*Prop1*^{df}/*Prop1*^{df}) and Snell (*Pit1*^{dw}/*Pit1*^{dw}) dwarf mice are extremely long-lived presumably partially because of decreased IIS, their metabolic characteristics must be interpreted with caution. This is because profound deficiency in anterior pituitary function results in other hormonal changes and negatively affects fertility, metabolic fitness, adiposity, glucose tolerance and insulin secretion, despite considerable life extension[24,25]. In humans, hypothyroidism is also characterized by diminution of the acute insulin response, resulting in impaired glucose tolerance[26], but the effects of this condition on human longevity are unclear. Thus, the lifespan extension of reduced IIS models must be uncoupled from their growth delay or hormonal imbalances to isolate clinically relevant therapeutic targets.

Deletion of TRPV1 (transient receptor potential vanilloid 1) pain receptors in peripheral nerves innervating pancreatic β cells extends mouse longevity by improving metabolic flexibility and in particular elevating prandial insulin secretion with age, without the corresponding growth delay or altered body weight observed in decreased IIS models[7]. In humans, metabolic syndrome is linked to dementia and increased incidence of multiple cancers[27,28]. Strong correlations between insulin sensitivity and cancer formation have also been found in the mouse, and reduced cancer incidence is observed in mice with improved energy expenditure at old age[7,29]. Mice carrying a TRPV1 mutation have both reduced cancer incidence and delayed onset of cognitive decline with age. Other genetic manipulations that reduce IIS meet these criteria to some extent. Long-lived Ames dwarf mice and GHR-BP (growth-hormone receptor binding protein) knockout mice display a lower incidence and delayed onset of certain cancers and tumours, increased insulin sensitivity and improved cognitive function at old age[30–32]. Additionally, prolonged rapamycin treatment delays cancer formation in aged mice and extends lifespan through inhibition of mTOR (mammalian target of rapamycin), a major cellular nutrient sensing pathway that regulates cell growth[33,34] and integrates insulin signalling and cell stress signals[35]. The method by which rapamycin regulates the rate of ageing is complex, because short-term treatment results in immunodeficiency and favours glucose intolerance and insulin resistance[36,37]. However, prolonged treatment results in improved metabolic profiles, increased oxygen consumption and ketogenesis, and markedly enhanced insulin sensitivity[38]. Robust genetic inhibition of downstream mTOR complex 1 (mTORC1) components, including deletion of ribosomal protein S6K1 (S6 kinase 1) and enhanced autophagy, lead to increased longevity with improved glucose tolerance and insulin sensitivity at mid-age[39,40]. However, despite the consensus that dampening mTORC1 activity extends lifespan, several studies report that this beneficial effect is achieved mostly by slowing cellular growth and cancer incidence, without impacting insulin metabolism[36,41]. These reports suggest that disruption of the other major mTOR complex, mTORC2, mediates aspects of the impaired insulin metabolism associated with rapamycin treatment, in particular the inability to suppress hepatic gluconeogenesis following insulin release. It is therefore unclear how prolonged rapamycin exposure promotes metabolic health, despite the recent report that insulin sensitivity amelioration following rapamycin treatment might rely on inhibitory effects of both mTOR complexes[38]. Better resolution of the mechanism through which rapamycin positively modulates glucose homeostasis is required to design drugs that can recapitulate the longevity extension obtained by rapamycin treatment without its detrimental side effects.

Mitochondria at the core of metabolic flexibility during ageing

Even though cellular energy can be rapidly generated through anaerobic glycolysis, it mostly originates from aerobic oxidation of carbohydrates and fatty acids in the mitochondria, highlighting this organelle's critical role in regulating global metabolic homeostasis. Not only is insulin secretion from pancreatic β cells mitochondria-dependent, but insulin signalling also relies on mitochondria to metabolize glucose in energy-consuming cells. Mitochondrial activity regulates gluconeogenesis and triglyceride synthesis in the liver and lipolysis in adipose tissue, as well as many other vital processes[42]. Accordingly, mitochondrial dysfunction or damage can greatly perturb metabolic flexibility and insulin sensitivity, impacting metabolic human diseases and ageing[12,43,44]. During the ageing process, mitochondrial biogenesis, function and dynamics decline (Fig. 2)[45,46]. This is particularly obvious in skeletal muscle — a highly metabolically active tissue extremely rich in mitochondria — which undergoes sarcopenia, a critical loss in mitochondrial volume density and decline in physical function with age[47]. Accumulation of lipids in skeletal muscle, liver and adipose tissue is indicative of decreased metabolic flexibility with ageing and therefore demonstrates a global reduction in oxidative phosphorylation capacity and mitochondrial biogenesis.

Molecular targets that can restore mitochondrial function and metabolic homeostasis at old age have emerged over the past decade. A key regulator of mitochondrial biogenesis and energy metabolism is the transcriptional coactivator PGC1α (proliferator-activated receptor γ coactivator α)[48,49]. Decreased PGC1α expression has been implicated in muscle insulin resistance in humans and diabetic mouse models[50,51]. Increasing PGC1α content in mouse skeletal muscle preserves oxidation phosphorylation, preventing muscle wasting and improving metabolic fitness by inhibiting insulin resistance and fat accumulation with age[52]. Mechanistically, PGC1α acts by regulating the activity of several transcription factors — including the nuclear respiratory factors NRF1 and NRF2 and mitochondrial transcription factor A (TFAM) — that in turn directly or indirectly induce genes related to mitochondrial biogenesis and respiration[53,54]. AMP-activated protein kinase (AMPK)-mediated phosphorylation activates PGC1α in conditions of low cellular energy, which corresponds to a high AMP/ATP ratio, therefore inhibiting anabolic processes consuming energy and activating catabolic pathways producing energy, such as fatty acid oxidation and respiration[55,56]. Strategies to directly manipulate AMPK to improve mitochondrial function in skeletal muscle have proven successful and offer important avenues to treat age-associated metabolic decline. Pharmacological AMPK activation by AICAR (5-Aminoimidazole-4-carboxamide ribonucleotide) treatment enhances exercise endurance[57] and increases mitochondrial biogenesis and respiration in mouse models of cytochrome *c* oxidase (COX) deficiency, rescuing the limited exercise capacity of these mice[58]. Importantly, activating AMPK by metformin supplementation is also linked to lifespan extension[59], although metformin action on lifespan might depend on other targets such as mTOR (ref. 60). Under low energy conditions, AMPK also increases the ratio of nicotinamide adenine dinucleotide NAD^+/NADH, a metabolic signal that triggers the NAD-dependent deacetylase SIRT1 (sirtuin 1) and is required for AMPK-dependent PGC1α activation[61]. AMPK and SIRT1 can autoregulate each other, a mechanism that has been postulated to improve the cellular response to low-energy states[62], and that orchestrates a complex catabolic response that increases mitochondrial biogenesis, enhances antioxidant defence and improves fatty-acid oxidation.

Mitochondrial oxidative stress and ageing

Mitochondrial networks are vulnerable to oxidative stress, resulting in impaired cellular function and increasing the chance of cell death. During ageing, mitochondrial electron transport chain (ETC) efficiency decreases, reducing ATP generation through increased proton leakage. Higher metabolic intensities correlated with higher proton conductance across the inner membrane of skeletal muscle mitochondria were found in long-lived mice, supporting the 'uncoupling to survive' theory[11].

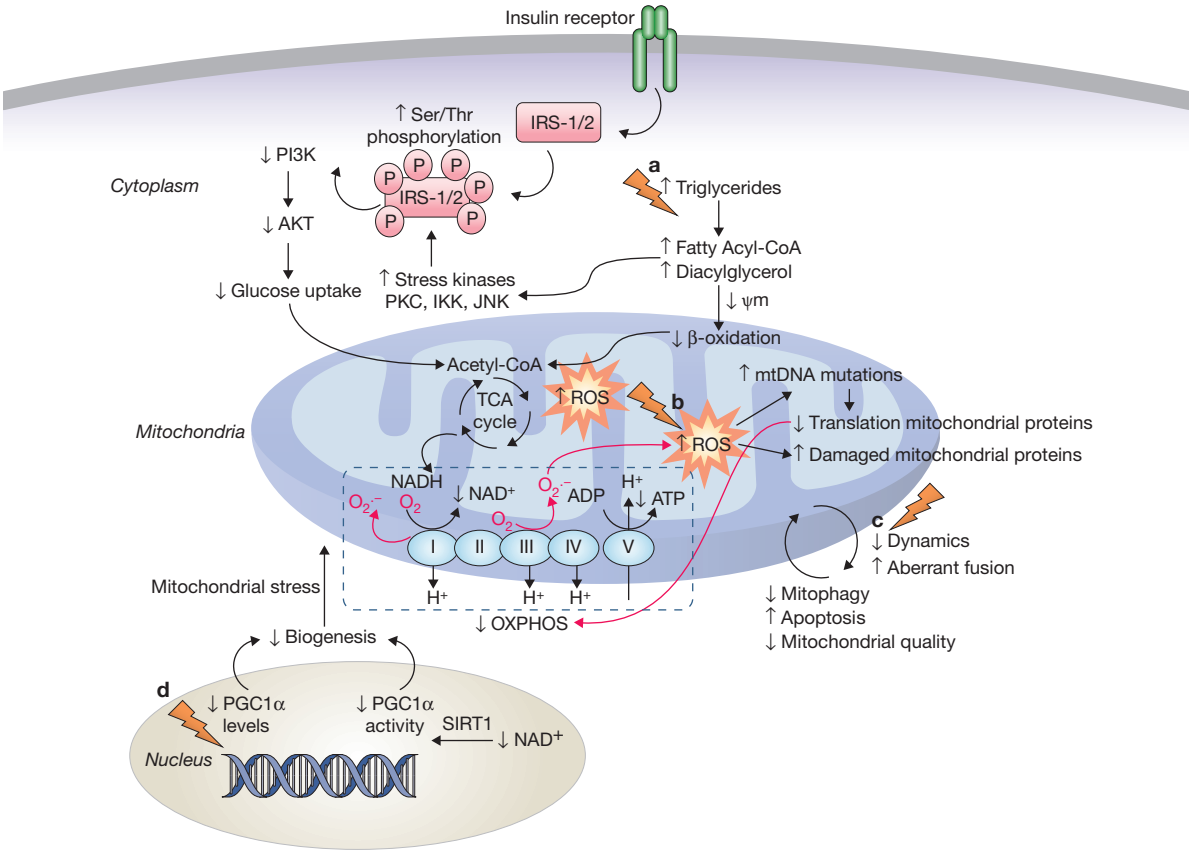

Figure 2 Effect of ageing on mitochondrial function. Multiple sources of damage (orange bolts, **a**–**d**) cause senescent mitochondrial phenotypes. (**a**) Nutrient surplus promotes the accumulation of lipid metabolite intermediates that overload and hyperpolarize the mitochondria. Incompletely oxidized lipid products from the tricarboxylic acid cycle (TCA) and reactive oxygen species (ROS) then build up, which causes oxidative stress to the respiratory system (OXPHOS; simplified electron transport chain shown for illustrative purposes). These intermediates also activate stress-sensitive kinases (protein kinase C, PKC; c-Jun N-terminal kinase, JNK; and IκB kinase, IKK), which phosphorylate insulin receptor substrates (IRS-1/2). This disrupts the insulin signalling pathway, and glucose uptake and glycolysis in response to insulin. (**b**) ROS accumulation causes mutations to the mitochondrial DNA (mtDNA) pool, affecting mitochondrial translation and damage to proteins vital to OXPHOS and mitochondrial dynamics. (**c**) Impaired dynamics and decreased mitophagy with age leads to aberrant mitochondria. (**d**) Mitochondrial biogenesis, controlled by PGC1α, declines with age. Insulin resistance contributes to the development of a vicious cycle of a negative feedback loop by impairing biogenesis in addition to the TCA cycle and OXPHOS.

However, proton leakage in the ETC also diminishes the amount of reactive oxygen species (ROS) produced, with ROS being by-products of mitochondrial ATP generation resulting from oxygen reduction. Thus, reduction of ROS levels seems to be beneficial by dampening oxidative damage to mitochondria. The recruitment of uncoupling proteins (UCPs) relieves mitochondrial ROS by uncoupling the proton gradient. In particular, UCP1, found in metabolically active tissues such as brown fat, is activated by fatty acids and dissipates the proton gradient as heat, with a low rate of ATP production allowing fast substrate oxidation and thermogenesis[63]. The production of superoxide (O_2^-) — a toxic by-product of respiration generated mostly by complexes I and III of the ETC — may also induce the uncoupling of mitochondria by activating UCP1 (or other UCPs) under certain conditions requiring fatty acids and inhibited by purine nucleotides[64]. Accordingly, increasing energy expenditure by UCP1 overexpression in mouse muscle has beneficial effects on metabolic health, extending median but not maximal lifespan, decreasing adiposity, increasing both core temperature and metabolic rate, and lowering lymphoma incidence[65].

The 'free radical' theory of ageing proposes a causative role for ROS in accumulated cellular damage over time, and in age-associated pathologies and functional decline of tissues. Supporting this theory, decreased ROS production from mitochondria has been observed in response to dietary restriction and linked to improved mitochondrial function in rodents. Despite the initial claim that increased respiration following dietary restriction is achieved by higher mitochondrial biogenesis[66], dietary restriction is now thought to preserve mitochondrial function by maintaining the integrity of existing cellular components and increasing the activity of oxidant scavengers such as catalase[67]. Mice expressing human catalase in mitochondria are protected from oxidative damage, cardiac dysfunction and cataract development, and exhibit extended longevity[69]. However, a conflicting study reports that overexpression of human catalase in all tissues is insufficient to extend lifespan[70]. PGC1α also minimizes the build-up of ROS through the transcriptional regulation of numerous ROS-detoxifying enzymes[68]. Other genetic models challenge the theory that a linear relationship exists between decreasing ROS and lifespan extension. Increasing mitochondrial ROS and oxidative damage do not cause progeria in mice[71,72], and multiple mouse models of increased antioxidant defences also do not extend lifespan[70]. Furthermore, mice expressing a defective mitochondrial polymerase develop mitochondrial DNA (mtDNA) mutations

that accelerate ageing by impairing mitochondrial function without increasing ROS[73–75]. Although the mtDNA mutator mouse studies initially challenged the free radical theory of ageing, recent conflicting evidence suggests that oxidative damage actually occurs in skeletal muscle of these mice[76]. Following these controversies, the role of ROS in ageing is being revisited, mainly supported by evidence obtained in *Caenorhabditis elegans* suggesting that high ROS levels may act as an important signal that triggers a gene expression pattern promoting survival under stressful conditions[77].

Activation of the mitochondrial UPR and longevity

Long-lived mouse models provide supporting evidence for the role of improving mitochondrial biogenesis and function in forestalling ageing. Paradoxically, in *C. elegans*, lifespan can be increased by reducing mitochondrial respiration through reduced function of nuclear genes encoding ETC components[78,79]. In particular, neuronal knockdown of COX activates the mitochondrial unfolded protein response (UPR^{mt}), a mitochondrial proteostasis mechanism that confers increased longevity to worms[80,81]. This pathway is part of the mitochondrial quality control machinery that detects misfolded proteins in the mitochondria, initiating a signalling cascade that leads to the transcription of protective genes[82]. Notably, 13 subunits of the oxidative phosphorylation complex are encoded by mtDNA, whereas the remaining mitochondrial proteins (of which there are more than 1,000) are encoded by nuclear DNA and imported into mitochondria to achieve proper folding. The UPR^{mt} senses imbalances in the stoichiometry of proteins in the mitochondrial compartment and relieves the stress by retrograde signalling to the nucleus through the degradation of misfolded proteins into peptides by the ClpP1 protease, consequently promoting transcriptional activation of nuclear-encoded chaperones residing in the mitochondrial matrix, such as heat shock proteins 60 (HSP60) and 70 (HSP70). This surveillance pathway is conserved in mammalian cells[83], but whether it plays a pro-longevity role in mammals is unknown. In support of its possible role in mouse lifespan regulation, low expression of a mouse mitochondrial ribosomal protein (Mrps5) triggers the UPR^{mt} and correlates with increased lifespan of inbred progenies of a cross between C57BL/6J and DBA/2J mice[84].

Evidence from mouse longevity studies suggests that impairing mitochondrial function can improve lifespan; however, it is unknown whether the UPR^{mt} is causal for the longevity increase. Constitutive ablation of SURF1 (surfeit 1), a putative assembly factor specific to COX, results in mild deficiency of COX in mice and significantly extended mouse longevity[85]. This is accompanied by a strong depletion of PGC1α levels, mtDNA content, citrate synthase activity and oxidative-phosphorylation-related genes in these mice, indicative of a decrease in respiration and mitochondrial biogenesis[58]. Reduction of COQ7 (coenzyme Q7, known as Mclk1 in mice), an enzyme responsible for the biosynthesis of ubiquinone (an ETC component), also extends mouse lifespan concomitant with ETC dysfunction and decreased mitochondrial coupling of respiration and ATP synthesis[86]. Although these studies confirm that decreased respiration has beneficial effects on mouse longevity, it is unknown whether the UPR^{mt} following mitochondrial dysfunction is required for longevity.

Strikingly, levels of NAD^+ were also decreased in the $Mclk1^{+/-}$ mice. It is well established that NAD^+ plays crucial roles in mediating mitochondrial energy metabolism by donating electrons to the ETC or by acting as a coenzyme for rate-limiting citric acid (TCA) cycle enzymes. Increasing NAD^+ levels, by supplementation of a NAD^+ precursor or through a drug such as resveratrol, improves mitochondrial function by activating SIRT1 in mice, and protects against the metabolic damage of high-fat feeding[87–90]. Thus, high NAD^+ levels correspond to mitochondrial biogenesis and metabolic flexibility, ultimately improving healthspan. Paradoxically, $Mclk1^{+/-}$ mice are long-lived despite their depleted NAD^+ levels. The mechanisms underlying this lifespan extension are unclear, and it is plausible that a different longevity paradigm independent of mitochondrial biogenesis, such as UPR^{mt}, is required to promote lifespan of these mice. The relationship between UPR^{mt} and mitochondrial biogenesis is, however, still under debate. Rapamycin and resveratrol treatment, which extend lifespan and metabolic fitness, increased respiration in mammalian cells and induced the UPR^{mt} (ref. 84). Thus, it is unclear whether UPR^{mt} in mammals predicts longevity and can be triggered as a cell survival mechanism following severe mitochondrial impairment, or as a response to mitonuclear imbalance that facilitates biogenesis. More investigation is necessary to elucidate the physiological implication of the UPR^{mt} in mammalian ageing.

Insulin resistance, mitochondrial perturbations and dynamics

Mitochondrial dysfunction has been implicated strongly in the onset of metabolic diseases. Cells cope with nutrient supply by increasing mitochondrial content, but persistent nutrient surplus can overload the mitochondria and cause dysfunction, a phenomenon also observed with ageing, as mitochondrial capacity declines (Fig. 2). This functional decline coincides with a global reduction in oxidative capacity of the skeletal muscle of patients with type 2 diabetes[91], and contributes to insulin resistance[51]. Energy excess overloads and hyperpolarizes mitochondria, leading to the accumulation of fatty-acyl-CoA and diacylglycerol, causing excessive production of ROS. These mitochondrial metabolites activate stress-sensing-kinases such as c-Jun N-terminal kinase and protein kinase C in the cytoplasm (which phosphorylate insulin receptor substrates), impairing insulin signalling in glucose-consuming tissues such as skeletal muscle and liver[92,93]. Insulin-stimulated glucose transport is inhibited in these tissues and reduces peripheral energy metabolism.

Furthermore, decreased brain insulin signalling is also observed in ageing and contributes to neurodegenerative disorders, as a consequence of insulin resistance and decreased insulin transport through the blood–brain barrier[94]. Abnormal insulin metabolism in the brain might originate from mitochondrial dysfunction, as the brain is highly vulnerable to oxidative stress and elevated ROS levels can aggravate insulin resistance[95,96]. Similar observations were made in the hypothalamus and hippocampus of mice fed a high-fat diet[97,98]. Downregulation of the mitochondrial chaperone protein HSP60 by excessive adipokine stimuli plays a central role in the onset of oxidative stress leading to mitochondrial dysfunction and hypothalamic insulin resistance[99]. Whether chaperone levels are also regulated by age-associated stress in the brain and confer mitochondrial dysfunction and deficits in brain energy metabolism remains undetermined.

Mitochondrial architecture is tightly regulated by the dynamic opposing processes of fusion and fission. Mitochondrial dynamics reflect bioenergetic adaptation to metabolic demand, facilitating mixing and exchange of mitochondrial contents such as mtDNA or metabolites[100]. Mitochondrial morphology and density are modulated

by variations in nutrient availability, with starvation triggering a fused state that facilitates autophagy[101]. These changes in mitochondrial architecture can affect oxidative phosphorylation complex assembly and ATP synthesis, allowing mitochondria to reorganize and dispose of damaged elements through mitophagy, an autophagic process that eliminates damaged mitochondria[102]. Mitophagy prevents cell apoptosis or necrosis following excessive ROS accumulation, pro-inflammatory signals or permeabilization of mitochondrial membranes, but the efficiency of autophagy declines with age[103]. Fission allows the segregation of damaged mitochondria and their recycling through autophagic processes, thereby ensuring mitochondria turnover and cellular viability, whereas fusion counterbalances functional defects and allows genetic complementation. Oversized abnormal mitochondria are present in senescent cells, as a result of dysfunctional mitochondrial degradation[104]. Youthful mitochondrial fragmentation can be restored in senescent cells by depleting critical mediators of fusion, the dynamin-related proteins FIS1 (fission 1 (mitochondrial outer membrane) homolog) and OPA1 (optic atrophy 1). Recently, lack of the pro-fusion mitofusins Mfn1 and Mfn2 has been implicated in regulation of whole-body energy homeostasis in distinct populations of hypothalamic neurons in mice[105–107]. Mfn2 deficiency prevents changes in mitochondrial dynamics and communication with the endoplasmic reticulum (ER), causing ER stress, insulin resistance in skeletal muscle and liver tissues, and hypothalamic leptin resistance, thus promoting obesity[105–109]. Mitochondrial health relies on a tight contact with the ER that provides critical resources to the mitochondria such as mitochondrial lipids and calcium ions, which are involved in mitochondrial dynamics and regulation of the TCA cycle. Although gradual impairment of mitochondrial dynamics with age is a proposed hypothesis for age-associated mitochondrial dysfunction, additional layers of regulation may exist, including ER-mitochondria tethering and impaired insulin signalling due to ER stress, resulting in cell-autonomous inflammation (as observed in type 2 diabetes)[110,111]. One of the main pro-inflammatory transcriptional programs induced following ER stress is the NF-κB (nuclear factor κB) signalling pathway[112]. Interestingly, restoring hypothalamic immunity upon ageing through the blockade of NF-κB signalling in microglia is sufficient to extend lifespan in mice[113]. It will be critical to define whether metabolic decline during ageing is mediated by the disruption of intricate connections between mitochondria and the ER, and the onset of ER stress and inflammation.

Conclusions and future perspectives

In light of the evidence reviewed here, progressive mitochondrial dysfunction is implicated in the deterioration of insulin sensitivity during normal ageing, consequently impairing insulin signalling in central and peripheral tissues, and contributing to a loss of metabolic flexibility. Thus, restoring mitochondrial biogenesis is likely to provide a therapeutic avenue to rejuvenate metabolism. However, in conflicting studies, reduced respiration and biogenesis throughout life can also increase lifespan, indicating an additional level of complexity. Epigenetic regulation is now emerging as a potent factor influencing mitochondrial biogenesis or integrity following the lack of exercise and overfeeding. Notably, DNA hypermethylation suppresses the expression of Pgc1α and Tfam in mice, and microRNAs have the ability to silence gene expression of Ucp2 and Mfn2, thus negatively affecting biogenesis, oxidative phosphorylation and mitochondrial dynamics[114]. Whether such epigenetic signatures are characteristic of the ageing process remains poorly understood; thus, it will be essential to assess the chain of events that leads to impaired transcriptional networks that maintain biogenesis (nuclear and mitochondrial transcription) as well as mitochondrial quality control machinery (dynamics, mitophagy and the UPR^{mt}). In particular, we are beginning to envision that these processes are likely to co-regulate each other, as highlighted by the reliance on fusion and fission events in order to remove damaged mitochondria through mitophagy and the dependence of biogenesis on protein translocation resulting from mitochondrial dynamics. These preliminary discoveries are identifying novel therapeutic perspectives in the effort to delay the onset of metabolic decline and age-associated metabolic diseases.

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